

Glutamin Enema Might Have Helped Reversing Intestinal Mucosal Injury Among Diarrheic Calves: Trophic Aminoacid For 'Microbiota-Gut-Brain Axis'

Deniz ALIÇ URAL

Plant and Animal Production Unit, Sultanhisar Vocational School, Aydın Adnan Menderes Univesity, TURKEY

ORCID: 0000-0002-2659-3495

ABSTRACT

Objective: Glutamine (Gln) has been shown to be an effective nutritional supplement for improving gastrointestinal barrier function in animals. In this study, the efficacy of Gln on intestinal morphology was evaluated by detecting diamine oxidase (DAO) levels, a valuable marker for detecting gastrointestinal toxicity and intestinal mucosal damage in calves with diarrhea. Method: A total of 20 calves with diarrhea of unknown etiology were included in this study. Following a physical examination by board-certified veterinarians who did not contribute other than any technical intervention, fecal rapid diagnostic test kits were applied when no responsible etiological agent could be identified. A total of 0.9 ml of blood was collected from each calf, and a sandwich ELISA was performed using commercially available bovine DAO test kits. Gln (Gut-cumin I Liquid Solution, Larek Tarım, Ankara, Türkiye) was administered rectally at a dose of 15 mg/kg for 7 days. Findings: The mean $\pm$ SE DAO (ng/mL) analysis on day 0 (start of the trial) and at the primary endpoint (day 7) were determined to be 9.12 ± 2.46 versus 28.19 ± 3.18 ($p < 0.01$), respectively. Conclusion: As determined by DAO levels, it would be reasonable to suggest that Gln, a trophic amino acid, may be helpful in reversing intestinal mucosal damage in calves with diarrhea. Gln may be a reparative molecule and feed additive in intestinal damage.

Key words: Damage, Diarrhea, Diamine oxidase, Glutamine, Intestine.

Glutamin Eneması İshalli Buzağlarda Bağırsak Mukozal Hasarının Tersine Çevrilmesine Yardımcı Olmuş Olabilir: 'Mikrobiyota-Bağırsak-Beyin Eksen'i' İçin Trofik Aminoasit

Öz

Amaç: Glutamin (Gln), hayvanların gastrointestinal bariyer fonksiyonunu iyileştirmek için etkili bir besin takviyesi olarak gösterilmiştir. Bu çalışmada, ishallerli buzağlarda gastrointestinal toksisite ve intestinal mukozal hasarı tespit etmek için değerli bir belirteç olan Diamin oksidaz (DAO) seviyelerinin tespiti sayesinde, intestinal morfoloji üzerinde Gln'nin etkinliği değerlendirilmiştir. Yöntem: Etiyolojisi bilinmeyen toplam 20 ishallerli buzağı bu çalışmaya dahil edildi. Teknik müdahale dışında herhangi bir katkıda bulunmayan kurul sertifikalı veteriner hekimler tarafından yapılan fiziksel muayenenin ardından, herhangi bir sorumlu etiyolojik ajan tespit edilemeyen, fekal hızlı tanı test kitleri uygulandı. Her buzağıdan toplam 0,9 ml kan alınarak ticari olarak temin edilebilen sığır DAO test kitleri aracılığıyla sandviç ELISA gerçekleştirildi. Gln (Gut-cumin I Sıvı Solüsyonu, Larek Tarım, Ankara, Türkiye) rektal yolla 15 mg/kg dozda 7 gün uygulandı. Bulgular: Ortalama $\pm$ SE DAO (ng/mL) analizi 0. günde (denemenin başlangıcı) ve birincil son noktada (7. gün), sırasıyla $9,12 \pm 2,46$ 'ya karşı $28,19 \pm 3,18$ ($p < 0,01$) olarak tespit edildi. Sonuç: DAO seviyeleriyle tespit edildiği üzere, ishallerli buzağlarda bağırsak mukozal hasarının tersine çevrilmesinde, trofik bir aminoasit olan Gln'nin yardımcı olacağını öne sürmek yerinde olacaktır. Gln bağırsak hasarında tamir edici bir molekül ve yem katkı maddesi olabilir.

Anahtar kelimeler: Bağırsak, Diamin oksidaz, Glutamin, Hasar, İshal.

INTRODUCTION

It has long been recognized that DAO as a metabolic enzyme that is increasingly exhibited at intestinal mucosa and placenta (Buffoni, 1966; Wolvekamp and de Bruin, 1994). Regarding human being DAO activity is elevated within the upper villi location of small intestine, in which it is participated for small intestinal mucosal epithelium maturation (D'Agostino et al., 1984; Shakir et al 1977). Given DAO activity through small intestinal location has been firmly in association, the latter might be a beneficial biomarker for the integrity/maturity belonging mucosae of small intestines (Luk et al., 1980; Wolvekamp and de Bruin, 1994). Enzyme-linked immunosorbent assay (ELISA) has long been issued in an attempt to calculate serum DAO concentration (Fukuda et al., 2020; Fukudome et al., 2014) and for estimation among newborn calves (Fukuda et al., 2020).

Gln, a well known and plentiful amino acid in free amino acid pool, has relationship with selected, relevant tissues (Calder, 1994). The latter classically a non-essential but "conditionally essential" amino acid participates in a) energy metabolism, and as a substrate for the synthesis of peptides and non-peptides (Albrecht et al., 2010; Amores-Sanchez, 1999; Coster et al., 2004), b) for detoxification of ammonia/systemic acid-base balance (Patience, 1990), and c) in involvement of immune systems (Calder, 1994; Newsholme, 1999; Newsholme et al., 1999)]. During certain catabolic conditions [i.e. trauma or sepsis (Lacey and Wilmore, 1990)], it was observed that intestinal, renal, and immune cells processed huge amounts of Gln (Wischmeyer, 2007; Karinch et al., 2001). In a prior work DAO was exhibited its value as a biomarker of intestinal mucosal injury among calves subjected to antibiotic treatment regimes (Alic Ural, 2024a). Another article determined the efficacy of rectal enema probiotic therapy to diarrheic calves in which mean DAO levels (ng/mL) among prior to and thereafter were presented as 8.48 ± 1.67 vs. 28.06 ± 3.51 , respectively, ($p < 0.001$) (Alic Ural et al., 2023). In the present research the hypothesis was to clearly indicate whether if a) DAO concentration might alter in relationship with intestinal mucosal injury during diarrheic episodes, b) and its concentrations would be conducted to physiological levels following Gln enema. It was also targetted Gln as

a nutraceutical support for the intestine, as this trophic aminoacid has long been well known fuel for enterocytes. Moreover regarding 'microbiota-gut-brain axis' concept mechanistic action of this trophic aminoacid, would have hasten and reverse intestinal mucosal injury, as it differs from previous research, and the specific novelty it brings compared to earlier studies. In the present study the aim was to validate and establish the efficacy of Gln enema for reversing intestinal mucosal injury among calves.

MATERIAL AND METHODS

Demographic Data

This study was conducted in accordance with the Declaration of Helsinki. The present original research was elucidated with written owner consent for each case enrolled and confirmed by Aydin Adnan Menderes University, Local Ethics Committee for Animal Researches (HADYEK) with report no: 64583101/2021/146 and date: October 27 2021. This was not an etiological study, in which the spatial distribution of responsible infectious or non-infectious reasons were not analyzed. Diarrhea was described as fecal consistency initially determined by Larson et al., (1977) and then was revised by McGuirk, (2008). Fecal consistency interpretation was based on a 3 points scale from 0 to 3 [scoring 0 normal - 3 watery] (McGuirk, 2008).

Research Algorithm

In a total of 20 calves enrolled randomly (at the age of 14 to 51 days of age), 0.9 ml blood was drawn from *V. jugularis* into anticoagulated tubes. Upon arrival to laboratory immediately (between cold ice packages) centrifugation was deemed available for separation of plasma. Commercially available DAO ELISA kit: Bovine Diamine Oxidase ELISA Kit (My Biosource, San Diego, United States) were thoroughly obtained (RDA Group, Istanbul). Sandwich ELISA methodology was similar to previous researches (Alic Ural et al., 2023; Alic Ural, 2024a, b).

Gln Dosaging

For dosaging Gln, selected literature was involved at, selected based upon previous research in rats (Avşar et al., 2001).

Thus, Gln involved in Gut cumin I Liquid Solution (Larek Tarım, Ankara, Türkiye) composed of also, L-arginine, threonine, curcumine, zinc, licorice root, ginger were given in rectal route as 15 mg/kg through usage of rectal enema catheters. All semi-interventional procedures were performed by board certified veterinary surgeons (K.U. and H.E.).

Statistical Analyses

Descriptive statistics were used to summarize the obtained data and the mean and standard error were presented in a table. The Mann-Whitney test was employed to compare DAO levels between groups. Statistical analyses and graphs were managed by using Graphpad Prism software (version 9.2, America), and results where the p-value was less than 0.05 were considered statistically significant.

RESULTS

Available DAO (ng/mL) results were shown in table 1. Prior to Gln enema $\bar{x} \pm SE$ values were deemed 9.12 ± 2.46 , which were significantly ($p < 0.01$) elevated (28.19 ± 3.18) thereafter treatment. Analytes were successfully performed without any error. Enema intervention was well accepted in entire calves participated at this study without any side effects.

Table 1. Presented DAO (ng/mL) Values in $\bar{x} \pm SE$ Before Treatment (bt) and After Treatment (at)

	BT	AT	p value
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	
DAO (ng/mL)	9.12 ± 2.46	28.19 ± 3.18	0.01

DISCUSSION

In the present study for elucidating the relationship between the serum concentration of DAO and Gln enema, Sandwich ELISA with commercially available species specific DAO test kits were preferred. Someone might principally aroused interest to this subject. Given L-glutamine is the fuel of colonocytes, intestinal mucosal injury as detected by DAO should thus be reversed, probably by Gln enema, as was the protocole at this study. This was a field study, in which obtained results should have helped changing treatment protocoles within the next future. This study was planned in

2023 and performed during March-May 2024.

Given the hypothesis of this study, investigating DAO concentrations (ng/mL) among diarrheic episodes, mean referreal values were 9.12 vs. 28.19 (table 1), after Gln enema. This results suggested that DAO levels probably linked within the small intestine, remained depressed (Debell et al 1987) at initial values, whereas Gln supplementation via recta route, improved DAO concentrations to an acceptable level in relationship with increased intestinal permeability (Alizadeh et al., 2022; Song et al., 2017). Therefore diminished DAO results prior to Gln might be denoting small intestinal mucosal injury (Alizadeh et al., 2022), whereas increased levels pointed out elevated intestinal permeability (Alizadeh et al., 2022; Song et al., 2017), herein at this study. A recently published study investigating even if Gln administration altered gastrointestinal and immune function in dairy calves, exhibited interesting results. In 3 repetitions, out of 30 Holstein heifer calves at the age of 1.5 ± 0.5 days old, 5 received Gln and other 5 did not. According to the results of that study Gln diminished gastrointestinal permeability among preweaning Holstein heifer calves (Ceja et al., 2023). The latter study was published prior to this article reported herein, which were independently and similarly aimed at similar targets (the present study was performed in 2024 as written above).

In a prior study researchers determined the participation of Gln and short-chain fatty acids [both through enema/oral administration] on mucosal recovery against experimental colitis model. According to the results of that study crypt depth was markedly elevated in both oral and enema Gln groups ($p < 0.05$). The mucosal DNA ingredient of the colitis and Gln enema groups were markedly higher than other relevant groups ($p < 0.05$). It was hypothesized that Gln enema might hasten mucosal recovery and regeneration in experimentally exhibited colitis among rats (Kaya et al., 2007). Similarly in the present study DAO (ng/mL) results as were shown in table 1, deemed 9.12 ± 2.46 vs. 28.19 ± 3.18 , prior to and after Gln enema, which were significantly ($p < 0.01$) elevated. This finding clearly indicate that increased DAO values reflected withdrawal of intestinal mucosal injury.

As a well known conditionally essential amino acid member,

Gln, is still under establishment for its availability as a probable feeding support for modification of gastrointestinal route and diminishing enteric disease existence. Gln administration through oral route among preweaning calves, presented diminished diarrhea-induced weight loss (Brooks et al., 1996). Wang et al. (2022) suggested modification for gastrointestinal morphology in preweaning calves that received Gln, given by intravenous route altered gastrointestinal integrity (Dong et al., 2019; Zhou et al., 2012). On the other hand different administration routes might probably alters Gln initial metabolic pathway (liver/kidney) instead of the small intestine, which could have cause unclear interpretation for gastrointestinal system health benefits (Wang et al., 2022). In the present study Gln was given in rectal route which could have been the potential for being a pioneer investigation. All calves accepted well, the enema procedure without any observable side effects. However this study did not investigate different routes of administration for Gln, which enabled the present author to conclude which route is superior to other. On the other hand Gln was given in its natural form directly to treat to target location, namely large bowel which could have influence on its distribution and kinetics. Moreover the beneficiary effect on this study should be further evidenced and proven by pharmacokinetic studies, highlighting mechanism of action and influence of rectal route comparatively to other administration techniques.

In a prior research the circulating levels and activity of DAO among calves with/without diarrhea under the age of 30 days showed highest level on day 0 following birth, whereas diminished particularly thereafter. On the other hand no variance was observed regarding DAO levels among diarrheic and control calves. In contrast, serum DAO activity was lowest on day 0 after birth, and gradually increased. Activity of sera DAO among diarrheic calves was diminished in contrast to control animal from exactly week 3 following birth. All aforementioned data denoted lack of correlation among activity and concentration of DAO in which the authors claimed that serum DAO activity is of beneficial for interpretation of small intestinal mucosal injury among calves (Ono et al., 2023). In the present study, as being supported with the

literature (Aliç Ural, 2024a,b; Fukudome et al., 2014; Luk et al., 1980; Ono et al., 2023). DAO analytes were deemed available, which is an established biomarker of intestinal injury. Moreover the present author would like denote that in this study for better understanding of the readers, DAO concentration was measured, not the activity. This was due to facility.

The serum concentration and activity of DAO in calves with or without diarrhea during the first month of life were examined. The serum DAO concentration was highest on day 0 after birth, and gradually decreased. No differences in serum DAO concentrations were observed between calves with diarrhea and control calves. In contrast, serum DAO activity was lowest on day 0 after birth, and gradually increased. Serum DAO activity in calves with diarrhea was lower than that in the control calves from day 20 after birth. These findings suggest that serum DAO activity and concentration are not correlated, and serum DAO activity is useful for assessing damage to the small intestinal mucosa in calves (Ono et al., 2023). In the present study solely concentrations of DAO were measured, however its activity was not the purpose (nor the present author has the availability for performing it). In the present study as was also partially reported above paragraphs, decreased (prior to Gln enema, probably related to intestinal mucosal injury) and increased concentrations of DAO (after Gln enema due to recovery) could reflect the importance of Gln for intestinal health.

The present study has some limitations. As was also aforementioned above DAO activity was not measured, in which solely concentration being evaluated. Someone might also criticise the lack of a control group within the methodology. Even if a control group was left, this could have helped preventing exclusion of time-dependent recovery and probable influence of other possible concurrent treatments. However due to economical values, ethical guidelines and certificate of attendance during approval, none of the animal owners accepted placebo or control group classification. Due to economical burden, increasing demands on dairy science and its relevant management (farming conditions, prices, husbandry, welfare etc.) the present author could not handle risk for loss could probably related to control group

inclusion. Moreover it was a relatively short term study, not a longitudinal one, enabling the interpretation of long-term DAO concentration levels.

CONCLUSION

In conclusion it should not be unwise to draw notification that serum DAO concentrations were elevated by use of Gln enema indicating reversed intestinal mucosal injury to those of calves with diarrhea.

CONFLICT OF INTEREST

The author has no conflicts of interest to declare.

FINANCIAL DISCLOSURE

The author declared that this study has received no financial support.

ETHICAL STATEMENT

The present original research was elucidated with written owner consent for each case enrolled and confirmed by Aydin Adnan Menderes University, Local Ethics Committee for Animal Researches (HADYEK) with report no: 64583101/2021/146 and date: October 27 2021.

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