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Original article

Evaluation of biomarkers of heat stress using automatic health monitoring system in dairy cows

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Abstract

The aim of this study was to evaluate biomarkers of heat stress (HS) from an automatic milking system (AMS), the relationships between measurements of the temperature-humidity index (THI), reticulorumen pH and temperature, and some automatic milking systems parameters in dairy cows (rumination time (RT), milk traits, body weight (BW) and consumption of concentrate (CC)) during the summer period. Lithuanian Black and White dairy cows (n=365) were selected. The cows were milked with Lely Astronaut® A3 milking robots with free traffic. Biomarkers were collected from the Lely T4C management program for analysis. The pH and temperature of the contents of the cow reticulorumen were measured using specific Smax-tec boluses. The farm zone's daily humidity and air temperature were obtained from the adjacent weather station (2 km away). According to this study, during HS, the higher THI positively correlates with milk lactose (ML), which increases the risk of mastitis and decreases CC, RT, BW, MY, reticulorumen pH, and F/P. Some biomarkers of HS can be milk yield, milk lactose, somatic cell count, concentrate intake, rumination time, body weight, reticulorumen pH, and milk fat – protein ratio. We can recommend monitoring these parameters in the herd management program to identify the possibility of heat stress.

Key words: heat stress, cow, automatic milking system, temperature, milk

Introduction

Heat stress (HS) is becoming a serious problem due to the negative impacts on ruminant performance (Conte et al. 2018), especially the milk yield of high producing animals (Kadzere et al. 2002), milk composition (Lambertz et al. 2014), reproductive performance, animal welfare and health (Renaudeau et al. 2012). Physiological responses, such as respiration rate, body temperature, sweating rate, appetite, water intake, and production performance, are affected by heat stress (Hansen 2007). Heat stress has adverse effects on milk production and reproduction in dairy cattle (West 2003, Hill and Wall 2015), also change their metabolism in response to ambient temperature change (Conte et al. 2018). As noted by Hansen (2007), heat stress is grow-

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ing because increases in milk yield result in greater metabolic heat production. Heat stress makes harms animal health, and a higher rate of clinical mastitis and higher milk somatic cell counts occurs in dairy cows exposed to a hot environment (Berman 2011).

The temperature-humidity index (THI) involves the collective impacts of air temperature and humidity related to different levels of thermal stress (Kendall et al. 2009, Nasr and El-Tarabany 2017), and which describes the thermal conditions which drive heat stress in dairy cattle (De Rensis et al. 2015). Despite not being formulated using bovine data, THI is related to the body temperatures of cattle exposed to heat stress (Gaughan et al. 2008). According to West (2007), environmental modifications should target the effects of high temperatures on cow body temperature and modify the environment at critical times when cows are stressed, including in the morning hours when ambient temperatures are typically cooler. The automatic monitoring of body temperature in dairy cattle could help identify illness, heat stress, and general physiological stress. Body temperature is associated with rumen content temperature, which may be monitored by reticular temperature sensors (Ammer et al. 2016) and rumination time (RT) may be assessed and recorded by RT sensors (Soriani et al. 2013). Ambient temperature, milk production, and breed influence the reticulorumen temperature (Herbut and Angrecka 2018). The majority of earlier studies were aimed at milk, protein, and fat production alterations during HS. Nasr and El-Tarabany (2017) found that high THI is associated with higher SCC and reduced milk yield, and quality, potentially affecting the animal welfare and farm economy. Indicators of heat stress can be directly measured on the animals (behavioral, physiological, productive, and reproductive indicators) and on those environmental parameters that can be considered risk factors (Coon et al. 2018). Early forecasting of heat stress risk makes it possible to limit its negative impact on cow welfare (Coon et al. 2018). The aim of this study was to evaluate biomarkers of HS from AMS, the relationships between temperature-humidity index (THI) and reticulorumen pH and temperature and some automatic milking systems parameters in dairy cows (rumination time (RT), milk traits, body weight (BW) as well as consumption of concentrate (CC) during the summer period.

Materials and Methods

Location, animals and experimental design

The study was conducted during the summer period from 2018-07-01 to 2018-08-31. Lithuanian Black and White breed dairy cows (n=365) were selected based on the following criteria; having had a two or more lactations (on average 2.9±0.13 lactation), from 1 to 200 (on average 98±6.15) days in milk (DIM), and being clinically healthy. The cows were kept in a loose housing system and were fed total mixed ration (TMR) throughout the year at the same time, balanced according to their physiological needs.

The cows were milked using Lely Astronaut® A3 milking robots with free traffic. They were fed a TMR consisting of 25% corn silage, 15% grass silage, 4% grass hay, 50% grain concentrate mash, and 6% mineral mixture. Composition of ration: dry matter (DM (%) 48.8; neutral detergent fiber (% of DM) 28.2; acid detergent fiber (% of DM) 19.8; nonfiber carbohydrates (% of DM) 38.7; crude protein (% of DM) 15.8; net energy for lactation (Mcal/kg).

Measurements

Average daily milk yield, rumination time, body weight, milk fat, and protein ratio were collected from the "Time 4 Cows" Lely milking management software. The pH and temperature of the cows' reticulorumens contents (n=20) were measured using specific Smax-tec boluses providing real-time data such as ruminal pH and temperature. The data were measured with specific antennas (Smaxtec animal care tech-nology®), collected using an analog to digital converter (A/D converter), and stored in an external memory chip. Calibration of the pH-probes was performed at the beginning of the experiment using pH 4 and pH 7 buffer solutions. The data were read every 10 minutes daily. All data were obtained using Smaxtec messenger® computer software.

The farm zone's average daily humidity and air temperature were obtained from the adjacent weather station (2 km away). Measurements were made every hour. The collected data were used to compute the daily temperature-humidity index using the formula described by Kendall and Webster (2009). $THI=(1.8*AT+32)-((0.55*0.0055*RH)-(1.8*AT-26))$, where AT = Ambient temperature (°C), RH = Relative humidity (%).

Data analysis and statistics

The records (n=33480) of the tested cows (n=365) in the summer season (92 days) were analyzed using IBM SPSS Statistics Version 20.0 for Windows. The evaluated indicators were normally distributed (were tested for normality using the Kolmogorov-Smirnov test; p>0.05). The somatic cell count (SCC) data were transformed (SCC = log2 (SCC/100) 3) to achieve a normal distribution for tests of parametrical statistics. The descriptive statistics (mean and standard

Table 1. Evaluated characteristics in dairy cows at different levels of temperature-humidity index during the research period.

THI (daily average)		RT	BW	MY	F/P	SCC	ML	CC
<72	M	447.53[a]	677.37[a]	36.94[a]	1.13[a]	165.95[a]	4.59[a]	3096.29[a]
	SEM	3.24	1.22	0.25	0.01	10.37	0.001	17.45
≥72	M	433.57[b]	672.11[b]	35.4[b]	1.13[a]	152.14[b]	4.59[a]	3043.00[b]
	SEM	14.91	5.65	1.97	0.03	26.21	0.001	18.12

[a,b] Values within a column with diferent superscripts differ significantly at p<0.05. RT – rumination time (min./day); BW – body weight (kg); MY – milk yield (kg/day); F/P – milk fat protein ratio; SCC – somatic cell count (thousand/ml); ML – milk lactose (%); CC – consumption of concentrate (g/day)

Table 2. Correlation between THI and risk level of mastitis and ruminal acidosis in dairy cows.

THI	SCC (thousand/ml)		F/P		RT	
	<200	≥200	>1.15	1.15-1.20	<432	432-473
<72	90.7%	9.3%	85.7%	14.3%	2.3%	97.7%
≥72	85.7%	14.3%	84.9%	15.1%	42.9%	57.1%
χ^2 test	p=0.049		p=0.867		p=0.000	

SCC – somatic cell count (thousand/ml); F/P – milk fat protein ratio; RT – rumination time (min./day)

error), one-way ANOVA test and Pearson correlation coefficient between the evaluated traits of cows (MY –milk yield, BW, CC, RT, ML – milk lactose, SCC and F/P -milk fat and protein ratio) and the metrological parameter (THI) were calculated. The relationship between the categorical variables (classes of THI, RT, milk SCC and F/P) were evaluated using the χ2 test. The results were considered reliable under p level <0.05.

We evaluated the data based on daily averages and grouped the cows according to the level of risk of ruminal acidosis by rumination time (min/d.): very high risk of ruminal acidosis (RT<432) and high risk of ruminal acidosis (432≤RT≥473) (Armstrong 1994). We grouped SCC data into two classes: healthy cows – milk SCC<200 thousand/ml and cows at risk of subclinical mastitis – SCC≥200 thousand/ml.

The THI was used to determine the influence of heat stress on dairy cows, and is divided into 2 categories: THI <72 (comfort zone; 92.5% of cows records) and THI ≥72 (higher risk of thermal stress; 7.5% of cows records) (Collier et al. 2008).

Results

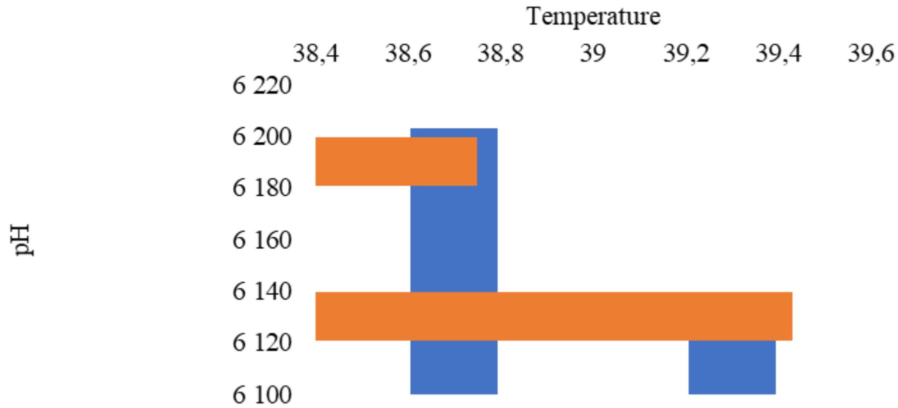
Relationship between THI with milk traits, body weight, consumption of concentrates, and rumination time in dairy cows

During the summer period, we recorded set 7 days (7.5%) of THI≥72. The average value of THI was 64.58±0.272, the average AT was 18.92±0.191°C, and RH was 70.62±0.620%. The milk SCC was negatively correlated with THI (r=-0.351, p<0.001). The ML was positively related to THI (r=0.120-0.124, p<0.01). The THI was negatively related to CC (r=-0.065, p<0.01) and milk F/P (r=-0.009; p<0.01).

There was a significant difference in daily MY (reduced by 4.17% from low THI to high THI). The BW of cows was slightly affected by THI, with the lowest BW (-5.26 kg) found at the highest THI level (Table 1). The heat stress decreased to an average of the CC (53 g, p<0.001) (Table 1).

Although this study showed that the mean of SCC in the risk class of THI was lower (Table 2), there was on the other hand a 5% higher number of cows with an increased risk of mastitis (SCC≥200 thousand/ml) at a higher THI level (Table 2).

A binary regression analysis of milk SCC showed that cows with THI≥72 were more at risk of mastitis (OR=1.625; 95% CI=1.444-1.828, p<0.001). It was also found that significantly more cows (40.6%) had a THI (≥72) and were more prone to a very high risk of rumen acidosis depending on the rumination time (when RT <432). The increased THI was associated with a significant increase in the probability of rumen acidosis, indicated by changes in RT level in cows (OR=31.500; 95% CI=28.272-35.096, p<0.001). Interestingly, the logistic regression model did not show a statistically significant relationship between increased THI levels and another indicator of rumen acidosis – milk F/P.



	THI<72	THI≥72
Reticululum pH	6 203	6 136
Reticulum temperature °C	39,43	38,75

Fig. 1. Reticulum pH and temperature characteristics in dairy cows at different levels of THI.

Relation of THI with reticulorumen pH and temperature

In the present study, the average reticulum pH of cows was 6.17±0.003, and reticulum temperature was 39.12±0.003°C. There was a negative correlation between reticulum pH and THI (r=-0.381 p<0.001) and between reticulum temperature and THI (r=-0.439, p<0.001). The increase in THI is related to a decrease in reticulum pH (from 6.25 to 6.09) (p<0.05). The lower (1.72%, p<0.05) reticulum temperature was estimated at a higher level of THI≥72 (Fig. 1).

Discussion

According to Nasr and El-Tarabany, thermal stress may influence animal welfare and milk yield and composition. THI is calculated from ambient temperature and relative humidity and can help to evaluate the impacts of the environment on cow performance and health (West 2003, Morton et al. 2007). High THI (higher than 72) might irritate the neuroendocrine system, and consequentially influence energy and water balance, hormonal equilibrium, and body temperature, which ultimately disturb the growth, reproduction, milk production, and the immune system (Vitali et al. 2015). These investigators reported that a daily afternoon maximum THI of 87 and a minimum morning THI of 77 should be considered the upper and lower daily THI values for maximum risk of dairy cow death due to heat stress (Richoux et al. 2014). According to Collier et al. (2008), the indirect effects include impacts of the environment on food and water availability, pest and pathogen populations, and the immune system's resistance to immunologic challenges. Supplemental rumen-active fat has advantages over starch-based concentrate to increase the diets' energy density for lactating cows during the warm summer months. The addition of fungal cultures to dairy diets has been shown to reduce respiration rate and body temperature during thermal stress in several studies. Rumen acidosis is often increased under heat-stress conditions associated with reduced forage intake and increased high energy feed intake (Vitali et al. 2015).

Heat stress and SCC

Somatic cells are an important constituent in the milk used to mark healthy mammary gland and milk quality (Allen et al. 2013). In our study, we found that the mean of SCC in the THI risk class was lower; on the other hand, there was a 5% higher number of cows with an increased risk of mastitis at a higher THI level. According to Nasr and El-Tarabany (2017), high THI (higher than 72) was associated with higher SCC (SCC significantly increased by 36%) and production losses in Holsteins, and SCC was within the normal level for healthy mammary gland.

SCC was higher in milk from Holstein dairy cows suffering from heat stress than dairy cows in comfortable conditions (Bouraoui et al. 2002). In summer, the growth and count of bacteria are amplified in the animal bedding stuff due to suitable temperature and humidity, facilitating increased exposure of teat ends to these bacteria. Elevated SCC was coupled with a reduction in the mammary gland's synthetic performance (Amamou et al. 2019).

Heat stress and MY

Climate is the biggest single factor affecting animal production and welfare (Vitali et al. 2015, Cowley et al. 2015). Reduced cow milk production is the most negative effect of heat stress as its economic results are usually visible after a few days (Zimbelman et al. 2009). Nasr and El-Tarabany (2017) found that daily milk production was higher and composition was better with the low THI when compared with a higher THI. In dairy cattle, heat stress decreases milk yield (Gao et al. 2017), which has been traditionally attributed to the heat--induced reduction in DMI (West 2003). However, the use of pair-fed thermal neutral controls demonstrated that reduced DMI only partially (about 50%) explains the decrease in productivity, suggesting that hyperthermia itself directly affects milk production (Gao et al. 2017). The high-yielding dairy cows started to reduce milk yield at a THI of approximately 68 (NRC 2001). Dairy cows were exposed to conditions well above this threshold (average THI=82.4), causing hyperthermia, tachypnea, and marked DMI reductions (Polsky and von Keyserlingk 2017).

When a cow becomes heat stressed, an immediate coping mechanism is needed to reduce DMI, causing a decrease in the availability of nutrients used for milk synthesis (West 2003). Simultaneously, there is an increase in basal metabolism caused by activation of the thermoregulatory system. Mild to severe heat stress can increase metabolic maintenance requirements by 7 to 25% (Von Keyserling et al. 2009), further exacerbating both the existing metabolic stress and the decrease in milk production. As a result, it is often assumed that milk production can be interpreted as a direct welfare indicator in that it offers producers the ability to monitor the animal's individual response to a challenging event (e.g., increasing ambient temperature or changes in nutrition) (Kohli et al. 2014). However, others have challenged the use of milk production as an acceptable welfare indicator (Wheelock et al. 2010). Despite the documented challenges with using milk production as an indicator of welfare in lactating dairy cows, very recent evidence suggests that milk composition changes may be more useful in assessing cows in immediate heat stress (Javed et al. 2009). Du Preez et al. (1990), in Southern African conditions, found that the productivity of cows was affected by heat stress when THI values were higher than 72 (which corresponds to 22°C at 100% humidity, 25°C at 50% humidity, or 28°C at 20% humidity). During the summer period, THI and AT showed similar correlation coefficients with cow productivity, BW, RT, and CC. Our study showed that, when THI reached 72, milk production of cows and feed intake decreased. We found that milk yield dropped by 0.22±0.001($R2=0.821$, $p<0.001$) kg per cow per day for each point increase in the value of THI above 72 to a level of THI=78. Heat stress influenced a decrease in CC's in dairy cows (1.72%, $p<0.001$). Besides changes in milk yield, heat stress could also decrease the rumination time (3.12%) (Table 1) and increase frequencies of mastitis (5%) (Table 2).

Heat stress and milk composition

Milk composition are also discordantly altered during hyperthermia, which indicates that HS regulates component synthesis in addition to its overall effect on milk yield (Gao et al. 2017). Milk F/P correlated negatively with THI (r=-0.009-0.020; $p<0.01$). Nasr and El-Tarabany (2017) revealed that percentages and yields of fat and protein were lower in the high THI compared to the low THI. The fat % in cool weather was higher than in hot weather (Berman 2011) but was lower than other researchers have reported (Bernabucci et al. 2014). The reduction in saliva $HCO3-$ content and the decreased amount of saliva entering the rumen makes the heat-stressed cow much more susceptible to subclinical and acute rumen acidosis (Shwartz et al. 2009). The milk lactose (ML) was positively correlated with AT and THI (r=0.120-0.124, $p<0.01$) but was negatively correlated with RH ($r=-0.208$, $p<0.01$). Nasr and El-Tarabany (2017) revealed that lactose % did not differ between low and high THI, which was in agreement with the findings reported by other researchers (Mishra et al. 1970).

Heat stress and RT, BW and CC

There was a negative relationship between RT, BW , and metrological parameters ($p<0.01$). The CC was positively related to RH ($r=0.117$, $p<0.01$) and negatively with AT and THI (r=-0.065-0.084, $p<0.01$). Moallem et al. (2010) indicated that THI's primary negative effect is a depression of RT, which subsequently led to a reduction in DMI, followed by a decline in milk yield. A statistically significant effect of THI on RT was identified, with RT decreasing as THI increased (Mishra et al. 1970). Acatincai et al. (2009) observed that with an increase in air temperature above 27 to 28°C, the rumination process was severely affected in Romanian Black and White multiparous cows. Moallem et al. (2010) indicated that the primary negative effect of the high THI is a depression of RT, which subsequently led to a reduction in DMI, followed by a decline in milk yield.

These authors suggested that rumination time plays a pivotal role in the negative effect of heat stress on DMI and, consequently, on milk production. However, Bernabucci et al. (2010) recently showed that reduced nutrient intake (an indirect effect of heat) accounts for only about 35% of the heat stress-induced decrease in milk synthesis. Moallem et al. (2010) indicated that the primary negative effect of the high temperature-

-humidity index (THI) was a depression of rumination time (RT), which subsequently led to a reduction in DMI, followed by a decline in milk yield. These authors suggested that rumination time plays a pivotal role in the negative effect of heat stress on DMI and, consequently, on milk production.

Energy intake is considered the most production-limiting nutrition component during summer (West 2003). According toRenaudeau et al. (2012), heat-stressed dairy cows reduced their feed intake to avoid excessive metabolic heat production while maintenance costs increased.

As rumination time decreases with increasing THI (Soriani et al. 2013), ruminal passage rate and DMI are reduced. For instance, heat stress animals reduce DMI, activity, and metabolic rate to decrease metabolic heat production (Von Keyserling et al. 2009). Earlierresearch (Armstrong 1994) showed that a decrease in RT is related to the prevalence of ruminal acidosis in dairy cows. We found that the risk of acidosis increased significantly (40.6%) when THI ≥72 because RT then reached the lowest level (RT<432).

Heat stress and reticulorumen pH and temperature

High environmental temperature adversely affects rumen health (Conte et al. 2018) for various biological and management reasons (Soriani et al. 2013). We found that an increase in THI is related to a decrease in reticulum pH. The lowest reticulum temperature was estimated at the highest AT and THI levels and the lowest level of RH. In agreement with Lefcourt et al. (1999), the daily maximum RT was significantly greater than the daily minimum RT in all seasons. The reticulorumen temperatures decreased after water intake and returned to the baseline temperature within 0.3 to 3.5 hr (Antanaitis et al. 2016). Season also profoundly affected the circadian body temperature rhythm, but there was little difference between dairy cow breeds (Kendall et al. 2009). This is important for assessment of the health condition by temperature. However, these changes may also reflect a biological change in the core body temperature associated with changing seasons. The seasonal body temperature baseline may be useful for interpreting the differences in body temperature and used for cow management by season. At a minimum, these differences indicate the need to adjust the thresholds used for management purposes by season (Herbut and Angrecka 2018). The summer months may be associated with heat stress. A heat-stressed cow is prone to rumen acidosis, and many of the lasting effects of warm weather (laminitis, low milk fats, etc.) can probably be traced back to a low rumen pH during the summer months (Bewley et al. 2008). According to Antanaitis et al. (2016), the reticulorumen temperature in lactating cows was influenced by circadian rhythm and season (the lowest temperature observed in the springtime was 38.81±0.001, and the highest was in autumn, 39.17±0.001).

Sievers et al. (2004) suggested that an intraluminal measuring system would be advantageous because it is independent of external disturbing factors, cannot be manipulated from the outside, and is less likely to be lost. Rumen temperatures have been established as effective measures of core body temperature (Bewley et al. 2008). Because of the activity of heat-producing rumen microorganisms, ruminal or reticular temperatures are generally approximately 0.5°C greater than the core body temperature (Bewley et al. 2008a,b).

According to Gao et al. (2017), ruminal pH was reduced (9.5 and 6% before and after feeding, respectively) during heat stress. Additionally, the intraruminal temperature may affect rumen metabolism (Gengler et al. 1970). These authors showed that an increase in intraruminal temperature implies a reduction in feed and water intake (water intake increased only when needed for the thermoregulation of the whole body), implying a decrease in volatile fatty acid production and a shift in fatty acid composition with a significant decrease in the acetate to propionate ratio (Conte et al. 2018).

Conclusions

According to this study, during HS, the higher THI positively correlates with ML, which shows tendencies to increase mastitis risk and decrease CC, RT, BW, MY, reticulorumen pH, and F/P. During the summer, some biomarkers of HS can be milk yield, milk lactose, somatic cell count, concentrate conception, rumination time, body weight, reticulorumen pH, and milk fat/protein ratio. We recommend monitoring these parameters in the herd health management program to identify heat stress.

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