

Zonulin as a Noninvasive Selected Biomarker of Gut Barrier Function Identify and Debug Calves Suffering from Diarrhea

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Abstract

Zonulin elucidated as a thoroughly known protein, is capable of modulating the gut integrity of intercellular connections. Intestinal permeability and its modulation by zonulin have been well-defined. Zonulin levels could increase in response to several stimuli, i.e. infection/gluten ingestion. Even if the latter occurs, zonulin signals into the body for elevating the permeability of the gut lining, permitting larger molecules to pass through. All aforementioned conditions initiate inflammation. In the present prospective field study, the aim was to determine the specificity of zonulin as a noninvasive selected biomarker of gut barrier function to identify and debug calves suffering from diarrhea. Furthermore, another purpose was to define the appearance of leaky gut (LeaG) among calves with diarrhea. By use of commercially available Bovine Zonulin ELISA test kits with a well-designed methodology all 11 diseased and other relevant healthy calves gave positive test results. Circulating zonulin levels (ng/mL) expressed as ($\pm$ SEM), there were significant differences ($p < 0.001$) between healthy (26.43 ± 3.528) and diarrheic calves (57.97 ± 4.250). As a preliminary conclusion, it should not be unwise to draw the hypothesis that zonulin levels debug diarrheic calves from healthy ones. Further studies are warranted.

Keywords: Calves, diarrhea, leaky gut, zonulin.

INTRODUCTION

To date, with positive vibes recorded at farm animals and both agricultural sciences and veterinary field, the relationship among the intestinal barrier, microbiota along with the immune system has paid growing attention. The significance of bona fide intestinal barrier functioning has gained proper attention, thus with growing body of evidence within relevant literatures, in ruminants residing to excessive vulnerability to microbial antigens imminent from pre-gastric fermentation chambers (Mani et al., 2012). Recently intestinal health on animal productivity and well-being are paid attention. To those of various factors coincided within actual management measurement interact with the animal's intestinal health, capable of altering barrier function and leading to leaky gut (LeaG). Related factors involved heat stress (Aliç Ural et al., 2021a; Aliç Ural et al., 2021b; Aliç Ural 2022; Baumgard and Rhoads, 2013; Pearce et al., 2013; Moeser et al., 2007; Wood et al., 2015), diminished feed intake (Zhang et al., 2013; Kvidera et al., 2017), weaning (Moeser et al., 2007; Wood et al., 2015) and ruminal acidosis (Emmanuel et al., 2007; Khafipour et al., 2009; Minuti et al., 2014) along with other relevant factors. On the other hand, interpretation of confounding factors contributing to LeaG is not very easy as the disorders responsible for diminished intestinal barrier integrity could also influence the immune response and metabolism of other relevant tissues (Kvidera et al., 2017). Examples of these confounding situations include the periparturient period in dairy cows and heat stress, both of which are accompanied by marked homeorhetic adaptations to support a new dominant physiological state (Bauman and Currie, 1980; Baumgard and Rhoads, 2013). Evaluating the physiological and

phenotypical consequences of leaky gut in isolation would provide insight into its contribution to the pathophysiology of common on-farm disorders.

MATERIALS AND METHODS

Study demographic data and variables

This prospective field study was performed (with the guidance of veterinary surgeons, whom withdrawn blood samples, with written owner consent) in a commercial farm located in Aydın Municipality at Egean Region of Turkey. This prospective field study was approved by the local ethic committee of Aydın Adnan Menderes University- HADYER on 27.10.2021 with no: 64583101/2021/146. A total of 22 calves were enrolled ($n=11$ each in diseased and healthy calves). Diarrhea was defined based on fecal consistency as shown in Table 1. The fecal consistency scoring was conducted using a 4-level scoring scale: 1=soft (does not hold form, piles but spreads slightly); 2=runny (spreads readily); and 3=watery (liquid consistency, splatters).

Table 1. Diarrhea was defined based on fecal consistency as first described by Larson et al., (1977) and then was revised by McQuirk, (2008).

Fecal consistency scoring	
0	normal (firm but not hard)
1	soft (does not hold form, piles but spreads slightly);
2	runny (spreads readily);
3	watery (liquid consistency, splatters).

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Sampling and trial era

One ml blood was withdrawn (by responsible veterinary surgeons) from *Vena jugularis* into anticoagulated tubes. Sera samples were then thoroughly sent to laboratory work by trained personnel. Commercial value Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) were purchased previously. Testing methodology was deemed similar to prior works by the present author involved (Alic Ural et al., 2021a; Alic Ural et al 2021b).

Statistical analysis

Descriptive statistics of the obtained data were performed and were tabulated as mean and standard error. Non-parametric Mann-Whitney U test was used for comparisons between groups, and cases with a p value less than 0.05 were considered statistically significant. All analyzes were performed using the Graphpad 9.0 (Prism, USA) program.

RESULTS

Demographic values

All diseased calves (n=11) from both sexes, at the age of 2 to 40 days old with diarrhea (based on fecal consistency. As McGuirk, (2008) proposed a revised 0 to 3 scale, and classified diarrhea between scores of 2 (loose however enough consistency to lie on bedding) or 3 (watery feces lying through the bedding), solely calves with a score of 2 or 3 were enrolled. 7 out of 11 calves presented a score of 2 and other relevant ones as score 3.

Serum zonulin levels expressed

As detected by commercially available Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) with a well-designed methodology (Alic Ural et al., 2021a; Alic Ural et al., 2021b), all 11 calves gave positive test results (Fig. 1 and Table 2). As shown both in fig. 1 (as box plot analytes) and table 2 (with Zonulin levels (ng/mL) expressed as ($\bar{X} \pm \text{SEM}$), there were significant alterations ($p < 0.001$).

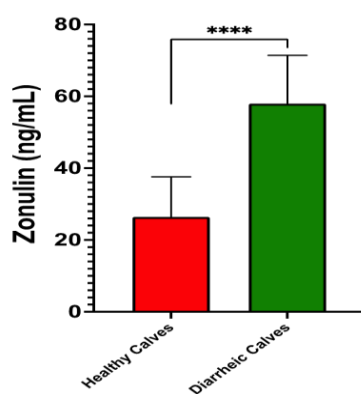


Figure 1. Boxplot analytes for circulating serum zonulin levels among healthy and diarrheic calves. Asterix showed statistically significant differences ($p < 0.001$) among healthy and diarrheic calves.

Table 2. Circulating serum zonulin levels (ng/mL) among healthy and diarrheic calves.

	Zonulin (ng/mL) ($\bar{X} \pm \text{SEM}$)	P value
Healthy Calves	26.43±3.528	0.001
Diarrheic Calves	57.97±4.250	

DISCUSSION AND CONCLUSION

The present field research (with multidisciplinary accession i.e. agriculture engineering, department of animal production and veterinary internal medicine) study, which is the foremost first to investigate correlations between circulating LeaG biomarker level, namely zonulin and diarrhea among calves, in which revealed that increased intestinal permeability might be associated with noninvasive selected biomarkers of gut barrier function. This issue needs to be addressed that, noninvasive biomarkers should have helped identify and debug calves suffering from diarrhea. Old and unfashioned biomarkers should be removed out and novel biomarkers should participate and be on the list of diagnosis at field conditions also. This is why this study was performed at field conditions as original research.

Elevated gut permeability has been suggested to be a structural constituent, besides with genetic background and ecological setoff, participating in chronic inflammatory burden [i.e. autoimmune, allergic, and metabolic disorders] (Arrieta et al., 2006, Fasano and Shea-Donohue, 2005, Sapone et al., 2006). To the vast majority of field veterinary surgeons, understanding the issue of increased intestinal permeability rely upon observable parameters. Oral presentation surveys revealed that most of the veterinary surgeons are unaware of field experience and performing analytes regarding intestinal permeability. Therefore, this field study should have the capacity of attracting the audiences, and highlight the importance of zonulin detection among calves with diarrhea. The author of this manuscript evolved and participated with zonulin analyses in several documented reports (Alic Ural et al., 2021a; Alic Ural et al., 2021b; Alic Ural, 2022). As obtained data relevant to this study as a preliminary observation denoted that LeaG was evident to those of diarrheic calves. Evidence of proof for LeaG was supported by circulating zonulin levels detected. As shown above on in fig. 1 (box plot analytes) and table 2 [with Zonulin levels (ng/mL) expressed as ($\bar{X} \pm \text{SEM}$)], significant differences ($p < 0.001$) debug healthy calves from those of diarrheic ones (also were shown in Fig. 2).



Figure 2. a and b) A 2-days old calf with diarrhea presenting zonulin level of 37.143 ng/mL and a fecal consistency score of 2 and d) a 15-days old calf with diarrhea presenting zonulin level 65.980 ng/mL with a fecal consistency score of 3.

Zonulin, as an analogue for *Vibrio cholerae* zonula occludens toxin, has been detected as ascetic regulator of epithelial tight junctions (eTj), which was shown to be transcribed in several disorders. In the present article with a multidisciplinary approach, the author hypothesized that probable loss of intestinal barrier function, could be attributable to i) secondary activation of the zonulin

pathway by any environmental triggers or alterations in gut microbiota, ii) involvement of intestinal innate immunity, iii) upregulation in gastrointestinal disease (herein non-differentiated diarrhea, with unknown etiology), and iv) diarrhea predominant gastrointestinal alterations might increase intestinal permeability among calves herein involved. Hence due to the hypothesis that zonulin might be an important factor involved in the pathogenesis of diarrhea a total of 11 diseased calves patients were studied. ELISA assay was performed in an attempt to make interpretation of zonulin serum levels. Due to significant alterations in serum zonulin levels detected, it should not be unwise to draw the conclusion that this methodology debugs healthy and diarrheic calves. Further researches are warranted, which would be the purpose of the next study.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: D.A.U., Design: D.A.U., Data Collection or Processing: D.A.U., Literature Search: D.A.U., Writing: D.A.U.

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