RESEARCH ARTICLE

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Alterations in Gut Integrity Due to Heat Stress Among Dairy Cattle of Aydin City: Analytical Interpretation of Zonulin Levels within Repetitive Measurements

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Abstract

Animal health and production are impaired with heat stress inducing dysfunction of intestinal barrier. In this study, it was aimed to determine that the effect of heat stress on gut health via circulating zonulin concentrations. Holstein dairy cows (n=7, age of 2-5 years) were housed in Aydin municipality with higher temperatures in summer. Blood sample for serum zonulin levels were collected at 12 pm and 00 am with temperature records of 44°C and 31°C respectively. Serum zonulin levels were found to be statistically significant (p=0,012) at night in contrast to daylight analytes. With this study it was appeared that heat stress had a negatively impact on intestinal integrity among cows.

Keywords: Intestinal dysfunction, heat stress, lactation cattle, zonulin.

INTRODUCTION

Climatic conditions and thereof alterations are proposed to pursue in an upward trend (Karl and Trenberth, 2003). Furthermore, beginning with 2001, it has been recorded as 16 out of 17 hottest years (Karl and Trenberth, 2003). Even if this increasing trend endure, livestock industry would be strucked (Tellez Jr et al., 2017). Heat stress (hS), denoting hyperthermia, might be caused by thermoregulation breakdown existing even if animals invent or take in more temperature than it depletes (Lara and Rostagno, 2013). Given adverse efficacy of hS mild discomfort, severe stress, multiple organ damage or death could exist. On the other hand, as the gut participates a major induction for digestive process, absorption of nutrients, and transportion, yet it is quite reactive and perceptive to hS.

Gastrointestinal lumen has a relationship with a responsive for digestion and absorption of most nutrients across to the transcellular area under normal thermal situations (Salzman, 2011). Intestinal epithelial cells arrange a barrier wall between peripheral and internal environment (Elson and Cong, 2012). Damage of gastrointestinal epithelium made changes in gut permeability and many microorganisms finds a way to translocate via portal vein to activate chronic inflammation (Ilan, 2012). Numerous researches has been focused on the effect of acute and chronic stress on intestinal permeability modifications which is caused by tight junction breakdown (Maejima et al., 1984, Koh et al., 1996, Assimakopoulos et al., 2011).

In the present study the researcher group of multidisiplinary arms (agriculture engineering, animal breeding and veterinary internal medicine) analyzed the effect of hS on tight junction (tJ) proteins and gut health via circulating zonulin concentrations.

MATERIALS AND METHODS

Study era and Climatic conditions during field trial

The study was performed in a private farm located in Aydin city of Turkey. The latter farm is dominated by Mediterrinean climatic conditions. In the province, where there is no significant difference between the sub-regions, summers are hot and dry, and winters are warm and rainy. The highest temperature in the province is observed in July and the lowest temperature is observed in January. Annual average temperature is 17.6°C (Kaya and Aydın, 2017).

Seven Holstein dairy cattle with 2-5 years of age at early lactation stage were admitted to study (Figure1). Cattle were feed twice daily with TMR ration composed of 125 kg concentrate including 19% protein with 400 kg corn silage, 200 kg straw, 150 kg barley silage and 50 kg clover for 50 animal/per.

Figure 1. Photographic records where the study was performed.

Equivalent temperature index for cattle reported previously (Wang et al., 2018) was used as a reference tool, for performing this field trial, which was shown a table 1 (Anonymous 1, 2021; Anonymous 2, 2021). According to this Table, present data obtained at 12 pm and 00 am with temperature records of 44°C and 31°C respectively, on 3 August 2021 (Anonymous 1, 2021; Anonymous 2, 2021), indicated that cows participated in this study were severe and emergency affected, respectively, by hS. Table 2 showed climatic conditions where the trial was performed, denoting Aydin Municipality.

Table 1. Equivalent temperature index used as reference for climatic conditions (Wang et al., 2018).

Equivalent temperature index for cattle	
Heat stress	Temperature
Mild (may not be hearmful)	23° C ≤ETIC<26 ° C
Moderate (may be harmful)	26° C ≤ETIC<31 ° C
Severe (triage must be involved)	31° C ≤ETIC<37 ° C
Emergency (triage and management)	ETIC>37 ° C

*ETIC: Equivalent Temperature Index for cows

Table 2. Equivalent temperature index used as reference for climatic conditions (Anonymous 1, 2021; Anonymous 2, 2021).

	July 2021	August 2021	September 2021
Mean temperature (°C)	26.2	29.8	30
Min. Temperature (°C)	19.8	22.6	23.1
Max. temperature (°C)	32.3	36.6	36.9

*Data was relevant to what was obtained (Anonymous 1, 2021) during field trial denoting weather conditions. Data was obtained at 12 pm and 00 am with temperature records of 44°C and 31°C (Anonymous 2, 2021).

Sampling and laboratory work up summary

Blood was withdrawn from *Vena jugularis* into anticoagulated tubes twice a day at 12 pm and 00 am at 03.08.2021. *Serum samples were stored at -20 ° C until the analysis was performed.* Bovine zonulin ELISA (MyBiosource ELISA kits, USA) were performed according to the procedure described by manufacturer.

Statistical analysis

All data was tabulated as mean and standard deviation. Normality tests of the data within the hours in same animals were determined by Shapiro-Wilk test. Comparisons between different measurement times were conducted by Mann-Whitney U test. All statistical analyses were performed by program SPSS 22.0 (IBM, USA) and $p < 0.05$ was accepted as statistically significant.

RESULTS

Circulating serum zonulin levels (ng/mL) was shown on bar graphic (Figure. 3) and Table 3, along with statistical analytes. Serum zonulin levels were deemed statistically (significantly altered) (p=0.012) at night in contrast to daylight analytes.

Figure 2. ELISA platform used in this study presenting commercially available test kits.

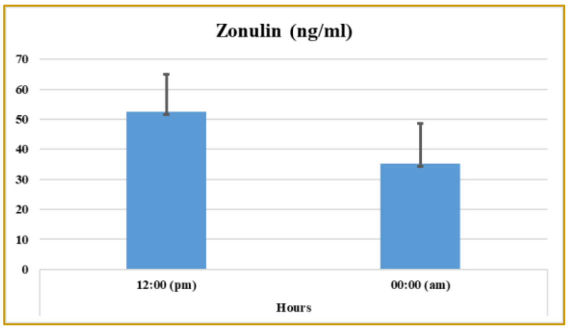

Figure 3. Circulating serum zonulin levels

Table 3. Serum zonulin levels as shown with $\bar{X} \pm$ SD (mean ±standart deviation).

	Hours		
Zonulin (ng/ml) $\bar{X} \pm$ SD	12:00 (pm)	00:00 (am)	*p value*
	52.52 ± 12.34	35.26 ± 13.25	0.012

DISCUSSION AND CONCLUSION

Under hot, such as where the present study performed, namely Aydın province in Turkey, humid environment, (i.e. summer) dairy cow cannot deplete enough body heat for preventing an increase in body temperature. Elevated air temperature, temperature-humidity index and increased rectal temperature raising censorious thresholds causes diminished milk yield and dry matter intake (West, 2003). The present study was performed during 03 August 2021, and related data was obtained at 12 pm and 00 am with temperature records of 44°C and 31°C respectively. Therefore, inclusion criteria were optimal for interpretation of hS on cows, denoting that this field study might be a role model for further studies. According to our knowledge there were no other documented reports in our country, analyzing zonulin levels in relation with hS among cows.

Hyperthermia and thereof subsequent hypoxia not only give rise to reactive oxygen species production, at the same time disrupt the antioxidant defense system (King et al., 2016). Apart from those alterations among cells of the intestinal barrier system, hS also influence the gut microbiota (Suzuki et al., 1983). Even if the gut microbiota ingredient changes, along with the hS induced the barrier function ruination, opportunistic intestinal infections might exist (Lian et al., 2020) in which colonization of enteric pathogens (Farag and Alagawany, 2018), and intestinal inflammatory responses (Ribet and Cossart, 2015). To the present authors' knowledge although involved cow in this study apparently seemed to be healthy, altered intestinal barrier integrity via hS presented as zonulin levels, aroused our interest even if those cows are diseased, or presenting systemic inflammation, which would be one of our target in the next study.

Intestinal barrier (iB) composed of biochemical and physical blockade against pathogenic and commensal ingredients belonging to the intestinal lumen. Given the iB prevents passage of bacterial toxin and pathogens whereas promotes nutrient absorption, intestinal epithelial cells are vital for the rectitude and functionality (Goto and Ivanov, 2013). Given intestinal epithelial cells preserve iB via incorporation of microbiome signals and integrate proper immune response (Goto and Ivanov, 2013), hyperthermia arbitrate intestinal epithelial cells along with their tJ resulting in intestinal permeability (Lambert et al., 1985). As a consequence physiologically, toxicologically or pathologically impaired or disturbed intestinal epithelial cells or tJ breakdown prevention of access of specific molecules into circulation resulting in systemic inflammatory response (King et al., 2021). Regarding thermal neutral environment, paracellular junction are diligently altered (Di Pierro et al., 2001). On the other hand, due to hS circumstances, the tJ barrier arbitrated and luminal content leak into the circulation, denoted as leaky gut (Bosenberg et al., 1998) accompanied by chronic systemic inflammation negatively influencing the performance of the animals. Furthermore altered gut permeability has been linked to bacterial translocation (Ilan, 2012). Performance analytes would be enrolled in our subsequent study.

Given the hS derange tJ in the intestine, along with increased intestinal permeability among humans, pigs, and rodents (Hall et al., 2001; Dokladny et al., 2006; Pearce et al., 2013), accompanied by elevated penetration of toxic compounds, small particles and bacteria, evoke an inflammatory respond in the gut (Farquhar and Palad, 1963; Baker et al., 1988; Hollander, 1988). In a prior study the authors comperatively investigated pair-fed dairy cows (kept at thermal neutral environment) to those of animals under hS (continuoulsy kept at 28 °C for four days), denoted that hS was capable of directly altering jejunal tJ proteins proposing impaired intestinal barrier (Koch et al., 2019). According to the latter researcher group spiking of bacterial and toxic compounds at hS was capable of modulating immune repertoire; inducing antioxidative fortification for maintenance of homeostasis among commensal bacteria and the jejunal immune system (Koch et al., 2019). Their bovine model presented direct efficacy of hS on the jejunum at moderately increased ambient temperature. In line with the latter study, another supportive finding was documented in pigs under hS. According to that study hS and decreased feed intake contributed to declined intestinal integrity and elevated endotoxin permeability (Pearce et al., 2013). Circulating serum zonulin levels (ng/mL) $\bar{X} \pm$ SD (mean ± standart deviation) as was shown on bar graphic (Fig. 2) and Table 3, revealed significant decreases (p=0,012) at night 35.26 ± 13.25 in contrast to daylight 52.52 ± 12.34 analytes (Fig. 3 and Table 3).

In conclusion, present data obtained at 12 pm and 00 am with temperature records of 44°C and 31°C respectively, on early August 2021, indicated that cows participated in this study were severe and emergency affected, respectively, by hS. As a preliminary finding hS appeared to negatively impact intestinal integrity among cows in the present study.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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