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Intestinal Mucosal Damage and Intestinal Permeability In Non Infectious and Infectious Diarrheic Calves In Relation to Diamine Oxidase Activity

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ABSTRACT

This field study was designed as a cohort (prospective) and involved 71 calves within the borders of Aydın Province. Each calf in the study was from one of three different farms (Farm A with 12 non-infectious diarrheic, 15 infectious diarrheic, Farm B with 13 non-infectious diarrheic, 19 infectious diarrheic, and Farm C as the healthy control group n=12). Serum samples were separated into Eppendorf tubes after centrifugation and stored in a -80 °C freezer until analysis. The competitive enzyme immunoassay technique was applied using the Bovine Diamine Oxidase ELISA test. Diamine Oxidase (ng/mL) measurements in infectious, non-infectious, and healthy calves were determined to be 6.52 ± 2.85, 7.16 ± 3.40, and 17.05 ± 2.63 ($p< 0.001$), respectively, in terms of mean ± standard error. The data obtained suggest that determining circulating diamine oxidase enzyme levels under field conditions can support diagnosis as a biological marker of intestinal permeability, and more prominently, diamine oxidase activity may decrease in both non-infectious and infectious diarrheic calves in relation to small intestinal mucosal damage. This could represent a step closer to the diagnosis of leaky gut in calves.

INTRODUCTION

In the intestine, the monolayered epithelial cells that separate the lumen, where microorganisms are densely located, from the largest immune compartment in the body; It acts as a protective barrier by preventing microorganisms, toxins, inflammatory metabolites and antigens from entering the systemic circulation (Mani et al., 2012). When the intestinal barrier has been injured, infiltration of toxic luminal antigens and bacteria can elicit aggressive immune activation leading to persistent systemic inflammation. Such inflammation causes changes in tissue function that ultimately alter the metabolic priorities of the animal to support the increased energy demands of the immune system, which negatively affects growth and productivity (Kvidera et al., 2017; Liehr et al., 2017). Diamine oxidase (DAO) is a predominant antihistamine intracellular enzyme exhibited within the mucosal segments belonging small intestine in an attempt to prevent enterocytes through histamine (Kovacova-Hanuskova et al., 2015). It has been elucidated that DAO participated within the solidarity of intestinal barrier and the grade for mucosal villous injury (Fukudome et al., 2014). The latter enzyme has been positioned at edged closing of fully-gorwn villous cells, mainly with elevated swithced on position, and its occupation denotes the rectitude and full growth for small intestinal mucosae (Honzawa et al., 2011). Even if injured mucosae or impoverised integrity for intestinal wall, DAO ooze via jejunal villous era through the systemic circulation (Zhang et al., 2016). It has been also postulated previously that DAO has been denoted as a biomarker for intestinal permeability (Alizadeh et al., 2022). In addition, injured small intestinal mucosae might deduce DAO activity (Alizadeh et al., 2022). Increased DAO serum levels and decreased DAO activity are associated with increased intestinal permeability and therefore lower

intestinal development (Alizadeh et al., 2022; Song et al., 2017). Regarding the hypothesis of this study, diamin oxidase as a valuable biomarker for intestinal injury, was analyzed in an attempt to detect its exitance in calves with non-infectious diarrhea. In this study, DAO levels were investigated for the detection of intestinal permeability and intestinal mucosal damage related to non-infectious or infectious diarrhea in 71 calves from 3 different farms located within the borders of Aydın Province.

MATERIALS AND METHODS

This field study was planned as a cohort (prospective), and 71 calves within the borders of Aydın Province were included. The ages of the calves were ranged beteeen 26 days to 67 days. Veterinarians with ethics committee certificates were involved in the collection of blood samples. The calves were included in the study after obtaining written consent forms from all 3 farm owners within the Aydın Adnan Menderes University Experimental Animals Local Ethics Committee [HADYEK] document no: 64583101/2024/21.

Each calf included in the study was collected from 3 different farms (Farm a: 12 with non-infectious diarrhea, 15 with infectious diarrhea, farm b: 13 with non-infectious diarrhea, 19 with infectious diarrhea and farm c: n=12 from calves without any diarrhea, referred to as the healthy control group). The veterinarians with the above-mentioned ethics committee certificate obtained a 0.7 ml blood sample from each calf via V. jugularis and quickly shipped it in the cold chain to be taken to the laboratory for processing. At this stage, our doctoral and graduate students provided assistance and logistic support on a voluntary basis. After centrifugation in the laboratory, the serum samples were separated into Eppendorf tubes and stored in a -80 °C freezer no longer than one mont until analysis was performed. Then, the samples were analyzed with the Bovine Diamine Oxidase ELISA test. Diamine Oxidase ELISA kit, competitive enzyme immunoassay technique. In this method, polyclonal anti-DAO antibody and DAO -HRP conjugate were used. The detection range was between 0.312 ng/ml and 20 ng/ml. Serum samples and buffer were added to pre-coated plates and incubated with DAO -HRP conjugate for 60 minutes. Following incubation, the wells were emptied and washed on 5 sessions. Obtained wells forwarded to incubation by use of a substrate for the HRP enzyme. The enzyme-substrate reaction leads to the formation of a blue-colored complex. A stop solution was then administered for stopping, which turns the blue-colored enzyme-substrate complex into yellow. The color shading was analyzed spectrophotometrically at 450 nm in a microplate reader.

Statistical analysis

Nonparametric methodology based on ranking was preferred with Kruskal Wallis one-way ANOVA test. P value was established as =0.001. Even if data was not normally distributed, other relevant methodology was deemed available.

RESULTS

As was given in table 1 dAo (ng/mL) values were deemed detected as 6.52 ± 2.85 vs. 7.16 ± 3.40 and 17.05 ± 2.63, regardind infectious, non-infectious and healthy calves, respectively ($p<0.001$). Achieved ata and relevant results were shown on table 1 and figure 1. There was no anaytical ELISA error during testing. Entire samples were correctly virtualized. All sample interpretation was performed at RDA Group Facilities in Tekstilkent, Istanbul by a specialized ELISA technician. Mean DAO (ng/mL) values comperativel was shown on table 1 and figüre 1 below.

Table 1. Table showing descriptive statistical analysis of dAo DAO values

	Infectious	Non-Infectious	Healthy	p Value
DAO (ng/mL)	6.52 ± 2.85[a]	7.16 ± 3.40[a]	17.05 ± 2.63[b]	< 0.001

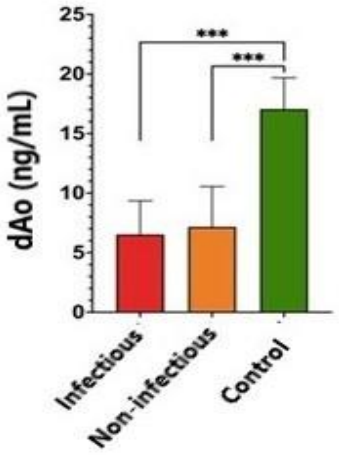

Figure 1. Comparative views of bar charts of dAo levels in infectious, non-infectious and healthy control group calves.

DISCUSSION AND CONCLUSION

As was given in table 1 dAo (ng/mL) values were deemed detected as 6.52 ± 2.85 vs. 7.16 ± 3.40 and 17.05 ± 2.63, regardind infectious, non-infectious and healthy calves, respectively ($p<0.001$). Intestinal permeability has been well recognized as a valuable biomarker for elucidating functioning of the intestinal barrier. Taking into account inflammatory bowel disease, permeability of the intestine has been a determinant component for interpretation of normal/abnormal conditions for gastrointestinal route (Usuda et al., 2021). Various studies in humans and animals have shown that increased intestinal permeability is positively correlated with plasma DAO concentration and negatively correlated with DAO activity. In this study, DAO concentration was measured in calves.

DAO, as an enzyme, catalyzes the oxidation of diamines histamine, putresin and cadaverine (Shakir, 1977). Regarding humanbeing and rodent models, DAO is uniquely located within the apical edges of mature villous cells and shows elevated activity. The latter venture denotes rectitude and full growth in the small

intestine. Many studies in humans and animals have shown that DAO activity is inversely correlated with increased permeability in the small intestine (Ayuso et al., 2007, Palacios et al., 2009). This study has the potential to be a cornerstone in the detection of intestinal mucosal damage in calves.

In a prior study in which calves with diarrhea were classified based on fecal consistency and blood pH, calves with severe or moderate diarrhea were compared to the control groupin which it was determined that plasma dAo DAO activity was significantly lower in the severe group compared to the moderate group. Quanz, (2022) reported that plasma DAO activity clearly decreased in the weeks when clinical signs coinciding with markers of dysbiosis indicated gastrointestinal distress in the cows in the study group. Increased dAo DAO and IL-6 levels are shown in a study examining the effect of early pathogenic *Escherichia coli* (*E. coli*) infection on the intestinal barrier and immune function of newborn calves (He etz al., 2022). In this study, DAO (ng/mL) measurements in healthy control calves compared to calves with both infectious and non-infectious diarrhea were determined as 6.52 ± 2.85, 7.16 ± 3.40 and 17.05 ± 2.63 ($p< 0.001$) in terms of mean ± standard error, respectively (table 1 and figure 1). The data obtained may show that the relevant enzyme may be of diagnostic benefit and that possible intestinal mucosal damage develops parallel to diarrhea. Obtained data is capable of updating veterinary surgeons at field conditions for their treatment protocoles.

Heat stress is also a factor that impairs intestinal function by inducing excessive production of reactive oxygen species (ROS) and proinflammatory cytokines along with increased intestinal permeability (Cheng et al., 2019; Song et al., 2017). It has been demonstrated that serum DAO activity increases in chickens induced with heat stress (Lan et al., 2020). In a mouse model, as a result of induction of leaky gut with *E. coli*, in addition to the observation of villus damage in histological examination; DAO and zonulin levels were found to be significantly higher than in control mice (Ren et al., 2022). Increased DAO and endotoxin levels after administration of methotrexate, which is used for its antitumoral activity but is also likely to have toxic effects on other cells, for chemotherapy in children are associated with increased intestinal permeability (Meng et al., 2016). In a study conducted on 69 humans with inflammatory bowel disease, increased levels of DAO associated with intestinal permeability and intestinal damage and levels of the intestinal bacterial metabolite D-Lactate were determined compared to after treatment (Song et al., 2009). In a pig model, decreased DAO activity following LPS-induced damage to the intestinal mucosa was increased due to increased villus height-crypt depth ratio when pigs were given fish oil (Liu et al., 2012). Decreased DAO activity in the intestine has been reported in Crohn's disease patients, which is associated with the severity of histological changes (Thompson et al., 1988). Takimoto et al., (2014) reported that decreased DAO activity in anorexia nervosa patients suggests the presence of intestinal structural disorder as one of the physical complications of malnutrition. In this study, it was thought that it would be useful to review hygiene and nutritional conditions under the supervision of a specialist veterinarian in all 3 farms.

In a previous study, 22 sick Japanese Black Calves were divided into equal groups and exposed to probiotics or antibiotics. Within 8 days of treatment, serum DAO activity increased significantly only in calves receiving probiotics ([64.4 ± 7.2 on day 1 vs. 76.3 ± 5.1 IU/ml on day 8)], indicating that probiotics were effective on serum DAO activity in calves with diarrhea (Fukuda et al., 2019b). In an important study conducted in our country, multi-strain probiotic treatment was applied rectally to calves with diarrhea; in terms of mean DAO levels (ng/mL), before and after values were determined as 8.48±1.67 vs. versus 28.06±3.51, respectively, with statistically significant changes ($p<0.001$). According to the results of the relevant study, it has been suggested that intestinal mucosal damage may develop in connection with diarrhea, and plasma DAO activity will increase sequentially. However, it has been thought that 10-day rectal enema probiotic treatment reverses this situation and mucosal healing is achieved with proportional feedback regulation (Alic Ural et al., 2023).

In this study, under field conditions, the determination of DAO enzyme activity with statistical significance ($p<0.001$) compared to the control group animals in calves with infectious and non-infectious diarrhea may be an indicator of intestinal mucosal damage. As a result, DAO enzyme maintains its potential to be a useful biomarker.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: D.A.U., Design: 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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Ethical Approval

The study was conducted with the approval of the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee under permit number 64583101/2024/21.

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