

Association between selected metabolic parameters and chronic digital dermatitis in Holstein dairy cows

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ABSTRACT

The objective of the present study was to investigate the association between chronic digital dermatitis (DD) and the selected metabolic and hematological parameters in Holstein dairy cows. For this purpose, thirty-eight parturient dairy cows investigated. Of all, 19 cows were suffering from chronic digital dermatitis, and 19 cows were clinically healthy. Cows were examined clinically and those with chronic DD were selected. Subjective evaluation of cows with DD was performed by one person and the score was recorded. Blood samples were obtained from diseased and control cows for measurement of metabolic profile. β -HB showed a significant increase in cows with DD in comparison to control health cows ($p < 0.01$). But glucose showed a significant decrease ($p < 0.05$). Regarding the macro- and micro elements, there was a significant decrease of calcium ($p < 0.0$) and phosphorus ($p < 0.01$) level in cows with DD in comparison with healthy control cows. while serum Mg showed non-significant changes between groups. Serum mineral values of Zn, and Cu were statistically significantly decreased ($p < 0.01$) in cows with chronic DD in comparison with the control cows. Total leukocytic counts, neutrophils and neutrophils lymphocyte ration were significantly increased in diseased cases compared with control ($p < 0.01$). But lymphocytes were significantly decreased ($p < 0.001$). In conclusion, the results of the present study indicate that DD may affect greatly the metabolic status of the diseased cows with consequent occurrence of other metabolic diseases. The biochemical and hematological changes may highly be related to inflammatory stress.

Introduction

In dairy cattle, the postpartum period is usually associated with a high incidence disease and a high risk of removal from the herd. These risk factors may trigger a cascade of other diseases, such as ketosis, retained placenta, metritis, mastitis, milk fever, displaced abomasum, lameness, and impaired fertility (Ingvartsen *et al.*, 2003).

Lameness in dairy herds continues to represent a serious issue, detrimental to animal welfare, milk production, and farm economics globally. In dairy cows, the main cause of lameness is claw lesions, which are either non-infectious (white line disease, sole ulcer, sole hemorrhage, interdigital hyperplasia) or infectious, including digital dermatitis (DD), interdigital dermatitis, heel erosion, and interdigital phlegmon (Van Nuffel *et al.*, 2015).

Digital dermatitis (DD) is one of the principal causes of lameness and hence presents serious welfare problems and economic loss on dairy farms worldwide. Despite many years of research, the etiology of DD is still not completely understood (Bruijnjs *et al.*, 2010). DD affects the interdigital skin, frequently causes lameness in dairy cattle. In addition to the reduction of animal welfare, DD affects the animal culling rate and leads to production losses such as decreased milk yield and a decline in milk quality (Plummer and Krull, 2017).

An investigation underscored the multifactorial nature of DD, implicating a strong bacterial component, particularly *Treponema* spp., and highlighted the environmental factors, such as moisture, as significant risk contributors. The same authors emphasized that despite extensive research over the past four decades, the precise pathogenesis of DD remains incompletely understood (Refaai *et al.*, 2013).

It has been stated that mineral deficiencies intensely affect the immune system as their subsequent deficiencies lead to reduced immunity, which increases the risk of any infectious disease (Libera *et al.*, 2021). Minerals interact differently with the immune system; however, their defi-

ciencies consistently increase the risk of mastitis occurrence in dairy cows. Others reported that an adequate supply of available trace minerals, such as Zn, Mn, Cu, or I help in improving disease resistance of cattle (Miller and Madsen, 1992).

Research has shown that DD is associated with elevated serum concentrations of metabolites of inflammation, as well as housing factors and environmental conditions (Pirkkalainen *et al.*, 2022). Although the clinical lesions and the associated microbiota of DD lesions have been widely investigated, the role of biochemical parameters in the pathogenesis of DD and the progression of the clinical events related to DD have not been clarified. Therefore, the purpose of our study was to describe the changes in selected biochemical and hematological parameters in cows diagnosed with digital dermatitis

Materials and methods

Cows

Nineteen dairy cows of 450-550 kg body weight at 60.0 ± 14.0 days in milk (DIM) with an average year milk yield of 7.500 kg/year were included in the study. An additional, 19 healthy cows were selected as control group. The farm feeding management was based on TMR, and the dairy cows were housed in free stalls with straw bedding. The present study was carried out in the period between February to April 2023. All procedures in terms of blood sampling and veterinary examinations followed the animal care and welfare legislation criteria of Mansoura University, Egypt. The approval was provided by the Mansoura University ethical committee (MU-ACUC-VET: 11. 12.23)

Clinical examination

Clinical examination of affected cows was carried out according to

standard technique (Plummer and Krull, 2017).. Cows with a previous history of digital dermatitis (DD) characterized by chronic painful proliferative growth of the epithelium on the planar foot surfaces that speeded into the interdigital space were included.

Samples

Two blood samples (2 ml) were collected from each cow via coccygeal venipuncture. The first sample was added to a tube containing Ethylene Diamine Tetra acetic Acid (EDTA) as anticoagulant for hematological examination that was done by using an automated method using hematology auto analyzer ADVIA 120i-five pot instrument the automated analysis was done following the manufacturer’s operational guidelines. The second sample was collected into a clean dry tube without anticoagulant for serum separation and was centrifuged at 2000 × g for 15 min within 2 h of collection. Only clear non hemolyzed serum was harvested and kept frozen at – 20°C for further biochemical analysis of BHBA determined by using relevant kits (Randox, UK) with the Biotek brand spectrophotometric reading device available at the enterprise according to the method, glucose was assessed by colorimetric method using test kits (SPINREACT, S.A., Girona, Spain), calcium (Ca) photometrically at a wavelength of 660 nm using an automatic biochemistry analyzer (Indiko, Thermo Fisher Scientific, Waltham, MA USA), phosphorous (P) using commercially available reagents (Linear Chemicals, Spain), and the absorbance values of samples and standard were recorded by using UV spectrophotometer. Serum magnesium, copper, and zinc were determined by atomic absorption spectrophotometry, using commercially available kits according to the manufacturer’s instructions.

Statistical analysis

Data analyses for cows with chronic digital dermatitis were performed using a statistical program (GraphPad Prism for windows Ver. 5.0, USA). The Shapiro test was applied to assess the normal distribution of the data. Data passed the normality test and were found to be normally distributed. So means values and standard deviation for each assessed variable were calculated. Independent t-test was used to compare control cows with those with DD. Pearson correlation was applied to identify the correlations among variables.

Results

In cows with DD, there was a significant decrease of serum glucose in comparison with control ones ($p < 0.01$), but there was a significant increase of β -HB ($p < 0.01$) (Figures 1-2).

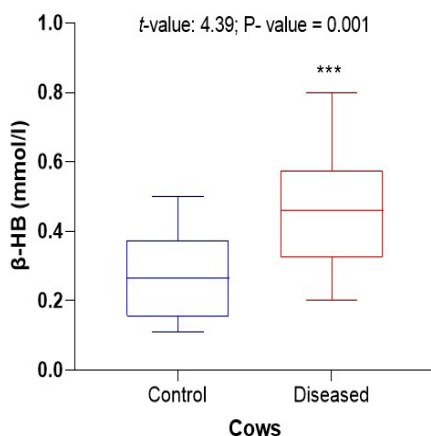


Figure 1. Serum level of β -HB in healthy cows and in cows with chronic digital dermatitis.

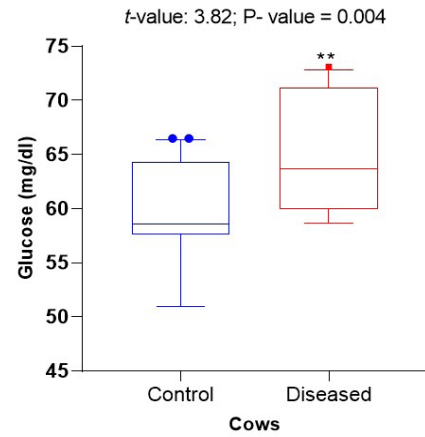


Figure 2. Level of serum glucose in healthy cows and in cows with chronic digital dermatitis.

Figures 3 and 4 illustrate the significant decrease of calcium ($p < 0.01$) and phosphorus ($p < 0.01$) level in cows with DD in comparison with healthy control cows. while serum Mg showed non-significant changes between groups.

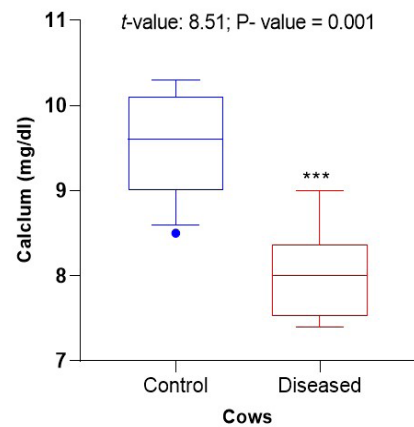


Figure 3. Level of serum calcium in healthy cows and in cows with chronic digital dermatitis.

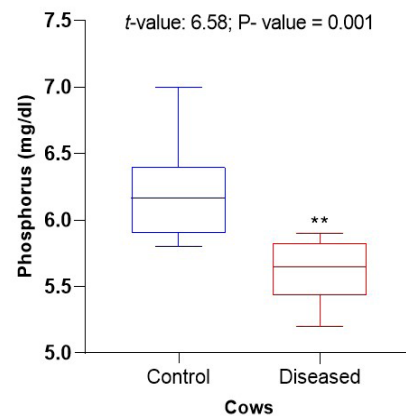


Figure 4. Level of serum phosphorus in healthy cows and in cows with chronic digital dermatitis.

Regarding trace elements, serum mineral values of Zn, and Cu were statistically significantly decreased ($p < 0.01$) in cows with chronic DD in comparison with the control cows (Figures 5-6).

Total leukocytic counts were significantly increased in diseased cases compared with control (Figure 7). Similarly, neutrophils were increased (6.05 ± 2.07 vs $3.71 \pm 0.69 \times 10^9/L$), but lymphocytes were significantly decreased (1.79 ± 0.56 vs $3.38 \pm 1.28 \times 10^9/L$). NLR were significantly increased in cows with chronic DD compared to controls (Figure 8).

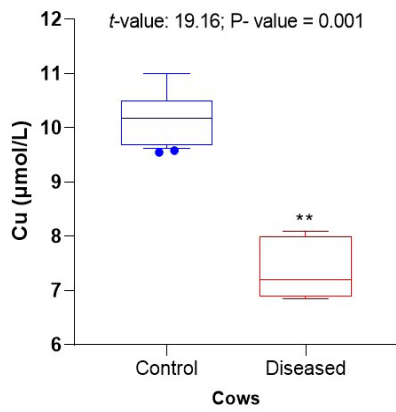


Figure 5. Level of serum copper in healthy cows and in cows with chronic digital dermatitis.

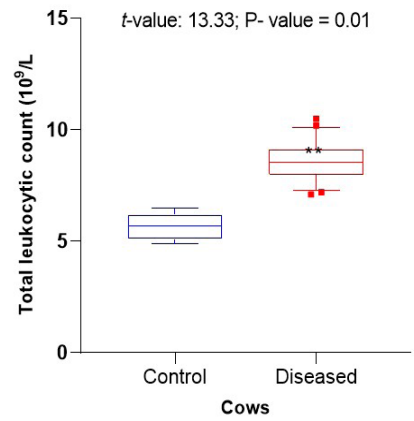


Figure 7. Total leukocytic count in healthy cows and in cows with digital dermatitis.

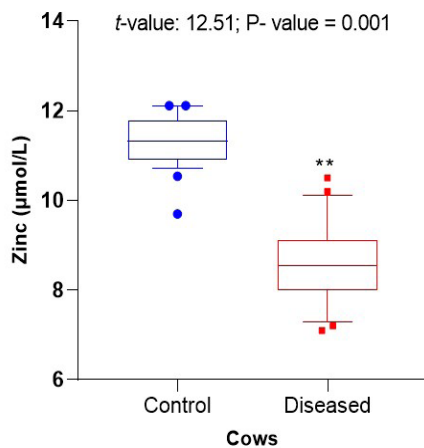


Figure 6. Level of serum Zinc in healthy cows and in cows with chronic digital dermatitis.

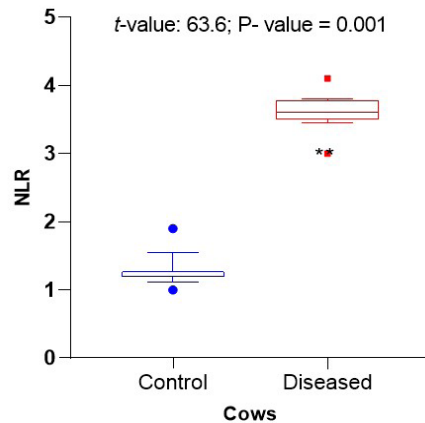


Figure 8. Neutrophil lymphocyte ratio in healthy cows and in cows with chronic digital dermatitis.

Discussion

Digital dermatitis (DD) is a very important cause of lameness in dairy cattle. It is a polybacterial disease with an increased prevalence of *Trepnema*, which is closely related to lameness. Lameness in dairy cows is the cause of appetite loss, a fall in milk production, reproduction performance, and an increase in culling rates, which also causes serious animal welfare issues (Amory *et al.*, 2008).

In present findings, a mild decrease of glucose level was documented in the DD affected cows compared to the healthy control cows. In contrast, an increase of serum glucose levels was recorded (Sun *et al.*, 2015). However, glucose concentrations were found similar in healthy and lame cows (Bhadauria *et al.*, 2020; Ristevski *et al.*, 2017). The explanation of an increase of glucose level in DD affected cows was attributed to that lame cows showed an extreme trend of energy supply, which was accompanied by decreased milk production of dairy cows suffering from different degrees of lameness (Sun *et al.*, 2015). An obvious increase of serum β -HB concentration was detected in the DD affected cows compared to clinically healthy ones. Similarly, previous finding suggest and increase of β -HB (Collard *et al.*, 2000), who stated that an extensive negative energy balance leads to lameness and metabolic problem. This could be attributed to that in lame cows food consumption was not affected by the health condition of the animals, and decreased milk production (Luz *et al.*, 2020). Dairy cows suffering from lameness can meet their energy demand with a normal feed intake due to the low milk production, and therefore, the incidence of negative energy balance was low for lame cows (Sun *et al.*, 2015). However, other report recorded a significant reduction (Sun *et al.*, 2015). Digital dermatitis and ketosis are two prevalent dairy cow diseases that increase the risk of production and health adverse

outcomes. Characterized by multifactorial etiologies, this study hypothesizes a linkage between digital dermatitis and ketosis (Relun *et al.*, 2013; Takci *et al.*, 2023). A shared association between these two diseases is possible due to shared risk factors alongside fiber and protein digestion, and the potential underlying associations between inflammation and metabolism. In fact, findings from recent studies have shown that both an excessive energy supply or a high portion of nonstructural carbohydrates in the diet seem to be predisposing factors in the development of digital dermatitis and ketosis (Krull *et al.*, 2016). In the present result, an increase of serum β -HB and mild decrease of glucose level support the association between ketosis and DD. This finding is supported by that reported previously in European dairy cows (Suthar *et al.*, 2013).

A marked decrease in serum calcium (Ca) in the affected group was documented compared to controls. This finding was similar in part to the previous study (Sun *et al.*, 2015; Paiano *et al.*, 2019; Bhadauria *et al.*, 2020). This could be attributed to its vital role in the keratinization and cornification process, and also it is crucial for the final steps in the production of the mature horn cell (Mülling *et al.*, 1999). Insufficient Ca provided to maturing keratinocytes may cause the formation of dyskeratotic horns (Rude *et al.*, 2003).

Significant reductions in serum zinc concentrations were recorded in our study of the digital dermatitis-affected group compared to controls. Our findings were in agreement with those reported previously (Kilic *et al.*, 2007; Zhao *et al.*, 2015). Zn and Cu are vital elements that maintain healthy hoof tissues (Tomlinson *et al.*, 2004), in addition to the crucial role of Zn in the keratinization process so insufficient supplies of Zn may predispose cows to the production of inferior horn tissue (Mülling *et al.*, 1999). Several researchers found that Zn aids in the regulation of a wide variation of immune system components, as well as those of T lympho-

cytes, natural killer cells, IL-2, IL-6, and TNF α (Pinna *et al.*, 2002; Ibs and Rink, 2003). Serum Cu levels showed a significant decrease in affected dairy cows compared to the control healthy group. This finding was in agreement with Sun *et al.* (2015) and Zhao *et al.* (2015). Many trace elements, such as zinc (Zn), and copper (Cu), are essential cofactors for a large number of enzymes (Zhao *et al.*, 2015; Burger, 2017). Cu deficiency leads to great depression in both cell-mediated and humoral immunity (Prohaska and Failla, 1993).

In the present study, hematological findings revealed that there was a significant increase of total leucocytes (TLC), neutrophils, ($p < 0.01$) in affected cows in comparison to the control healthy group, while lymphocyte count showed a significant decrease ($p < 0.01$). Our findings were in agreement with those given with that of previous findings (Meimandi Parizi and Khalafizadeh, 2006; Dogra *et al.*, 2020), leukocytosis, neutrophilia, and monocytosis are owing to stress and chronic inflammatory response (Pirkkalainen *et al.*, 2022). Neutrophil-lymphocyte ratio (NLR) showed a significant increase ($P < 0.05$) in the digital dermatitis-affected group compared with controls. Peripheral blood cell count ratio is a relatively new inflammatory indicator, including neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and lymphocyte-monocyte ratio (LMR). Such indicators can reflect systemic inflammation very well, in addition to they are easy to obtain and low in cost (Li and Xie, 2021).

Conclusion

In this study, a possible association between the serum markers and DD indicated a systemic change of these biochemical and hematological variables in association of digital dermatitis. Further investigation is necessary to determine the contribution of infection to these changes.

Acknowledgments

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Conflict of interest

The authors have no conflict of interest to declare.

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