RESEARCH ARTICLE

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Heat Stress and Seasonal Dissipation of Circulating Zonulin Levels Among Calves in Aydın Region

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Abstract

Zonulin, a well-recognized protein, is influencing the integrity of intercellular connections in the intestines. It has also been evidenced that heat stress (hS) might alter intestinal permeability. In the present retrospective field research the aim was to investigate the gastrointestinal permeability of calves exposed to hS (especially in summer) by determining serum zonulin levels and their relationship to seasonal dissipation among appearently healthy calves. As two different months represented 2 seasons, serum zonulin concentrations in January 2022 (at 20:00 pm 4°C and 08:00 am 10°C) and June 2022 (at 20:00 pm 19°C and 08:00 am 27°C) exhibited significant differences. Inter-group and intra-group comparison showed statistically significant differences (p=0.012) both in seasonal distribution and spatial distribution at different day/night times. When compared between the January and June groups, the mean zonulin levels (ng/mL) at 12 pm and 08:00 am in the January group were 28.04±12.49 vs. 11.55±8.45, respectively (p=0.012). On the other hand at June group the mean zonulin levels at 12 pm and 08.00 am were 58.54±19.16 vs 24.03±9.9, respectively (p=0.012). Obtained results should be cautiously subjected to interpretation, in which hS affect intestinal integrity and seasonal dissipation of circulating zonulin levels should be taken into consideration for intestinal health of calves.

Keywords: Calves, dissipation, heat, seasonal, stress, zonulin.

INTRODUCTION

Calves are prone to consequences of heat stress (hS) even if temperatures reach up to 26 ºC. As hS give rise to energy usage of calf in an attempt to disperse overabundance heat from its body, reduced weight gain, health disorders, and unsatisfactory growth performance ('a calf does not mean a great cow at all time'). Furthermore, hS can also lower blood serum immunoglobulin levels, leading to a greater risk of disease and death (Tao et al., 2012).

Given intercontinental warming (Schär et al., 2004), dairy industry is over against hS in which Aydin City, in Turkey has long been well known with its very hot summer conditions to those of animals more exposed to those alterations (Schüller et al., 2014; Duru, 2018; Demirhan and Şahinler, 2019). It has been well elucidated within several literature (Ruff et al., 2019; Lian et al., 2020; Nanto-Hara et al., 2020; Alic Ural et al., 2021a, b) that hS induce leaky gut and intestinal integrity disruption.

Thermoneutral zone (tNz) might be denoted as a unique milieu temperature range in which livestock perpetuate a constant body temperature for maximum productivity (Kadzere et al., 2002). As was shown Figure 1. in which adopted from original data of Hahn (1997), tNz for calves and heifers were between 13 to 25 °C, and 0 to 15 °C, respectively, (Hahn, 1997). Relevant hS exists i) through body temperature of the livestock animal elevates and even if could not evaporate sufficiently for maintenance of thermal equilibrium, ii) probably in relationship with terrain temperature above tNz together with elevated humidity/slow air movement (Morrison, 1983; Bernabucci et al., 2010). Relevant heat build-up could be resulted in performance and reproduction alterations along with elevated mortality of livestock. An estimation of yearly economic waste to livestock via hS was reported nearly $1.69-$2.36 billion within the United States (Stpierre et al., 2003). Given hS is a significant summons within the animal husbandry, the present research as field retrospective study was performed in an attempt to detect seasonal dissipation of serum zonulin levels among apparently healthy calves.

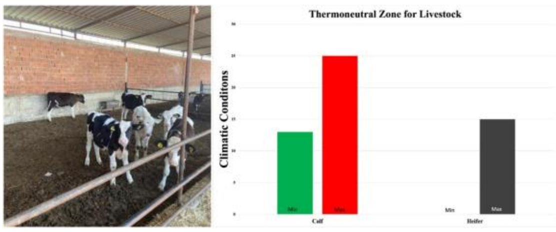

Figure 1. Calves were involved in the present study (the present authors' archive) under thermoneutral zone (tNz), b) Climatic conditions relevant to tNz for calves [13 to 25 °C] and heifers [0 to 15 °C] adopted from Hahn, 1997 and self-drawn.

MATERIALS AND METHODS

Study Demographics

This retrospective field study was performed (with the support of 2 veterinary surgeons taking blood samples) in a commercial farm located in Aydin City. All involved calves were apparently healthy, were self-control, to those of blood sampling was deemed available twice by the

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referring veterinary surgeons on January and June, 2022. This study was approved by the local ethic committee of Aydin Adnan Menderes University- HADYEK on 27.10.2021 with no: 64583101/2021/146.

Sampling and Field Research

One ml blood was withdrawn (by experienced veterinary surgeons) from *Vena jugularis* into anticoagulated tubes. Sera samples were immediately forwarded to laboratory work. Commercially available Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) were used. Testing methodology was similar to prior works by the present author and her teammates involved at the relevant researches (Alic Ural et al., 2021a, b).

Statistical analysis

Descriptive statistics of the obtained data were performed, they were tabulated as mean and standard deviation. It was determined that the distributions of the data that did not show homogeneous distribution before and after the logarithmic transformation processes. Wilcoxon test was used to compare the measurements at different times of the same season. In the comparison of the measurements of different seasons in the same time period, the independent sample Kuruskal-wallis test was used. All statistical analyzes and graphs SPSS 26.0 (IBM, USA) and Graphpad (Prism, USA) programs were used and the p value was below 0.05 were considered statistically significant

RESULTS

Available and obtained results were deemed available at Table 1 and Figure 2-3 showed a calf involved at the present study in comparison to other 2 calves. As two different months represented 2 seasons, serum zonulin concentrations in January 2022 (at 20:00 pm 4 °C and 08:00 am 10 °C) and June 2022 (at 20:00 pm 19 °C and 08:00 am 27 °C) exhibited significant differences. Inter-group and intra-group comparison showed statistically significant differences (p=0.012) both in seasonal distribution and spatial distribution at different day/night times. When compared between the January and June groups, the mean zonulin levels (ng/mL) at 12 pm and 08.00 am in the January group were 28.04±12.49 vs. 11.55±8.45, respectively (p=0.012). On the other hand at June group the mean zonulin levels at 12 pm and 08.00 am were 58.54±19.16 vs 24.03±9.9, respectively (p=0.012) (Table 1 and Figure 2).

Table 1. Circulating serum zonulin levels (ng/mL) among healthy calves in relationship with seasonal dissipation (mean± SD).

Score	January 2022		June 2022	
	20:00	08:00	20:00	08:00
Zonulin levels (ng/mL)	28.04 ± 12.49[a]	11.55 ± 8.45[c]	58.54 ± 19.16[b]	24.03 ± 9.9[cd]
Wicoxon test results	0.012		0.012	

[a,b,c,d]:Data shown with different letters on the same line are statistically different.

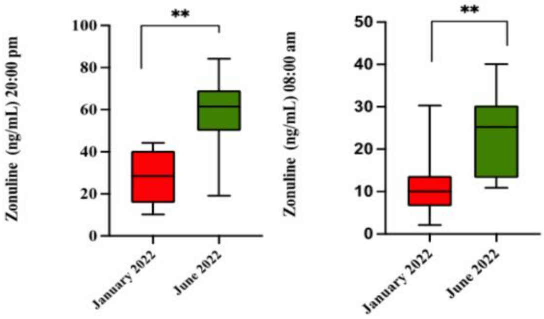

Figure 2. Boxplot analyses for circulating serum zonulin levels related to seasonal dissipation.

Figure 3. A calf exposed to hS and two other calves (not involved at the present study) without hS.

DISCUSSION AND CONCLUSION

The present author participated and took an active role for performing 2 previous researches. In first one which took place in August 2021 with climatic conditions involving 41.1 ^{0}C with a 36 % humidity, serum zonulin levels were analyzed by use of commercially available ELISA test kits (very similar to this study) to those of calves under hS. Serum zonulin (ng/ml) levels were detected (60.07±21.20) at mid night 00.00 am. On the other hand mid-day analyses at 12.00 pm (34.60±10.90) (p=0.018) were also determined. According to the latter field research altered zonulin concentrations suggested intestinal barrier disruption associated probable intestinal permeability increase due to hS (Ural et al., 2021a). Second study performed previously composed of cows exposured to hS whether developed intestinal barrier dysfunction. In that study, Holstein dairy cows aged 2-5 years (n=7) kept in Aydin with elevated temperature in mid-end summer were subjected to field analysis for serum zonulin concentration detection. Serum zonulin levels were markedly (p=0.012) altered (at 12 pm -44 °C and 00 am -31 °C). In the latter investigation hS negatively changed intestinal integrity in cows (Ural et al., 2021b). According to the results of the present study, taking into account serum zonulin concentrations in January 2022 (at 20:00 pm 4 °C and 08:00 am 10 °C) and June 2022 (at 20:00 pm 19 °C and

08:00 am 27 °C) there were statistically significant differences. Inter-group and intra-group comparison revealed statistically significant differences (p=0.012) both in seasonal distribution and spatial distribution at different day/night times. When compared between the January and June groups, the mean zonulin levels (ng/mL) at 12 pm and 08.00 am in the January group were 28.04±12.49 vs. 11.55±8.45, respectively (p=0.012). On the other hand at June group the mean zonulin levels at 12 pm and 08.00 am were 58.54±19.16 vs 24.03±9.9, respectively (p=0.012) (Table 1).

In conclusion as lactating dairy cow has been implicated as a compassionate model for interpretation of hS as because of their in-depth metabolic warm manufacturing along with small surface:volume ratio (Kocha et al., 2019), calves were also taken into consideration in relationship with hS (Alic Ural et al., 2021a), as was also evidenced of proof in this study. Intestinal health monitorization should be deemed available via serum zonulin levels measured with seasonal dissipation, as evidenced in this study.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: D.A.U. Data Collection or Processing: D.A.U. Analysis or Interpretation: D.A.U. Literature Search: D.A.U. Writing: D.A.U.

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