RESEARCH ARTICLE

PROBIOTIC ENEMA PROTECTS INTESTINAL MUCOSA AND ALTERS PLASMA DIAMINE OXIDASE ACTIVITY AMONG CALVES WITH DIARRHEA

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ABSTRACT

Diamine oxidase (dAo) (alternatively histaminases,in under the old denomination), as a natural enzyme present in high levels, promotes the integrity and maturation of the small intestinal mucosa. In the present study, we sought to investigate whether the plasma levels of diamine oxidase activity denote mucosal injury during diarrhea among calves, and we attempted to use rectal enema probiotic therapy against it. Upon arrival at a private and commercial milk-fed calf facility, calves were subjected to scoring on fecal consistency on a scale from 0 to 3. Calves exhibiting a fecal score of 2 (loose feces)-or 3 (watery feces) were enrolled as diarrheic. As solely calves with diarrhea were enrolled. All diarrheic calves received rectal enema multi strain probiotic treatment (Farm Rumin Probiotic Powder). The mean dAo levels (ng/mL) among diarrheic calves before and after probiotic enema treatment were detected as 8.48±1.67 and 28.06±3.51, respectively, presenting statistically significant alterations ($p<0.001$). In summary, it should not be unwise to draw a preliminary conclusion that plasma dAo activity was decreased in response to intestinal mucosal injury in relationship with diarrhea, which was reversed with rectal enema probiotic treatment for 10 days, reflecting a feed-back regulation of dAo activity connected with mucosal recovery, as was proposed.

Keywords: Bovine health, growth performance, intestinal barrier function, intestinal biomarker

INTRODUCTION

With the development of novel gastrointestinal and antimicrobial drugs also involving molecular targeted drugs the usage of several uncontrolled and random drugs has been increasing. However, gastrointestinal mucosal damage might be conducted through administration of several drugs, being capable of consequently causing gastrointestinal toxicity among calves. There have been no data on the relationship with polypharmacy among calves experiencing diarrhea and/or other relevant gastrointestinal clinical signs. However, several drugs could influence diamine oxidase (dAo) activity, with an old-fashioned article reporting that the antimalarial drugs (amodiaquine, quinacrine and chloroquine) could be capable of inhibiting the catabolism of putrescine, which was attributed to action of the latter drugs on dAo (Ma and Sourkes, 1980). Another study denoted that chloroquine, clavulanic acid, cimetidine, verapamil isoniazid, metamizole, acetyl cysteine, amitriptyline, diclofenac, metoclopramide, suxamethonium and thiamine have varying degrees of inhibition potential efficacy of interaction on dAo (Leitner et al., 2014). There exist no relevant data on the efficacy of commonly used drugs among calves, even if interacting with dAo (however, our subsequent study would thus be aimed at this issue, probably). Neonatal diarrhea, a significant economic concern in the cattle industry, leads to mucosal inflammation and villous atrophy in calves, resulting in dehydration and electrolyte imbalances (Mosier and Oberst, 2000; Smith, 2009). While many aforementioned treatments rely on indirect markers, the state of the intestinal mucosa is still often overlooked. Diamine oxidase is a well recognized cytoplasmic enzyme present dominantly within the small intestine villi, playing a pivotal role in the degradation of histamine (Kitanaka et al., 2002). As well recognized data indicate, dAo acts within the mucosa, mostly in the top villi, while its activity occurrs increasedly in the small intestine (Biegański, 1983). There has been a pragmatic relationship between plasma dAo activity and the matureness/unity of the intestinal mucosa (Wolvekamp and de Bruin, 1994). Taking into account the latter data, as mucosal damage intensified, mucosal/plasma dAo activity exhibited decline. It should not be unwise to mention that regarding the prior investigations, plasma dAo activity plays a pivotal role in predicting intestinal mucosal injury (Tanaka et al., 2003; Miyoshi et al., 2015) and in rats (Akimoto et al., 2006). Fukuta et al. (2019a) indicate that plasma dAo activity might serve as a reliable biomarker for assessing intestinal mucosal disorders stemming from diarrhea.

In the present study the objectives were (1) to examine the action of rectal enema probiotic containing multistrain probiotic bacterial species, (2) to approve it for the treatment of unclassified diarrhea, at least clinically (as a substitute to frequently used empiric antibiotic therapy), and (3) to examine whether the serum dAo activity in calves is related to diarrhea responding to probiotic enema treatment.

MATERIAL AND METHODS

Approval of the ethics committee

This retrospective field study was performed at a commercial milk-fed calf facility in the Eagean Region of Türkiye and was approved by the local ethic committee of Aydın Adnan Menderes University Ethical Commitee on 27/10/21 under ref. no. 64583101/2021/146. All participated calves were enrolled in the present research with a written owner's consent.

Study design

In a total of 8 calves participating, 0.5 ml blood was drawn from *V. jugularis* into anticoagulated tubes. Following centrifuge, plasma was seperated. Commercially available dAo ELISA kit: Bovine Diamine Oxidase ELISA Kit (My Biosource, San Diego, United States) was purchased by RDA Group, Istanbul and became available. The latter assay exhibits elevated sensitivity and excellent specificity for detecting dAo. There has been no known cross-reactivity/interference between dAo and analogues, as was previously described. Plasma samples were analyzed with an available

assay Quantitative Competitive via Sandwich ELISA. Sensitivity was1.0 ng/mL with a detection range between 0.312-20 ng/mL. All samples were stored at suitable degree prior to analytes, and all reagents were kept at 2–8 degree C. Fecal scoring system was previously described (Graham et al., 2018) (Table 1). Diarrhea was unclassified due to the limited financial budget.

Table 1 Fecal scoring system adopted (Graham et al. 2018)

Fecal Score	
0	normal consistency
1	semiformed or pasty
2	loose feces
3	watery feces

Rectal enema probiotic usage

Rectal probiotic enema was shown in Figure 1. For this purpose, probiotic support (Prebio, Farm Rumin, Turkey) was applied by the way of the rectum roughly with a catheter once a day during 10 days . This product contains *Lactobacillus plantarum* (1k1604) 5 x 109 CFU, *Sacchoromyces cerevisiae* (CNCM-1-1077) (4b1711) 1 x 109 CFU, fermentated product of *L. acidophillus*, *L. helveticus*, *L. fermentum* 15 x 109 CFU, a protein-rich product derived from *Bacillus subtillis* 5 x 109 CFU. In addition to above content, the supplement also includes 470 mg/packet inulin, 20 mg/packet colloidal silica (E 551b) and 60 mg/packet calcium propionate (1a282).

Statistical analysis

Descriptive statistics was utilized to analyze the obtained data, and the resulting values were presented as the mean and standard error. The Mann-Whitney analysis was performed to compare the diamine oxidase levels between the different groups. Graphpad Prism software (v. 9.2, America) was used for conducting the statistical analyses and generating the graphs. Statistical significance was defined as situations where the p-value was less than 0.05.

RESULTS

All tabulated data along with statistical values were given in Figure 2 and Table 2 with box plot analytes. During ELISA analysis there was no error, and all samples were investigated. A well-educated laboratory staff performed ELISA and followed the instructions from the commercial kits. Mean dAo (ng/mL) values prior to (BT) and after treatment (AT) showed statistical significance ($p<0.01$).

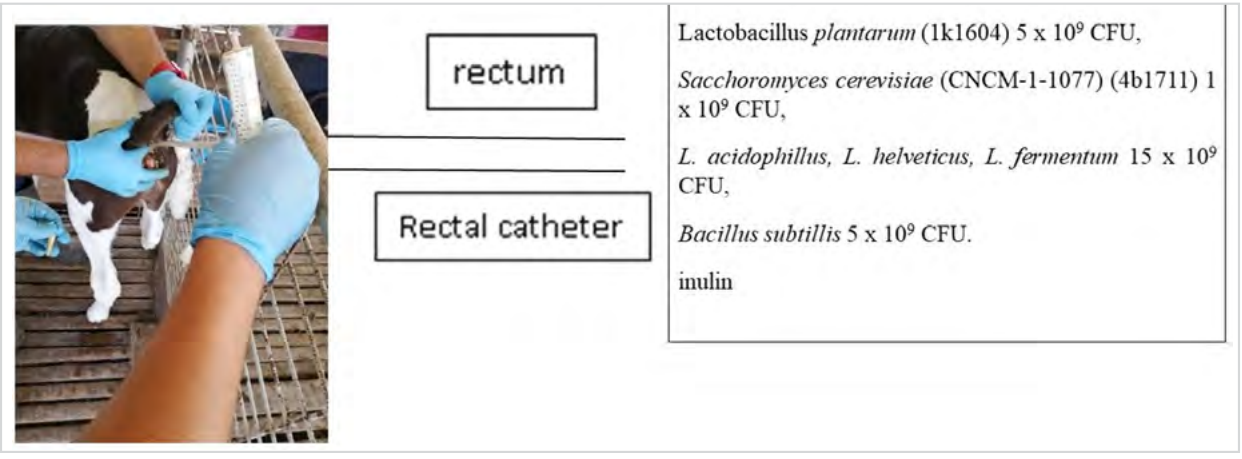

Figure 1 Photographic record and schematic representation of rectal enema probiotic (Farm Rumin) administered 15 cm within the rectum for each calf enrolled

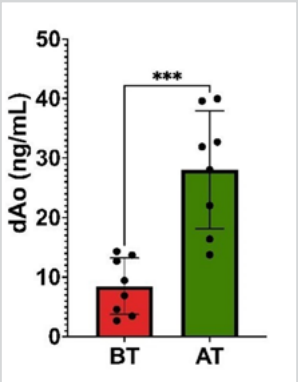

Figure 2 Box plot analytes for mean dAo values before (BT) and after treatment (AT)

Table 2 Statistical analytes for mean dAo values

	BT (x̄)	AT	p value
dAo (ng/mL)	± SE	± SE	0.001
	8.48 ± 1.67[a]	28.06 ± 3.51[b]	

[a,b]: Values indicated with different letters on the same line are statistically significant. BT: before treatment, AT: after treatment.

DISCUSSION AND CONCLUSION

Diamine oxidase is expressed specifically within the intestine, kidney and placenta, where it is kept in vesicular structures for secretion activity (Luk et al., 1980; Schwelberger et al., 1998; McGrath et al., 2009). dAo is conveyed and stored in epithelial cells villi along the intestine, constituting a primary defense barrier against dietary histamine (Schwelberger et al., 1998). On the other hand, pending whether the existing intestinal dAo activity sufficiently degrades the selected amount of histamine, it can translocate into the circulation resulting with histamine-related clinical signs (Schwelberger et al., 1998; McGrath et al., 2009). The latter data should be discussed in depth. Histamine that is released within the gastrointestinal system might be quickly subjected to detoxification via amine oxidases in health, and it could also be produced by epithelial cells (Wagner et al., 2003). Hence, acid-induced alterations of epithelial barrier function induce translocation of histamine from the gastrointestinal route to the bloodstream (Aschenbach and Gabel, 2000).

In a prior research aimed to determine plasma dAo activity alterations, an attempt was made to mirror the severity of intestinal mucosal disorder in 36 out of 50 Holstein calves with diarrhea (14 calves without diarrhea were assigned to the control group). The plasma dAo activity was markedly diminished ($p < 0.01$) in the calves with severe or moderate diarrhea in contrast to control group and was significantly decreased ($p < 0.05$) in the severe group in comparison to the moderate group. Obtained results denoted that plasma dAo reflects the degree of intestinal mucosal disorder in association with diarrhea (Fukuda et al., 2019a). Another interesting article seeked the association among serum dAo activity, postnatal days and the plasma copper (Cu) values among diarrheic and healthy calves. In healthy calves, the serum dAo activity was markedly higher at 2 Postnatal Day in contrast to ≥ 7 Postnatal Day, without any significant alterations detected after 7 Postnatal Day. The serum dAo activity in 14 diarrheic calves (66.78 ± 14.37 IU/ml) was diminished significantly in contrast to 19 healthy calves (170.33 ± 97.83 IU/m, $p<0.01$) (Fukuda et al., 2020). In the present study, the mean dAo levels (ng/mL) among diarrheic calves before and after probiotic enema treatment were detected as 8.48 ± 1.67 and 28.06 ± 3.51, respectively, presenting statistically significant alterations ($p<0.001$). Altered dAo levels following rectal enema probiotic treatment could reflect mucosal healing and mucosal integrity restoration among calves enrolled.

Twenty-two diarrheic Japanese black calves, equally divided, received probiotics (n=11) or antibiotics (n=11) treatment limited to 8 days in which serum dAo activity markedly elevated only in probiotic treatment (64.4 ± 7.2 on Day 1 vs. 76.3 ± 5.1 IU/ml on Day 8). According to the latter study, probiotics could be capable of influencing serum dAo activity in diarrheic calves (Fukuda et al., 2019b). This finding was also supported by our study, as decreased dAo levels were restored and returned to elevated levels in contrast to the prior era of probiotic enema treatment.

Our valuable field data suggested that multistrain probiotic enema treatment involving *L. plantarum, S. cerevisiae, L. acidophillus, L. helveticus, L. fermentum* and *B. subtillis* could have easily substituted empiric antibiotic therapy and polypharmacy, specifically in non-bacterial infectious diarrheic calves. Some might speculate as to why, as an etiological algorithm was not available, we preferred probiotic usage. This may be briefly explained by our preliminary field investigation and fecal smear cytology (which was not necessary to be reported) providing us with preliminary evidence of proof of bacterial origin of enteritis. Furthermore, we also noted that serum dAo activity decreased in calves with diarrhea, in which rectal enema probiotic usage could have reversed serum dAo activity and cause its increase, Further warranted research is required in larger calf populations in an attempt to extrapolate the association between intestinal mucosal damage and serum dAo activity in diarrheic calves.

Following injury to the intestinal mucosa capable of affecting gut microbiota in relationship with the existence of great amounts of D-lactate, it could quickly send it off to circulation because of elevated intestinal permeability (Ficek et al., 2017). Furthermore, intestinal mucosal damage and elevated intestinal permeability influence dAo activity (Meng et al., 2016). In a prior study, D-lactate and dAo were among the selected biomarkers of intestinal permeability (Wu et al., 2019). In that study, the levels of D-lactate and dAo, both in the observation and control groups, were significantly diminished after treatment in contrast to prior values ($p<0.001$). According to that study, probiotic combination therapy reversed the stress response and intestinal permeability of term neonates (Wu et al., 2019). In the present study, restored dAo values could easily be attributed to altered intestinal permeability, toward which our subsequent study would be directed.

In conclusion, serum dAo activity sensitively indicated gastrointestinal mucosal damage prior to diarrhea onset (probably) and could be a useful biomarker of intestinal mucosal damage. Rectal enema probiotic usage could be capable of reversing this damage. Furthermore, decreased fecal scoring obtained could also reflect the efficacy of probiotic enema treatment and could be in relationship with altered dAo levels.

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The first (DAU) and the last authors were the second and first advisor, respectively, of the PhD student (ET).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – KU, DAU.; Design – KU, DAU.; Supervision – KU, DAU.; Resources –HE, SE, NK, ET.; Materials – HE, SE, NK, ET.; Data Collection and/or Processing – HE, SE, NK, ET.; Analysis and/or Interpretation – KU, DAU, HE, NK, ET.; Literature Search – KU, SE.; Writing Manuscript – KU, DAU, HE, SE, NK.; Critical Review – KU, DAU.

REFERENCES

Akimoto T, Takada M, Ichihara T, Kuroda Y. 2006. Molecular analysis for differential diagnosis of small bowel obstruction: expression of proinflammatory cytokines and diamine oxidase activity. Int J Biomed Sci, 2(2), 160.

Allard FD, Stelow EB. 2019. Review of drug-induced injury in mucosal biopsies from the tubular gastrointestinal tract. Adv Anat Pathol, 26(3), 151-70. https://doi.org/10.1097/PAP.0000000000000230

Aschenbach JR, Gabel G, 2000. Effect and absorption of histamine in sheep rumen: significance of acidotic epithelial damage. J Anim Sci, 78, 464-70. https://doi.org/10.2527/2000.782464x

Biegański T. 1983. Biochemical, physiological and pathophysiological aspects of intestinal diamine oxidase. Acta Physiol Pol, 34, 139-54.

Ficek J, Wyskida K, Ficek R, Wajda J, Klein D, Witkowicz J, et al. 2017. Relationship between plasma levels of zonulin, bacterial lipopolysaccharides, D-lactate and markers of inflammation in haemodialysis patients. Int Urol Nephrol, 49, 717-25. https://doi.org/10.1007/s11255-016-1495-5

Fukuda T, Otsuka M, Nishi K, Nishi Y, Tsukano K, Noda J, et al. 2019a. Evaluation of probiotic therapy for calf diarrhea with serum diamine oxidase activity as an indicator. Japan J Vet Res, 67, 305-11. https://doi.org/10.14943/jjvr.67.4.305

Fukuda T, Tsukano K, Nakatsuji H, Suzuki K. 2019b. Plasma diamine oxidase activity decline with diarrhea severity in calves indicating systemic dysfunction related to intestinal mucosal damage. Res Vet Sci, 126, 127-30. https://doi.org/10.1016/j.rvsc.2019.08.027

Fukuda T, Tsukano K, Otsuka M, Murakami Y, Kitade Y, Nakatsuji H, et al. 2020. Relationship between postnatal days, serum Cu concentration and plasma diamine oxidase activity in Japanese Black calves. J Vet Med Sci, 82, 1488-91. https://doi.org/10.1292/jvms.20-0278

Graham AN, Renaud DL, Duffield TF, Kelton DF. 2018. Calf cleanliness does not predict diarrhea upon arrival at a veal calf facility. J Dairy Sci, 101, 3363-6. https://doi.org/10.3168/jds.2017-14113

Ishikawa H, Watanabe S. 2011. Cattle bile aggravates diclofenac sodium-induced small intestinal injury in mice. Evid Based Complement Alternat Med, 315858. https://doi.org/10.1155/2011/315858

Kitanaka J, Kitanaka N, Tsujimura T, Terada N, Takemura M. 2002. Expression of diamine oxidase (histaminase) in guinea-pig tissues. Eur J Pharmacol, 437, 179-85. https://doi.org/10.1016/S0014-2999(02)01302-X

Leitner R, Zoernpfenning E, Missbichler A. 2014. Evaluation of the inhibitory effect of various drugs / active ingredients on the activity of human diamine oxidase in vitro. Clin Transl Allergy, 18, 23. https://doi.org/10.1186/2045-7022-4-S3-P23

Luk GD, Bayless TM, Baylin SB. 1980. Diamine oxidase (histaminase). A circulating marker for rat intestinal mucosal maturation and integrity. J Clin Invest, 66, 66-70.

Ma K, Sourkes TL. 1980. Inhibition of diamine oxidase by antimalarial drugs. Agents Actions, 10, 395-8. https://doi.org/10.1007/BF01968035

McGrath AP, Hilmer KM, Collyer CA, Shepard EM, Elmore BO, Brown DE, et al. 2009. Structure and inhibition of human diamine oxidase. Biochemistry, 48, 9810-22. https://doi.org/10.1021/bi9014192

Meng Y, Zhang Y, Liu M, Huang YK, Zhang J, Yao Q, et al. 2016. Evaluating intestinal permeability by measuring plasma endotoxin and diamine oxidase in children with acute lymphoblastic leukemia treated with high-dose methotrexate. Anticancer Agents Med Chem, 16, 387-92.

Miyoshi J, Miyamoto H, Goji T, Taniguchi T, Tomonari T, Sogabe M, et al. 2015. Serum diamine oxidase activity as a predictor of gastrointestinal toxicity and malnutrition due to anticancer drugs. J Gastroenterol Hepatol, 30(11), 1582-90. https://doi.org/10.1111/jgh.13004

Mosier DA, Oberst RD. 2000. Cryptosporidiosis: a global challenge. Ann N Y Acad Sci ANN, 916, 102-11. https://doi.org/10.1111/j.1749-6632.2000.tb05279.x

Panarelli NC. 2014. Drug-induced injury in the gastrointestinal tract. In Seminars in diagnostic pathology (31(2), pp.165-75). WB Saunders. https://doi.org/10.1053/j.semdp.2014.02.007

Schwelberger HG, Hittmair A, Kohlwein SD. 1998. Analysis of tissue and subcellular localization of mammalian diamine oxidase by confocal laser scanning fluorescence microscopy. Inflamm Res, 47, 60-1. https://doi.org/10.1007/s000110050273

Smith GW. 2009. Treatment of calf diarrhea: oral fluid therapy. Vet Clin North Am Food Anim, 25, 55-72. https://doi.org/10.1016/j.cvfa.2008.10.006

Tanaka Y, Mizote H, Asakawa T, Kobayashi H, Otani M, Tanikawa K, et al. 2003. Clinical significance of plasma diamine oxidase activity in pediatric patients: influence of nutritional therapy and chemotherapy. Kurume Med J, 50(3-4), 131-7. https://doi.org/10.2739/kurumemedj.50.131

Wagner W, Ichikawa A, Tanaka S, Panula P. 2003. Mouse mammary epithelial histamine system. J Physiol Pharmacol, 54, 211-23.

Wolvekamp MCJ, De Bruin RWF. 1994. Diamine oxidase: an overview of historical, biochemical and functional aspects. Dig Dis, 12, 2-14. https://doi.org/10.1159/000171432

Wu J, Zhang J, Chen J, Han Y. 2019. Probiotics decrease the stress response and intestinal permeability of term neonates with low Apgar scores. Exp Ther Med, 18, 4322-8. https://doi.org/10.3892/etm.2019.8107

PROBIOTIČKA ENEMA ŠTITI INTESTINALNU SLUZNICU I SMANJUJE AKTIVNOST PLAZMATSKE DIAMINOOKSIDAZE KOD TELADI SA DIJAREJOM

SAŽETAK

Diaminooksidaza (DAO) (stari naziv histaminaza) je prirodni enzim prisutan u visokim koncentracijama koji djeluje na cjelovitost i sazrijevanje sluznice tankog crijeva. Cilj našeg istraživanja je ispitati da li aktivnost diaminooksidaze odražava stepen oštećenja crijevne sluznice kod teladi sa dijarejom i mogućnost upotrebe rektalne probiotičke eneme u liječenju iste. Nakon dolaska u privatni komercijalni objekt za uzgoj teladi na prehrani mlijekom, telad su ocijenjena prema konzistenciji fecesa na skali od 0 do 3. Telad ocijenjena sa rezultatom 2 (rijedak feces) ili 3 (tečan feces) su uključena u istraživanje kao telad sa dijarejom. Sva telad sa dijarejom su primila rektalnu enemu koja je sadržavala višesojnu probiotsku terapiju (Farm Rumin Probiotic Powder). Srednje vrijednosti DAO (ng/mL) kod teladi sa dijarejom prije i nakon terapije enemom su iznosile 8.48±1.67 i 28.06±3.51 i označene su statistički signifikantnim ($p<0.001$). U sažetku, nije nerazumno preliminarno zaključiti da je aktivnost plazmatske DAO snižena kao odgovor na oštećenje intestinalne sluznice kod dijareje koja je izliječena s enemom probiotika apliciranom rektalno u trajanju od 10 dana. Ovim se oslikava povratna regulacija DAO aktivnosti povezana s oporavkom sluznice, kao što je i pretpostavljeno.

Ključne riječi: Funkcija intestinalne barijere, intestinalni biomarker, performansa rasta, zdravlje goveda.