

## Open Access

Berl Münch Tierärztl Wochenschr 127,  
177–182 (2014)  
DOI 10.2376/0005-9366-127-177

© 2014 Schlütersche  
Verlagsgesellschaft mbH & Co. KG  
ISSN 0005-9366

Korrespondenzadresse:  
przemyslaw.cwynar@up.wroc.pl

Eingegangen: 05.12.2013  
Angenommen: 11.02.2014

Online first: 14.03.2014  
[http://vetline.de/open-access/  
158/3216/](http://vetline.de/open-access/158/3216/)

### Summary

### Zusammenfassung

U.S. Copyright Clearance Center  
Code Statement:  
0005-9366/2014/12705-177 \$ 15.00/0

Department of Environmental Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, Wrocław, Poland<sup>1</sup>

Department of Animal Physiology and Biostructure, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland<sup>2</sup>

## Effect of heat stress on physiological parameters and blood composition in Polish Merino rams

### *Einfluss von Hitzestress auf physiologische Parameter und die Blutzusammensetzung von polnischen Merino Widdern*

Przemyslaw Cwynar<sup>1</sup>, Roman Kolacz<sup>1</sup>, Albert Czerski<sup>2</sup>

The Merino sheep represents the most prevalent sheep breed in Poland and is one of the most genetic-stable sheep races. Therefore, the aim of the study was to determine the responses of Polish Merino sheep to three thermal conditions: thermoneutral (16.5°C, group I), mild heat stress (30°C, group II), and severe heat stress (50°C, group III). During the experiment heart rate (HR; beats/min), respiratory rate (RR; breaths/min), rectal temperature (RT) and skin temperatures (ST) were measured daily. The ST measured at four points of the animal body was mostly statistically different ( $p < 0.01$ ) between the treatment groups. It was found that the highest HR occurred in group II (30°C), and that it was higher than in groups I (16.5°C) and III (50°C) ( $p < 0.01$  for groups I, III). The RR was different ( $p < 0.01$ ) in all experimental groups (I, II, III) with the highest increase in group II. There was also a clear and significant ( $p < 0.01$ ) increase in RT during the heat stress phases. Blood analysis including morphology, biochemistry and hormones (adrenaline, noradrenaline, ACTH, and cortisol) was also performed. Significant differences in cortisol level in group II ( $p < 0.01$ ) and group III ( $p < 0.05$ ) were observed. There was an upward trend in adrenaline and ACTH as a result of increasing temperature. Differences in noradrenaline levels ( $p < 0.01$ ), with the highest level recorded in group III, were also observed. Additionally there was a strong upward trend in blood parameters, especially in hormone concentrations (cortisol, noradrenaline).

**Keywords:** Merino, heat stress, rectal temperature, skin temperature, blood

Das Merino Schaf ist die häufigste Schafrasse in Polen und ist eine der Rassen mit der höchsten genetischen Stabilität. Daher war es das Ziel der Studie, die Reaktionen polnischer Merino Schafe bei drei verschiedenen Temperaturbedingungen zu untersuchen: einer neutralen Temperatur (16,5 °C, Gruppe I), bei mildem Hitzestress (30 °C, Gruppe II) und bei sehr großem thermischem Stress (50 °C, Gruppe III). Während des Experimentes wurden die Herzfrequenz (HR, Schläge/min), die Atemfrequenz (RR; Atmenzüge/min), die rektale Temperatur (RT) und die Hauttemperatur (ST) täglich gemessen. Die Hauttemperatur (ST), gemessen an vier Punkten des Tierkörpers unterschied sich meist signifikant ( $p < 0,01$ ) zwischen den Gruppen. Die höchste HR wurde in Gruppe II (30 °C) gefunden, sie war höher als in den Gruppen I (16,5 °C) und III (50 °C) ( $p < 0,01$ ). Die RR war in allen Versuchsgruppen (I, II, III) unterschiedlich ( $p < 0,01$ ) mit dem höchsten Anstieg in der Gruppe II. Es gab einen signifikanten ( $p < 0,01$ ) Anstieg der RT während der Hitzestress-Phasen. Eine Blutanalyse einschließlich Morphologie, Biochemie und Hormonbestimmung (Adrenalin, Noradrenalin, ACTH und Cortisol) wurde ebenfalls durchgeführt. Es wurden hoch signifikante Unterschiede zwischen den Cortisolspiegeln in Gruppe II ( $p < 0,01$ ) und Gruppe

III ( $p < 0,05$ ) beobachtet. Ein Aufwärtstrend beim Adrenalin und ACTH als Folge der steigenden Außentemperatur war zu beobachten. Die Noradrenalin-Level unterschieden sich signifikant ( $p < 0,01$ ), wobei der höchste Wert in der Gruppe III auftrat. Es gab einen starken Aufwärtstrend bei den Blutparametern, vor allem bei den Hormonkonzentrationen (Cortisol, Noradrenalin).

**Schlüsselwörter:** Merino, thermischer Stress, Rektaltemperatur, Hauttemperatur, Blut

## Introduction

The phenomenon of mammalian sensitivity to heat, especially in small ruminants such as the Polish Merino, is widely reported in the literature (Caroprese, 2008; Hargreaves, 2008). In many studies a tolerated temperature range from  $-12^{\circ}\text{C}$  to  $32^{\circ}\text{C}$  is reported for an adult sheep (Silanikove, 2000; Srikandakumar et al., 2003; Caroprese, 2008). The extent of this tolerance largely depends on various factors, mainly on the physiological state of the organism and anionic-cationic compounds in the blood (Srikandakumar et al. 2003). Moreover, Rensis and Scaramuzzi (2003) similarly to Korczynski et al. (2004) strongly recommend to control the heat stress syndromes in animals when high environmental temperatures occur. Therefore, an animal's skin temperature (ST), as well as rectal temperature (RT) should be measured, as a fundamental indicator of thermal homeostasis of the body. The RT parameter is an excellent indicator of thermal balance of the body and is recommended as a research method by Rensis and Scaramuzzi (2003). The hyperthermia syndrome also causes respiratory alkalosis as an effect of hyperventilation or well known heat stress symptoms with the disturbance of the mineral balance of the animal's body, which should provide the blood control. Experiments conducted on Omani and Australian Merino confirmed a correlation between the environmental temperature and blood parameters (Srikandakumar et al., 2003). Unfortunately, the variety and severity of the stressors require to make not only basic blood analysis, but also complicated hormone or neurotransmitter tests, including cortisol and catecholamines (Nazifi et al., 1999; 2003). Detection of these stress hormones is a particularly important element in assessing the level of stress and has a direct relationship with the physiological state of the animals. Furthermore, EU legislation concerning farm animal welfare usually specifies only the minimum temperature for each species without the possibility of occurrence of hyperthermia and associated stress (Kiyatkin, 2005). Therefore, the aim of the present study was to determine the influence of heat stress in high temperatures ( $30^{\circ}\text{C}$  and  $50^{\circ}\text{C}$ ) on physiological parameters such as HR, RR, RT and ST in Polish Merino. Also, the endocrine and blood composition were examined to complement the knowledge of heat stress on farm animals.

## Material and Methods

### Animals and research location

15 mature Polish Merino rams (age 1 year  $\pm$  2 weeks, body weight  $40.1 \pm 1.4$  kg) from a national breeding farm certified by the Polish Sheep Association were used in

this study. The Polish Merino represents the most prevalent sheep breed in Poland and according to Nowicki et al. (2001) is one of the most genetic-stable sheep races, which was the main argument to use it in the study. Rams were maintained separately in three experimental groups (five sheep per group). The animals were kept in climatic chambers with thermal and acoustic isolation. The sheep were fed grain oats in doses of 0.2 kg per head (100% of intake), grass hay (Tab. 1) and water were provided ad libitum. Feeding and watering during the entire experiment were maintained at a constant level. A 14-hour period of electric daylight (08:00–21:00) was programmed. The chambers were equipped with autonomous vacuum ventilation systems composed of a thermostat, air conditioning and heating ventilators. Both temperature and humidity were measured by stationary and mobile electronic thermo-hygrometers. The relative humidity of 70% ( $\pm$  3%) was constant during every part of the experiment. Air movement in the chambers was controlled by anemometers and the mean velocity of air movement was 0.2 m/s ( $\pm$  0.1 m/s). All of the microclimatic parameters such as temperature, humidity and gases were under constant monitoring by electronic sensors using Visual System Scada Pro (Microbe, Poland). In order to ensure full control of the conditions in the chambers, a video monitoring system was also installed. Animal handling as well as experimental procedures were reviewed and accepted by the 2<sup>nd</sup> Local Ethical Committee of Experimental Procedures on Animals in Wroclaw, Poland (protocol no. 82/2009). The study was carried out in the Experimental Farm of Wroclaw University of Environmental and Life Sciences in Wroclaw (Poland).

### Experimental procedures

The experiment was divided into three identical parts for every group, each lasting 14 days: an adjustment period (group I) at  $16.5^{\circ}\text{C}$  ( $\pm$   $1^{\circ}\text{C}$ ), when the mean temperature-humidity index (THI) was 61, heat stress at  $30^{\circ}\text{C}$  ( $\pm$   $1^{\circ}\text{C}$ , THI 82) (group II) and heat stress at  $50^{\circ}\text{C}$  ( $\pm$   $1^{\circ}\text{C}$ , THI 112) (group III). The adaptation time to heat stress in sheep is rarely referenced in the literature; moreover it is usually unspecified. Rennefeld et al. (2010) pointed out that the mechanism of habituation, especially to painful factors, is still not well recognized. Therefore, a 14-day break between experimental phases was applied

**TABLE 1:** The chemical composition of feed for Polish Merino during experiment

Item	Dry matter (%)	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Ash (%)
Grain oats	88	11.8	8.9	4.1	3.1
Hay	91	20.4	21.3	3.8	9.4

in order to avoid the phenomenon of habituation to stress reactions, when all of the tested aspects of the animal health suggested its homeostasis. During the daily research (6:00–20:00; 14 hours/day), at temperatures of 30°C (THI 82) and 50°C (THI 112) the heating cycle was interrupted for 10 hours (08:00–16:00; 10 hours/day), during the night period the temperature was 16.5°C (THI 61). The physiological parameters such as heart rate (HR; beats/min), respiratory rate (RR; breaths/min), rectal temperature (RT) and skin temperatures (ST) were measured daily. The internal body temperature in sheep was measured per rectum using electronic thermometer (Diagnostic, Poland). Measurements of ST, using a touch point thermometer (Marco, Poland), were taken at two thermostable points, on the back (BT) and on the groin (GT), and at two thermolabile points, on the head (HT) and on the rear left leg (LT).

**Blood sampling and laboratory analysis**

Blood was collected from the jugular vein at 11:00 o'clock every day to avoid taking into account the daily fluctuations of biochemical elements, especially hormones. Whole blood, used in morphological and biochemical analysis, was collected into EDTA vacuum tubes (Sarstedt Monovette®, Germany). Furthermore, 500 µl of blood was collected into Eppendorf tubes to carry out the analysis for the presence of hormones such as adrenaline, noradrenaline, adrenocorticotrophic hormone (ACTH), and cortisol. The serum composition was collected into clotting tubes (Sarstedt Monovette®, Germany). Blood was centrifuged at 4000 rpm for 8 minutes. Determination of selected hormones was made by enzyme-linked immunosorbent assay (ELISA) using

IBL Hamburg GmbH (Germany) reagents. Blood tests were performed using the Veterinary Hematology Analyzer ABX VET (HORIBA ABX, Canada), a Pentra-400 (HORIBA ABX, Canada) biochemical analyzer, and the reader of fluorescence, luminescence and absorption produced by SYNERGY (Biotek, VT, USA). Laboratory analyses were performed in the biochemical laboratory of the Department of Environmental Hygiene and Animal Welfare (Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland), certified by RIQAS System.

**Calculations and statistical analysis**

The mean temperature-humidity index (THI) was calculated using the following equation: THI = 0.81 dry bulb (°C) + RH (dry bulb - 14.4) + 46.4 where the relative humidity (RH) is in decimal form according to Hahn (1997). Data were analyzed using Statistica software ver. 5.0. (OK, USA). Based on the minimum and maximum values of physiological parameters and blood components the reference intervals for Polish Merino were determined (Tab. 2–3). The arithmetic means as well as standard deviation were also calculated. Significant differences were detected using the analysis of variance (ANOVA). Evaluations of the heat stress effect on chosen parameters were analyzed by Tukey's test. Statistical differences were considered as significant at the level of p < 0.05 or as highly significant at a confidence level of p < 0.01.

**Results**

**Physiological parameters**

The results of the effect of heat stress on physiological parameters are presented in Table 2. It was found that the highest HR occurred at 30°C (when the mean THI was 82) and was higher than in group I (control) and III (p < 0.01). Analyzing the RR, it was found that the mean values in groups I, II and III were significantly different (p < 0.01). It should be noted that in the course of the experiment at 50°C (when the mean THI was 112) a deepening of breaths at the expense of a reduction in their frequency was observed. It was found that ST on the back as well as on the head was statistically higher in groups II and III (p < 0.01). It was noted that the GT was different in groups II and III (p < 0.01), while the statistical differences between groups I and II were lower (p < 0.05). There was a clear and significant increase in RT (p < 0.01) during the heating periods in groups II and III.

**Blood parameters**

The effect of heat stress on blood parameters is presented in Table 3. Sheep blood in morphological analysis showed a significant decrease in hematocrit (HCT) mean values, especially in group III (p < 0.01). There was also a statistical difference between groups I and II (p < 0.05). A significant decrease (p < 0.01) in the number of red blood cells (RBC) between groups I and III was observed. There were differences (p < 0.05) in Glu in group I vs. group III with a clear downward trend which was directly proportional to increasing temperature. The opposite trend was noted in Ca level, which significantly increased (p < 0.05) in group III. There was also a significant increase (p < 0.05) in K content in group III. Despite the fact of clear trends in other morphological and biochemical components in

**TABLE 2:** The effect of heat stress on physiological parameters in Polish Merino

Item	Control (n = 15, THI = 61)	Heat stress 30°C (n = 15, THI = 82)	Heat stress 50°C (n = 15, THI = 112)
HR/min	90.34 <sup>A</sup> ± 8.36	102.10 <sup>B</sup> ± 18.75	86.63 <sup>A</sup> ± 12.07
RR/min	57.91 <sup>A</sup> ± 10.79	146.30 <sup>B</sup> ± 32.80	126.10 <sup>C</sup> ± 31.17
BT (°C)	35.79 <sup>A</sup> ± 0.87	36.45 <sup>B</sup> ± 0.43	36.71 <sup>B</sup> ± 0.41
GT (°C)	36.72 ± 0.68	36.50 <sup>A</sup> ± 0.70	36.79 <sup>B</sup> ± 0.44
HT (°C)	32.63 <sup>A</sup> ± 0.77	35.00 <sup>B</sup> ± 1.71	35.53 <sup>B</sup> ± 0.25
LT (°C)	29.69 <sup>Aa</sup> ± 1.41	30.36 <sup>Ab</sup> ± 1.16	31.75 <sup>B</sup> ± 0.25
RT (°C)	39.05 <sup>A</sup> ± 0.30	39.46 <sup>B</sup> ± 0.24	39.90 <sup>C</sup> ± 0.16

HR: heart rate. RR: respiratory rate. BT: back temperature. GT: groin temperature. HT: head temperature. LT: leg temperature. RT: rectal temperature. The significance of differences between groups within a given parameter were determined with letters: A, B, C – at the level of p < 0.01; a, b – at the level of p < 0.05

**TABLE 3:** The effect of heat stress on blood and endocrine parameters in Polish Merino

Item	Control (n = 15)	Heat stress 30°C (n = 15)	Heat stress 50°C (n = 15)
RBC (T/l)	10.82 <sup>A</sup> ± 0.71	10.14 ± 0.61	9.63 <sup>B</sup> ± 1.19
HCT (l/l)	0.34 <sup>Aa</sup> ± 0.02	0.31 <sup>b</sup> ± 0.02	0.30 <sup>B</sup> ± 0.03
Glucose (mmol/l)	4.09 <sup>a</sup> ± 0.35	3.88 ± 0.27	3.75 <sup>b</sup> ± 0.31
Ca (mmol/l)	2.68 <sup>a</sup> ± 0.11	2.72 ± 0.07	2.77 <sup>b</sup> ± 0.08
K (mmol/l)	4.59 <sup>a</sup> ± 0.25	4.71 ± 0.15	4.88 <sup>b</sup> ± 0.41
Cortisol (ng/ml)	1.77 <sup>A</sup> ± 1.82	11.86 <sup>Ba</sup> ± 5.36	16.97 <sup>Bb</sup> ± 5.19
ACTH (ng/ml)	15.13 ± 18.99	17.58 ± 22.93	25.45 ± 48.25
Adrenaline (ng/ml)	5.41 ± 3.28	7.26 ± 4.61	7.42 ± 5.55
Noradrenaline (ng/ml)	9.91 <sup>A</sup> ± 8.94	11.49 <sup>A</sup> ± 10.01	24.71 <sup>B</sup> ± 15.30

RBC: red blood cells. HCT: hematocrit. The significance of differences between groups within a given parameter were determined with letters: A, B, C – at the level of p < 0.01; a, b – at the level of p < 0.05

blood, connected with the thermal factor, the statistical analysis showed no significant correlation ( $p > 0.05$ ). There was a clear and highly significant difference ( $p < 0.01$ ) in the cortisol in the blood between groups I and II, and a significant difference ( $p < 0.05$ ) between groups II and III. There was an upward trend in the ACTH level as a result of increased temperature (Tab. 3), but the differences could not be confirmed statistically ( $p > 0.05$ ). Similar trends were observed during the analysis of adrenaline, without statistical differences ( $p > 0.05$ ), which was probably the result of the hormone's short decay time. Significant differences in noradrenaline were found, with the highest level in group III ( $p < 0.01$ ).

## Discussion

### Heart rate

One of the first reactions correlated with the homeostasis of an organism is the HR, which is a direct response to stress factors, including higher ambient temperatures (Marai et al., 2007). It allows increased emission of absorbed heat by convection or radiation. It was found that the mean HR in Polish Merino was  $90.34 \pm 8.36$ . Taking into account other studies these results can give rise to the hypothesis that small changes in HR in sheep are the effect of breed variation, or a result of statistical error. The HR indicator was measured daily with numerous repetitions, so the authors strongly believe that the HR mentioned before is typical for the Polish Merino breed in thermoneutral conditions. The optimum thermal-humidity conditions for Finnish sheep, similarly to Suffolk, give a HR result in the range of  $82.50 \pm 6.70$  and  $88.00 \pm 11.00$ , respectively. The mean HR values in small ruminants may vary  $\pm 10\%$  in the neutral ambient temperature (Marai et al., 2007). Higher, statistically confirmed, mean HR in our work was a result of the temperature of  $30^\circ\text{C}$  and the constant relative humidity (THI = 82), which induced heat stress in Polish Merino. Nevertheless, higher temperature ( $50^\circ\text{C}$ ; THI = 112) did not confirm the expectations. In group III the HR decreased, which was strongly correlated with the RR results. In the authors opinion this unexpected effect was a result of animals' exhaustion in hard environmental conditions, especially when the THI was 112. This study may suggest that the temperature  $50^\circ\text{C}$  with the 70% of relative humidity is close to cause death of Polish Merino sheep.

### Respiratory rate

An increased RR trend is also one of the well-known signs of heat stress in sheep, as was confirmed in our study. Marai et al. (2007) pointed out that sheep lose up to 60% of heat through lung ventilation, which may reaffirm the results. The mean RR at  $30^\circ\text{C}$  (THI = 82), as a result of experimental conditions, was over two times higher than in the control group (Tab. 2), but according to the blood parameters, symptoms of respiratory alkalosis were not observed. Similar conclusions were also presented in a heat stress study in Omani and Australian Merino sheep (Srikandakumar et al., 2003). Experiments in different cattle breeds (Pereira et al., 2008) also confirmed the thesis of Marai et al. (2007) in small ruminant studies, that the RR may show different ranges among the animal breeds. In our experiment at  $50^\circ\text{C}$  (group III) the RR was higher than controls, but compared to  $30^\circ\text{C}$  this value statistically decreased, which was unexpected.

The breaths of sheep in group III became deeper than in previous groups (I and II), which in our view was also related to decreased HR. Nevertheless, the breath volume was not measured in the experiment, but in the authors' opinion this method should be recommended during heat stress studies in animals. The results of this study may suggest that these symptoms appear as an adaptive effect to strong heat stress or, similarly to RR results, may indicate an animal exhaustion because of high THI. Thompson et al. (1999) hold a similar opinion and argued that rising temperature may be inversely proportional to the number of breaths.

### Internal and skin temperature

The ST supports the assessment of animals' sensitivity to heat stress, especially in mammals. The skin surface performs an important role in heat exchange between the body and the environment. Absorbed heat is poorly tolerated, especially in the dense fleece sheep breeds. Good indicators of heat transfer through the skin in small ruminants are the ears, legs and scrotum (Talwar and Fahim, 1998; Marai et al., 2007). Lublin and Wolfenson (1996) have a similar opinion. Moreover, they suggest that the differences between the ST on the ears and the RT may reach  $\pm 30\%$ . In our study the temperature measurements on the skin were made at four body points, including two thermostable and two thermolabile. It was found that the ST fluctuations in Polish Merino were the direct effect of environmental conditions during the experiment. The experiment confirmed that the differences between the mean thermolabile temperatures were higher than at thermostable points. Nevertheless, the measurements at BT and GT points seem to be more reliable due to the minor temperature fluctuations. The ST results suggest that there is a necessity to control the surface temperature in thermal stress cases. Similar conclusions were reached in a heat stress study on calves (Korczynski et al., 2004), in which ST measurements were made, and the authors suggest its implementation in all heat stress research. It should be noted that another representative measurement of the physiological state in animals is RT and has gained the approbation of many authors over the years (Hahn, 1997; West, 1999; Silanikove, 2000; Srikandakumar et al., 2003; Marai et al., 2007). The RT in sheep, in a thermoneutral zone, varies between  $38.30$  and  $39.90^\circ\text{C}$ , and is directly dependent on the breed. Marai et al. (2007) have shown that this value in sheep kept in the same climatic zone was  $38.20^\circ\text{C}$  for the Rahmani breed,  $39.30^\circ\text{C}$  for the Barka breed, and  $39.50^\circ\text{C}$  for the Ossimi breed. Our research has shown that under thermoneutral conditions the mean rectal temperature in Polish Merino was  $39.05^\circ\text{C}$ , and it showed a significant increase – proportionally to the heating effect. It was observed that the RT statistically increased in both cases ( $30^\circ\text{C}$  and  $50^\circ\text{C}$ ), as presented in Table 2. These results according to numerous thermal experiments suggest that every  $20^\circ\text{C}$  of increased external (environmental) temperature may increase the RT by  $0.5^\circ\text{C}$  of internal (rectal) temperature in the Polish Merino breed, what is presently analysed in detail. Marai et al. (2007) confirmed that increasing temperature from  $18.00$  to  $35.00^\circ\text{C}$  causes a significant increase in RT, but approaching  $42^\circ\text{C}$  it begins to be a serious threat to the animal's life. Nevertheless, in ruminants, the most tolerant species are the camels, reaching an RT of up to  $45.00^\circ\text{C}$  (Silanikove, 2000).

### Morphological parameters of blood

It was shown that an important aspect of proper diagnosis of heat stress in sheep is the evaluation of the blood parameters, which was also confirmed by Djordjevic et al. (2004). There is a necessity to control both the immunological parameters as well as hormone levels, especially cortisol (Caroprese, 2008). Nazifi et al. (2003) and Ashutosh et al. (2001) noted that the thermal conditions, especially associated with the periodicity of the seasons, may directly affect the changes in blood parameters, as well as the fluctuations of cortisol. The blood results obtained in our experiment mainly coincide with the analysis carried out by Winnicka (2008). Significant differences that occurred in parameters such as HCT or RBC were inversely proportional to increasing temperature and similar to other researchers (Aganga et al., 1990; Pereira et al., 2008). The mean value of HCT and RBC decreased during heat stress, which was provoked by enlarged water intake.

### Biochemical parameters of blood

The level of aspartate aminotransferase (AST) in our work was even 35% higher in group III than the AST concentration in the control group, which was the result of high experimental temperature ranges. Unfortunately, the statistical differences were not confirmed because of the wide fluctuations of AST values. Ashutosh et al. (2001) argue that the AST level, in optimal thermal conditions, is approximately 146 U/l, which is consistent with the results obtained in our work. It was found in both cases that high temperatures brought an increase in mean AST, while this value in Iranian sheep (Nazifi et al., 2003) was more than twice as high. Analogous data were obtained in the analysis of urea and Ca, although only the latter parameter was statistically confirmed, and varied within the referential range. Nevertheless, there were also some differences between the Iranian sheep (Nazifi et al., 2003) and the Polish Merino in our study. The differences were mainly in the content of Glu in the Nazifi et al. (2003) work (3.27 mmol/l) versus 3.88 mmol/l (group II) and 3.75 mmol/l (group III) in our study. Plasma Glu decreased proportionally to increasing thermal conditions, when the mean THI was strongly uncomfortable and the RR, especially in group II, was over two times higher than controls. The same tendency was observed at 50°C (group III). This fact may suggest that the experimental animals were exhausted by heat stress, without any physical effort to provide thermal balance in the organism. Small ruminant studies in morphologic and biochemical blood parameters often give different results, depending on the authors' opinion as well as the species or animal breed. It is the main reason why this kind of study should be published. Nazifi et al. (2003) noted that the blood parameters in Iranian sheep during thermal stress (40°C) had similar results to the blood parameters in control groups. During sheep studies also the seasonal effect with high temperatures causes lower concentration of Glu in the blood (Rensis and Scaramuzzi, 2003), which was also mentioned in our results (Tab. 3).

### Endocrine parameters of blood

The effect of thermal stress on animals must also take into account the aforementioned stress hormone concentrations. The effect of thermal stress also gives a strong response in the levels of numerous hormones

and neurotransmitters (Chi-Wan and Trimble, 1997). The concentration of cortisol in the blood determines the physiological reaction of the animal organism as well as its emotional state (Pereira et al., 2008). The content of cortisol in a hot environment increases in less than 20 min and remains at a high level for over 2 h (Silanikove, 2000). Our study in Polish Merino rams and the early handling with the researchers' presence reduced strong hormonal reactivity in sheep for humans. This method allowed us to collect the blood samples, especially for endocrine analyses, with a low influence of emotional stress caused by the experimental procedure, as was also recommended by Pereira et al. (2008). We confirmed that the cortisol concentration increases in direct proportion to ambient temperature with highly significant differences. It was noted that the cortisol concentration in other studies (Nazifi et al., 2003), in thermoneutral conditions, is around 10.76 ng/ml in Iranian sheep and maintained an upward trend up to 19.32 ng/ml in higher temperatures than 40°C. In the present work the cortisol concentration increased nearly tenfold ( $p < 0.01$ ) at 50°C and the noradrenaline level increased over twofold ( $p < 0.01$ ), which is presented in Table 3. The endocrine reaction in Polish Merino was an adequate response to the intensive heat stressor as well as to the experimental conditions in the climatic chambers, especially high, strongly uncomfortable THI. Nevertheless, a study performed on Indian sheep (Ashutosh et al., 2001) suggested that higher cortisol levels may not be correlated with a high ambient temperature. However, this point of view has strong opponents, who argue that in small ruminants, heat stress is associated with increased cortisol concentration in the blood. (Nazifi et al., 2003). It was found that the stimulation of noradrenergic cells, such as the limbic system of neural connections (mainly the hippocampus), indicates a crucial role in stress reactions (Riniolo and Schimdt, 2006). It should be noted that fluctuations in noradrenaline concentration affect not only ACTH secretion but also the feeling of stress by the animal, reducing nerve sensitivity (Riniolo and Schimdt 2006). Unfortunately, the clearly increased trends in both ACTH and adrenaline were not confirmed by statistical analysis ( $p > 0.05$ ), mainly because of the parameter fluctuations. Despite the full unification of the animals in experimental groups and fast blood collection mentioned fluctuations were probably a result of animal stress associated with the precised time of blood sampling. Finally, the stress reactions were summarized by Minois (2000) and 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

Thermal comfort, not only in animals but also in humans, is a strong stress stimulus with long-term consequences and a serious health risk. The observations reported in this study suggest that the response to heat stress is largely dependent on the species, animal breed and health. It was found that both, the physiological test and the full blood analyses, perform a significant role in heat stress reactions in Polish Merino sheep. In spite of many different studies in the field of thermal stress, the physiological parameters HR, RR, RT and ST require further

investigation. Moreover, a strong, but not linear trend between thermal stress and blood parameters, especially hormone concentrations (cortisol, noradrenaline), gives the opportunity for easy detection of animal homeostasis or animal exhaustion in poor environmental conditions, what proves the practical applicability of our studies.

## Acknowledgement

The work was supported by the Polish Ministry of Science and Higher Education as a research project in the years 2010–2012 (grant N N311 279238). The authors would like to thank the reviewers for their helpful remarks and valuable comments.

Conflict of interest: The authors declare that they have no competing interests that might have influenced the content of this manuscript.

## References

- Aganga AA, Umunna NN, Oyedipe EO, Okoh PN Aduku AO (1990):** Response to water deprivation by Yankasa ewes under different physiological states. *Small Rumin Res* 3: 109–115.
- Ashutosh D, Dhanda OP, Kundu RL (2001):** Effect of climate on the seasonal endocrine profile of native and crossbred steep under semi-arid conditions. *Trop Anim Health Pro* 33: 241–252.
- Caroprese M (2008):** Sheep housing and welfare. *Small Rumin Res* 76: 21–25.
- Chi-Wan L, Trimble MR (1997):** Stress and Epilepsy. *J Epilepsy* 10: 177–186.
- Djordjević J, Cvijić G, Vučković T, Davidović V (2004):** Effect of heat and cold exposure on the rat brain monoamide oxidase and antioxidative enzyme activities. *J Therm Biol* 29: 861–864.
- Hahn GL (1997):** Dynamic responses of cattle to thermal heat loads. *J Anim Sci* 77: 10–20.
- Hargreaves M (2008):** Physiological limits to exercise performance in the heat. *J Sci Med Sport* 11: 66–71.
- Kiyatkin EA (2005):** Brain hyperthermia as physiological and pathological phenomena. *Brain Res Rev* 50: 27–56.
- Korczynski M, Rojkowski A, Janeczek W, Kupczynski R, Pogoda-Sewerniak K (2004):** The influence of microclimatic elements on selected indicators of physical thermoregulation and basic physiological factors in calves in their first weeks of life. *Scien Books of Rural Academy of Wroclaw* 501: 113–118.
- Lublin A, Wolfenson D (1996):** Lactation and pregnancy effects on blood flow to mammary and reproductive systems in heat-stressed rabbits. *Comp Biochem Physiol* 115A: 277–285.
- Marai IFM, El-Darawany AA, Fadiel A, Abdel-Hafez MAM (2007):** Physiological traits as affected by heat stress in sheep – A review. *Small Rumin Res* 71: 1–12.
- Minois N (2000):** Longevity and aging: beneficial effects of exposure to mild stress. *Biogerontology* 1: 15–29.
- Nazifi S, Gheisari HR, Poorabbas H (1999):** The influence of thermal stress on serum biochemical parameters of dromedary camels and their correlation with thyroid activity. *Comp Haematol Int* 9: 49–53.
- Nazifi S, Saeb M, Rowghani E, Kaveh K (2003):** The influence of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. *Comp Clin Pathol* 12: 135–139.
- Nowicki B, Jasek S, Maciejowski J, Nowakowski P, Pawlina E (2001):** Breeds of the farm animals. (in Polish). PWN, Warsaw, Poland, 67.
- Olsen A, Vantipalli MC, Lithgow GJ (2006):** Lifespan extension of *Caenorhabditis elegans* following repeated mild hormetic heat treatments. *Biogerontology* 7: 221–230.
- Pereira AMF, Baccari Fjr, Titto EAL, Almeida JAA (2008):** Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in Alentejana, Mertolenga, Frisian and Limousine cattle breeds. *Int J Biometeorol* 52: 199–208.
- Rennefeld C, Wach K, Scoell ED, Lorenz J, Bingel U (2010):** Habituation to pain: Further support for a central component. *Pain* 148: 503–508.
- Rensis De F, Scaramuzzi RJ (2003):** Heat stress and seasonal effects on reproduction in the dairy cow – a review. *Theriogenology* 60: 1139–1151.
- Riniolo TC, Schimdt LA (2006):** Chronic heat stress and cognitive development: An example of thermal conditions influencing human development. *Develop Rev* 26: 277–290.
- Silanikove N (2000):** Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest Prod Sci* 67: 1–18.
- Srikandakumar A, Johnson EH, Mahgoub (2003):** Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin Res* 49: 193–198.
- Talwar A, Fahim M (1998):** Circulatory response to hyperthermia during acute normovolaemic haemodilution. *Int J Biometeorol* 41: 169–174.
- Thompson JA, Brimacombe M, Calvin JA, Tomaszewski MA, Davidson TJ, Magee DD (1999):** Effects of environmental management on seasonal decrease in milk production in dairy cattle. *J Am Vet Med A* 24: 85–88.
- West JW (1999):** Nutritional strategies for managing the heat-stressed dairy cow. *J Anim Sci* 77: 21–35.
- Winnicka A (2008):** Reference values of basic laboratory tests in veterinary medicine, fourth ed. (in Polish). SGGW Press, Warsaw, Poland

### Address for correspondence:

Dr. Przemysław Cwynar  
Wrocław University of Environmental and Life Sciences  
Faculty of Biology and Animal Science  
Department of Environmental Hygiene and Animal Welfare  
Chelmonskiego 38C  
51–630 Wrocław  
Poland  
przemyslaw.cwynar@up.wroc.pl