Investigation of diamine oxidase as a biomarker of intestinal mucosal injury in calves exposed to antibacterial treatment

ABSTRACT

Diamine oxidase (DAO) (alternatively old-fashioned name histaminases), as being an enzyme in high concentrations, support the integrity and maturation of small intestine. Histamine intoxication and related issues are suggested to exist lacking enzyme diamine oxidase. Enrollment of the gastrointestinal mucosa in several diseases, along with influence of some medications seemed to diminish gastrointestinal DAO activity. The aim of this study was to investigate intestinal mucosal injury in sick calves exposed to antibiotic treatment. In accordance with the inclusion criteria, 20 sick calves with gastroenterologic or respiratory system problems exposed to antibiotic treatment for >3 weeks and 10 other healthy calves were evaluated. Commercially available DAO ELISA kit: Bovine Diamine Oxidase ELISA Kit and Sandwich ELISA-mediated DAO assays were performed. In this study, the mean DAO (ng/mL) level was determined as 5.552 in sick calves exposed to antibiotic treatment, while the mean value was determined as 16.48 in healthy calves in the comparative evaluation ($p<0.001$). The data obtained suggest that DAO activity may be affected in calves exposed to antibiotic treatment for at least 3 weeks.

Keywords: Antibiotics, calves, diamine oxidase, mucosal injury

INTRODUCTION

Diamine oxidase (DAO) is of great importance intracellular enzyme with antihistaminic property exhibited within the small intestinal mucosae for prevention of enterocytes against battling histamine (Kovacova-Hanuskova et al., 2015). DAO is the foremost biomarker for interpretation of gut mechanical barrier and the proportion of mucosal villus injury (Fukudome et al., 2014). The latter enzyme is exhibited at the apical border of mature villous cells with selectively increased activation and its activation denotes the rectitude and full growth of the small intestinal mucosa (Honzawa et al., 2011). In case of injured mucosa or underdeveloped gut wall rectitude, DAO leaks from the jejunal villus tips through central circulatoric vehicle (Zhang et al., 2016). DAO concentration through the circulation might denote intestinal permeability biomarker (Alizadeh et al., 2022). In addition, small intestinal mucosal injury can reduce diamine oxidase activity (Alizadeh et al., 2022). Elevated DAO serum levels and decreased DAO activity are associated with increased intestinal permeability and thus lower intestinal development (Alizadeh et al., 2022; Song et al., 2017). In Turkey, only one doctoral dissertation on intestinal mucosal injury in calves (Türk, 2023) and the author of this article served as the second thesis advisor within the scope of that thesis. Since this thesis work is at

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Research Article

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the publication stage and no other article has been encountered, it is necessary to better understand why this study was carried out in order to better understand the original value of the subject. Many different drugs reduce DAO activity (Finazzi-agro et al., 1979; Leitner et al., 2014; Maintz and Natalija, 2007; Tobajas et al., 2023) yet random drug use continues without this issue being on the agenda and possibly ignored. Regarding the hypothesis that intestinal mucosal injury should be related to drug usage among calves, in this study, we aimed to determine the intestinal mucosal injury mediated by DAO enzyme activity in calves with diarrhea exposed to antibiotic treatment for more than 3 weeks.

MATERIALS AND METHODS

This retrospective field study was conducted in commercial calf facilities located in the Aegean Region of Turkey. HADYEK certificate holders took an active role in the collection of blood samples in the field study. The study was approved by the local ethics committee of Aydın Adnan Menderes University Ethics Committee on 27/10/21 with the reference number 64583101/2021/146. All participating calves were included in the current study with the written consent of the owner.

In accordance with the inclusion criteria, 20 sick calves with gastroenterologic or respiratory system problems exposed to antibiotic treatment for >3 weeks and 10 other healthy calves were evaluated. Anticoagulated tubes were used to collect 0.5 ml of blood from the *V. jugularis*. Plasma was separated after centrifugation. Commercially available DAO ELISA kit: Bovine Diamine Oxidase ELISA Kit (My Biosource, San Diego, United States of America) (Alic Ural et al., 2023) was purchased and made available by RDA Group, Istanbul. The corresponding Sandwich ELISA exhibits high sensitivity and excellent specificity for detecting DAO. As previously described, there is no known cross-reactivity/interaction between DAO and analogs. Plasma samples were analyzed with an available Quantitative Competitive assay via Sandwich ELISA. The sensitivity was 1.0 ng/mL and the detection range was 0.312-20 ng/mL. All samples were stored at the appropriate temperature prior to analyses and all reagents were kept at 2-8°C. The stool scoring system has been described previously (Graham et al., 2018). Sera samples were picked up into relevant tubes and then forwarded to storage in -80°C freezer until analysis were performed. The samples were then analyzed by Bovine Diamine Oxidase ELISA assay. The Diamine Oxidase ELISA kit is a test based on the 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 one hour. Following incubation era, relevant pores were emptied and subjected to washing 5 times. The pores were then incubated with a substrate for the HRP enzyme. The enzyme-substrate reaction leads to the formation of a blue colored complex. A final liquid was added to finalize the reaction, which turns the blue colored enzyme-substrate complex yellow. The color vigour was measured spectrophotometrically at 450 nm in a microplate reader. For statistical interpretation, SPSS 22.00 (IBM, America) program was used and Kruskal Wallis one-way ANOVA test was applied to rank the non-parametric analytes. P value was set as 0.01.

RESULTS

It was learned that at least one antibiotic option was used for digestive system problems or suspected infectious diseases for more than 3 weeks in the anamnesis taken clearly under the control of a veterinarian under field conditions. For enrollment at ths study the calves were

selected to those of solely antibiotic prescribed, otherwise no other drug usage was deemed available. During blood collection, it was ensured that at least 21 days of treatment had been completed under the supervision of a veterinarian with the relevant certificate.

Table 1 shows the relevant data together with statistical values. During the ELISA analysis, there were no false readings that could be recorded, and all samples could be analyzed. Mean DAO (ng/mL) values showed statistical significance ($p<0.01$) in antibiotic treated calves compared to healthy control group.

Table 1. Circulating serum diamine oxidase levels among diseased and healthy calves.

DAO (ng/mL)	Sick calves exposed to antibiotic treatment	Healthy calves	P value
Average	5.552	16.48	0.001
Standard Error	0.577	0.683	
95% CI	(4.34 - 6.77)	(14.93 - 18.02)	

DISCUSSION

In the intestine, single-layered epithelial cells separating the microorganism-dense lumen and separated through the vast majority immune complex function as a protective barrier, preventing microorganisms, toxins, inflammatory metabolites and antigens from entering the systemic circulation (Mani et al., 2012). Even if the intestinal integrity is unsettled, permeation of toxic intraluminal foreign compounds and microorganisms could elucidate fired immune respond prone to low grade systemic inflammation. Such inflammation accompany alterations within the functioning of the tissue changing the animal's metabolic load to support the elevated energy requirement of the immune respond, in turns unwantedly influence productivity and growth (Kvidera et al., 2017; Liehr et al., 2017). In the present study the author investigated DAO, as a biomarker of intestinal mucosal injury to those of calves subjected to prior antibiotic treatment by field veterinarians.

Intestinal penetrability is frequently preferred as a biomarker for assessing intestinal barrier function. Given inflammation of intestines, penetrability is a vital condition for interpretation of the health or disease status for the gastrointestinal tract (Usuda et al., 2021) Several studies in humans and animals reveal that increased intestinal permeability is positively correlated with plasma DAO concentration and negatively correlated with DAO activity (Lackner et al., 2019). In this study, DAO analysis was performed to investigate intestinal mucosal injury in calves exposed to antibacterial treatment.

In a study in which calves with diarrhea were classified through fecal consistency and blood pH (Fukuda et al., 2019a), circulatory DAO concentration was markedly diminished in calves with severe/moderate diarrhea compared to the control group and in the severe group compared to the moderate group. Quanz (2022) reported that plasma DAO activity was clearly decreased in weeks when clinical signs coinciding with markers of dysbiosis indicated gastrointestinal distress in cows in the study group. In a study examining the influence of untimely pathogenic *Escherichia coli* (*E. coli*) invasion at gut barrier and immune functioning of newborn calves (He et al., 2022); increased DAO and IL-6 levels are shown. Although cytokine analysis was not possible in this study, it is planned to conduct these studies in the next study by establishing a consensus and multidisciplinary team with broad participation.

Heat stress is also a factor that impairs intestinal function by inducing overproduction of reactive oxygen species (ROS) and proinflammatory cytokines along with increased intestinal permeability (Cheng et al., 2019; Song et al., 2017). It was revealed that serum DAO

activity increased in heat-stressed broilers (Lan et al., 2020). As a result of the induction of permeable intestine by *E. coli in* a mouse model, in addition to the observation of villus injury in histologic examination; DAO and zonulin levels were found to be significantly higher compared to control group mice (Ren et al., 2022). Increased DAO and endotoxin levels are associated with an increase in intestinal permeability after the administration of methotrexate, which is used for antitumoral activity but is likely to be toxic to other cells, for chemotherapy in children (Meng et al., 2016). In a study in 69 humans with inflammatory bowel disease, increased levels of DAO and D-Lactate, an intestinal bacterial metabolite associated with intestinal permeability and intestinal injury, were found after treatment (Song et al., 2009). In a porcine model, DAO activity, which decreased after induction of intestinal mucosal injury by LPS, increased after administration of fish oil to pigs due to an increase in the villus height-to-crypt depth ratio (Liu et al., 2012). In Crohn's disease patients, decreased DAO activity in the intestine has been reported to correlate with the severity of histologic changes (Thompson et al., 1988). Takimoto and colleagues (2014) found decreased DAO activity in patients with anorexia nervosa, suggesting the existence of intestinal morphological disorder related to malnutrition. Since etiologically based assessment was not possible in this study, the presence/absence of infectious agents was not evaluated. Nevertheless, since the analyzes were collected in a temperate climate, it does not seem possible to mention heat stress.

In a previous study, DAO activity was measured in diarrheic calves to detect intestinal mucosal disorders. Based on stool composition (between 0-3) and blood pH (acidemia: blood pH<7.25) in 36/50 calves with diarrhea, DAO activity was lower ($p < 0.05$) in severely or moderately diarrheic calves compared to the control group; plasma DAO activity was significantly and negatively correlated with stool scores (Fukudo et al 2019a). Fukuda et al. (2019b) investigated whether probiotic administration could be an alternative to antibiotics in diarrheic calves and measured DAO activity related to diarrhea as a second objective in their study. Twenty-two evenly divided Japanese black calves with diarrhea were treated with probiotics (n=11) or antibiotics (n=11) limited to 8 days; serum DAO activity was found to be significantly elevated only in probiotic-treated calves. In the light of the data obtained, it was suggested that probiotics can affect serum DAO activity in diarrheic calves (Fukuda et al., 2019b). Very recently, another study comparable to the studies in the above paragraph aimed to establish even if plasma concentrations of DAO in calves indicate mucosal injury during diarrhea, against which rectal enema probiotic treatment was used. Following acceptance to commercial facility, calves were scored on a scale from 0 to 3 according to fecal consistency. Calves exhibiting a stool score of 2 (loose stools) or 3 (watery stools) were considered to have diarrhea and only calves with diarrhea were recorded. All calves with diarrhea were treated with rectal enema with multi-strain probiotics. The mean DAO levels (ng/mL) of diarrhea calves prior to and thereafter probiotic enema therapy were 8.48±1.67 and 28.06±3.51, respectively, which showed statistically significant changes ($p<0.001$). In summary, it was stated that plasma DAO activity was lowered in respond to intestinal mucosal injury associated with diarrhea and this was reversed by rectal enema probiotic treatment for 10 days and it was possible to draw a preliminary conclusion that DAO activity reflects a feedback regulation associated with mucosal healing as suggested (Alıç Ural et al., 2023).

It has been well postulated that even if physiological activity entire organs have been modified by the microbiota (Goyal et al., 2015; Marsland and Salami, 2015), gut mucosal lining and its immune accompanying components, are influenced by this symbiosis (Caballero et al., 2015). Moreover, microbiome manage intestinal developmental stages by modifying vascularization, thickening of villi, widening of mucosal location, existence of mucus, proliferation of cells and epithelial junctioning (Kelly et al., 2015; Sommer and Backhed, 2011; Reinhaedt et al., 2012). On the other hand, antibiotics, to the present author's knowledge are increasingly used at field conditions, rattle the balance between commensal microecology and could accompany to a diminished or changed interaction between the microbiota and the underlying mucosa (Becattini et al., 2016). In the present study DAO activity was analyzed for directly measuring intestinal mucosal injury and thus related mechanisms aforementioned above. In the present study although etiological interpretation was not deemed available, available data should help veterinary surgeons for planning treatment protocols.

CONCLUSION

In the present study, the mean DAO levels (ng/mL) in calves exposed to antibiotic treatment for at least 3 weeks were 5.552 vs. 16.48, suggesting that antibacterial treatment may contribute to decreased DAO activity and thus intestinal mucosal injury. Further prospective studies with larger populations and comparative analyses with other biomarkers are warranted.

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Author contributions: Motivation / Concept: DAU; Design: DAU; Control/Supervision: DAU; Data Collection and / or Processing: DAU; Analysis and / or Interpretation: DAU.

Availability of data and materials: Data and materials may be used subject to the author's permission.

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