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Original study

Effect of heat stress on acid-base balance in Polish Merino sheep

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Abstract

Effect of heat stress on changes in acid-base balance, physiological parameters and cortisol level were evaluated in Polish Merino sheep. Fifteen sheep were exposed to high temperature conditions (30 °C) in order to induce heat stress. All environmental parameters such as temperature, humidity and air movement were monitored. A decrease of partial pressure of CO_2 (pCO_2) in blood and concentration of total CO_2 (tCO_2) and an increase of pO_2 were observed. The cortisol level also significantly increased. In the next stage of the experiment the soothing effect of air movement was examined. An increased air movement led to reduction of thermal stress. An increase in pCO_2 and decrease in cortisol level were observed. The study showed that heat stress leads to changes in acid base balance and cortisol secretion. Air movement has a soothing effect on heat stress in Polish Merino sheep.

Keywords: sheep, heat stress, acid-base balance, cortisol, air movement

Abbreviations: pCO_2: partial pressure of CO_2, pO_2: partial pressure of O_2, tCO_2: concentration of total CO_2, THI: temperature-humidity index

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Introduction

Sheep are bred in variety of environmental conditions all over the world. During summer heat, in many countries, including most of Europe, short term heat stress can occur. In some countries it can be a serious economic problem. Sackett *et al.* (2006) estimated that the annual production losses in the Australian feedlot industry due to summer heat stress in animals can reach up to AUD 16.5 million.

Climatic factors, such as high temperature, high relative humidity, high solar radiation and low air movement can induce a heat stress response in animals. Under heat stress conditions a decrease of reproductive function, weight gain and milk yield is usually observed (Alhidary *et al.* 2012, Gaughan *et al.* 2009, Haheeb *et al.* 1992). The thermo-neutral zone for sheep is set between –12 and 32 °C but it depends on animal physiological state, age, weight, health and type of feeding (Finocchiaro *et al.* 2005, Gaughan *et al.* 2009). The severity of heat stressor should not be determined only by temperature factor. It is important to include also humidity level as it has a significant impact on perceptible temperature. To include both factors temperature-humidity index (THI) is used by numerous authors. 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 is compromised.

The change of environmental temperature has a significant impact on physiological processes. Heat stress results in a decrease of feed intake, disturbance in the metabolism of water, protein, energy and mineral balance, hormonal secretions and blood metabolites (Marai *et al.* 2003, 2004). Oxygen consumption increases in direct proportion to temperature. An increase in temperature of 10 °C causes a two- or even a threefold increase in oxygen consumption speed (Schmidt-Nielsen 1997). To meet the demand for oxygen, the organism is forced to increase gas exchange by an increased respiration rate. As thick fleece layer prevents effective heat loose trough evaporation from body surface in sheep, breathing becomes not only a method of O_2 supply but also a method of heat loss (Hales & Brown 1974). Daily temperature variation also has an influence on the level of thermal stress. If the temperature drops below 21 °C at night and remains at that level for at least 3-6 h, animal has sufficient opportunities to lose the heat accumulated during the day (Muller *et al.* 1994).

Acid-base balance is a complex physiological process. Its role is to maintain a stable pH in the body of an animal. It's regulated by both intracellular and extracellular buffers as well as the nephritic and respiratory system. Extracellular H^+ is one of the most dynamically regulated blood variables (Goel & Calvert 2012, Houpt 1989). Destabilization in this system can be dangerous for the animal's life. Structure of proteins and enzymes are dependent on organism's pH. Also cellular functions such as DNA synthesis, mitosis, glycolysis and gluconeogenesis are disrupted by significant pH changes (Greenbaum & Nirmalan 2005). Under heat stress conditions a number of respiratory and metabolic disorders occur. A pCO_2 above 40 mmHg as well as pH below 7.4 stimulates respiration, whereas pCO_2 lower than 40 mmHg and pH higher than 7.4 decrease respiration. A set of mechanisms to maintain body pH is used: chemical buffering, respiratory compensation (modifying pH by balancing the H^+ production with CO_2 excretion) and renal compensation (excretion of H^+ or HCO_3- by the kidneys) (Kadzere *et al.* 2002).

The aim of the study was to track physiological changes in sheep during acute heat stress, especially acid-base balance. An important part of the study was also to check whether increased air movement will mitigate acid-base balance disruptions.

Material and methods

Fifteen Polish Merino sheep selected for similar constitution and fleece length of average age: 12 months, average weight: 45 kg from local breeding centre (Poland) where used. All procedures for this experiment were approved by the 2nd Local Ethical Committee for Experiments on Animals in Wroclaw, Poland (No 82 / 2009, issued: 27.06.2009).

Sheep were placed in a closed experimental room equipped with heating unit, thermo hygrometers and device for measuring the concentration of ammonia (NH_3), carbon dioxide and hydrogen sulfide (H_2S). They had 14 hours of day light and free access to water and forage (hay). Feeding was made with nutritive fodder in the form of oats: 0.2 kg/head/day. Air temperature, relative humidity and air movement parameters were monitored by SCADA PRO software designed for this research by MicroB S.A., Poland.

The experiment consisted of three stages. The first experimental phase of neutral conditions including temperature and humidity lasted 14 days. Environmental conditions in this stage of experiment where set on optimal temperature and humidity for animals and the THI index did not exceed 72. Those conditions where maintained during day and night. The THI was counted according to Hahn (1997):

$$THI=0.81\ db\ {}^{o}C+RH\ (db\ {}^{o}C-14.4)+46.4 \quad (1)$$

where db °C is the dry bulb temperature and RH is the relative humidity RH% / 100.

The second experimental phase lasted seven days and started with a sudden rise in temperature up to 30 °C. It resulted in conditions that may be considered as heat stress environment: THI>79. In this stage of experiment conditions at night were comparable to those during the day. The third experimental phase lasted seven days and was to maintain the temperature of the second stage, along with increased air movement. The average temperature during the day was 30 °C, THI index was 78.30 and air flow at the level of 3.12 m/s.

Blood samples for acid-base balance and cortisol level tests were collected from the jugular vein (*vena jugularis externa*) every other day at 13:00. Whole blood was cooled at the temperature of approx. 4-6 °C and transported to the laboratory within 1 hour from drawing. The acid-base balance was evaluated with CIBA CORNING-248 (Ciba Corning Diagnostics Corp, Medfield, MA, USA) blood gas analyser. Meanwhile the following gasometric parameters were determined: pH, oxygen pressure (pO_2) and carbon dioxide pressure (pCO_2), actual bicarbonate (HCO_3– actual), base excess, total content of carbon dioxide (tCO_2) and percentage of haemoglobin saturation with oxygen. Cortisol level was detected with an enzyme-linked immunosorbent assay (ELISA) with Cortisol Assay Kit (Cat Combi Elisa, IBL Hamburg GmbH, Gemany).

Obtained data were subjected to the analysis of variance using general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model was:

$$Y^{ijk} = \mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\varepsilon_{ijk} \quad (2)$$

where Y_{ij} is the dependent variable, μ is the overall mean, a_i is the effect of treatment (i=1, 2, 3 stage), β_j is the series of analyses after experimental factor introducing, $\alpha\beta_{ij}$ is the effect of the treatment × time interaction and ε_{ijk} is the random residual error.

Differences among treatment means were tested for significance using Duncan's multiple range test. Effects were considered significant at a probability of $P\leq0.05$ and $P\leq0.01$.

Results and discussion

High temperature and humidity, in combination with a solar radiation and low air movement, can result in heat stress which leads to a loss of productivity and even the death of the animal. The temperature-humidity index (THI) could be used as an indicator of climatic conditions. THI values of 70 or less are considered comfortable for farm animals, 75-78 stressful and values greater than 78 cause extreme distress (Kadzere *et al.* 2002). Sevi *et al.* (2001) found that THI values of about 80 for only few hours during the day represent a stressful condition to lactating ewes.

Environmental parameters for each stage of this research are summarized in Table 1.

Table 1
Summary of environmental parameters for different stages of the experiment

Item		Temperature, °C	Relative humidity, %	NH_3, ppm	H_2S, ppm	THI	Air movement, m/s
STAGE I	day	20.9±0.4	74.2±4.3	0.87±0.29	0.14±0.02	70.49±0.33	0.26±0.00
	night	20.1±0.7	73.8±0.4	0.98±0.24	0.11±0.01	68.66±0.35	0.26±0.00
STAGE II	day	30.6±1	50.5±5.9	1.47±0.36	0.21±0.02	79.39±1.25	0.30±0.00
	night	28.6±0.9	47.8±1.6	1.65±0.17	0.17±0.00	76.77±0.19	0.30±0.00
STAGE III	day	30±0.5	48.5±2.0	2.60±0.23	0.20±0.03	78.30±2.80	3.12±0.01
	night	26.2±0.4	47.3±2.6	2.53±0.14	0.14±0.00	73.35±0.08	3.12±0.01

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

At the stage I of the experiment animals performed physiological body functions and were not affected by the environment. At stage II of the experiment THI index during the day was 79.39. Those conditions should be considered as severe heat stress, as a result of that heart rate had risen significantly and respiration rate had risen highly significantly at this stage (Table 2).

Table 2
The physiological parameters during the experimental proceedings

Item	STAGE $I_{(n=40)}$	STAGE $II_{(n=40)}$	STAGE $III_{(n=40)}$
Heart rate, beats/min	90.23 ± 12.42^{a}	107.79 ± 28.84^{Ab}	80.80 ± 12.17^{B}
Respiration rate, breaths/min	56.21 ± 16.01^{A}	96.43 ± 41.92^{B}	57.60 ± 23.27^{A}
Temperature, °C	39.36 ± 0.37^{A}	39.36 ± 0.33^{A}	38.97 ± 0.40^{B}

n: number of tested samples, Differences are statistically highly significant $^{ABC}P<0.01$, $^{abc}P<0.05$.

Those results coincide with the research of Alhidary *et al.* (2012). In their research lambs exposed to a high ambient temperature (37.4 °C) showed an 0.8 °C increase in body temperature, an increase in respiration rate by 110 breaths/min (3 do 9 day of experiment), and increase in water intake. Feed intake decreased by 22 %. Koluman & Daskiran (2011) suggest that providing ventilation during the warmest hours of the day may sustain the welfare and performance of crossbred lambs. This correlation has been tested at the third stage of the experiment as deliberate air movement was introduced. The results (Table 2 and 3) show that air movement mitigated the effect of heat stress. However, an increase in ammonia concentration can be seen (Table 1), but it has not reached the value which could have a negative impact on animals. According to Phillips *et al.* (2012) an ammonia concentration at level up to 16.5 ppm does not have an effect on haematological variables in sheep.

Table 3
The results of the acid-base balance and cortisol level in blood for the various stages of the experiment

Item	STAGE $I_{(n=40)}$	STAGE $II_{(n=40)}$	STAGE $III_{(n=40)}$
pH	7.41±0.04	7.43±0.03	7.41±0.03
pCO_2, mmHg	$42.64±4.88^{A}$	$36.49±2.65^{Ba}$	$40.73±3.77^{b}$
pO_2, mmHg	$34.74±6.82^{a}$	$43.89±10.97^{b}$	40.51±9.39
HCO_3 act., mmol/l	26.24±1.42	23.59±2.85	25.4±2.55
Base excess, mmol/l	1.36±1.29	-0.34±2.70	0.77±2.46
tCO_2, mmol/l	27.54±1.47a	24.71±2.79b	26.63±2.65
O_2 SAT, %	64.9±13.38	78.39±12.90	73.44±11.94
Na^+, mmol/l	139.4±2.80	136.5±5.24	136.44±5.83
K^+, mmol/l	4.23±0.28	4.32±0.16	4.21±0.13
Cortisol, ng/dl	5.88±2.75a	7.97±1.82Ab	4.48±2.56B

Differences are statistically highly significant $^{ABC}P<0.01$, $^{abc}P<0.05$.

Cortisol plays an important role in all types of stress. In conducted research at the stage II of the experiment the temperature was deliberately increased to the level of 30.65 °C. That resulted in thermal stress in animals. This is emphazised by the increase of the cortisol level (Table 3). Another research also confirms the increase of the cortisol level as a reaction to heat stress in animals (Caroprese *et al.* 2012). Supplementation of vitamin C and vitamin E with selenium decreased plasma cortisol levels, indicating that supplementation may have a negative effect on cortisol levels during heat stress, and showed that supplementation of antioxidants had mitigated the effects of heat stress in goats (Sivakumar *et al.* 2010).

In conducted research the appearance of thermal stress (stage II) in sheep resulted in an increased respiration (panting). That led to hyperventilation which resulted in a decrease of the CO_2 level in the body (hypocapnia). It can be seen in the decrease of pCO_2, and the increase of tCO_2 (Table 3). Such as the respiration increased, the pO_2 level increased as well (Table 3). That can lead to respiratory alkalosis. In this case we can talk about slightly compensated alkalosis. As the level of pCO_2 decreased, the organism to stay in physiological balance has led to compensation which resulted in decrease in HCO_3- actual and base excess. Blood pH and HCO_3-increased, but blood pCO_2 and base excess decreased when the animals were subjected to the heat stress (THI=93) (Srikandakumar *et al.* 2003). These results indicate that both breeds of sheep (Omani and Merino sheep) were able to maintain a normal acid-base balance when heat stress lead to an increase of body temperature and respiration rate. Omani sheep are more heat tolerant than Merino sheep. In Srikandakumar's (2003) research there was no sign of hypercapnia in heat stress conditions. However, in a cool environment the acid-base balance parameters suggested metabolic acidosis. That was probably a result of the diet.

The compensation mechanism balanced pCO_2 level which can be seen in stage III. Importantly, despite appearance of alkalosis there was no electrolyte imbalance (Na^+ and K^+ remained unchanged). This means that the heat stress occurred was not strong enough to disrupt the electrolytes balance. Heat stress leads to a decrease of Na^+ which results in a decrease of strong ion difference (SID) level in blood (Ondogo *et al.* 2006). The concentration of pCO_2 and Atot (from plasma protein) decreased which resulted in blood alkalization. Under heat stress pCO_2, tCO_2, pO_2 and oxygen saturation, the concentration of Mg^{2+}, glucose, and HCO_3- showed quadratic ($P<0.05$) responses with time (Ondogo *et al.* 2006)

The described study shows that severe heat stress (THI=79.39) in Polish Merino sheep is compensated by the respiratory alkalosis. The mentioned thermal factor also induced an increased cortisol concentration in the blood. Increased velocity of air movement caused the launch of alkalosis compensatory mechanisms, with increased level of HCO_3- actual and base excess, what resulted in a decrease of blood pH. The study suggests that a practical method of influencing the acid-base balance in the blood of sheep under heat stress is increased air velocity. The resolution proposed in our work undoubtedly positively affects the animal welfare, which was confirmed by lowered concentration levels of cortisol in the blood.

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