



NOTE

Internal Medicine

Relationship between postnatal days, serum Cu concentration and plasma diamine oxidase activity in Japanese Black calves

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ABSTRACT. The aim of study was to investigate the relationships among serum diamine oxidase (DAO) activity, postnatal days and the plasma copper (Cu) concentration, using calves with or without diarrhea. In healthy calves, the serum DAO activity was significantly higher at 2 postnatal days than at ≥ 7 postnatal days, and no significant changes were observed after 7 postnatal days. In addition, no significant correlation was found between serum DAO activity and plasma Cu concentration at all postnatal days in healthy calves. Although, the serum DAO activity in 14 diarrheic calves (66.78 ± 14.37 IU/ml) was lower than that in 19 healthy calves (170.33 ± 97.83 IU/ml, $P < 0.01$), plasma Cu concentrations in all calves remained within the normal range.

KEY WORDS: calf, copper concentration, diamine oxidase, diarrhea, postnatal day

Neonatal calf diarrhea remains the most common cause of death in beef and dairy calves, and continues to be a major cause of economic loss for the cattle industry [20]. Understanding the state of intestinal villi in diarrheic calves is important for successful treatment. Although indirect indicators, such as mortality, duration of treatment, and fecal characteristics, have been used to assess treatment effects [6–8], there are few studies concerning an indicator for the state of the intestinal mucosa. Diamine oxidase (DAO) is a copper (Cu)-containing cytoplasmic enzyme found mainly in small intestinal villus epithelial cells that plays an important role in the oxidative deamination of histamine in the small intestine [16, 25]. As DAO produced in the villi of the small intestine is metabolized in the liver soon after its release into the blood, serum DAO activity in the blood reflects the state of the villi of the small intestine in real time [4]. Akimoto *et al.* [2] suggested that changes in serum DAO activity reflect intestinal barrier damage in rats. Tanaka *et al.* [24] also demonstrated that plasma DAO activity can be a sensitive marker of intestinal function in children. We previously reported reduced DAO activity in calves with diarrhea [11], and that plasma DAO activity may reflect the degree of intestinal mucosal disorder caused by diarrhea due to the negative correlation between plasma DAO activity and fecal score [10]. Therefore, DAO may be a plasma and/or serum biomarker for intestinal damage and inflammation in not only humans, but also calves [10, 11].

However, it has been reported that DAO activity increases with growth in rats [18], but there is no report on how DAO activity changes with growth in calves. In addition, as DAO is a Cu-containing cytoplasmic enzyme, it must also be clarified whether DAO is affected by changes in the blood Cu concentration when calves develop diarrhea [16, 25]. Graham *et al.* [13] reported that blood Cu concentrations increased with growth in a study evaluating biomarkers of poor neonatal growth. In addition, as DAO is an enzyme containing copper, its usefulness as a biomarker for Cu deficiency has been reported and it is known that DAO activity is low even in Cu deficiency [19]. If the blood Cu concentration changes by postnatal days or presence of diarrhea, serum DAO activity may also change with these factors. Therefore, before using DAO activity as an indicator of intestinal villus damage due to diarrhea in calves, it is necessary to elucidate the relationships among blood DAO, postnatal days, and blood Cu concentrations. The aim of study was to investigate the relationships among serum DAO activity, postnatal days and the plasma Cu concentration, using calves with or without diarrhea.

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the School of Veterinary

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Medicine, Rakuno Gakuen University (Approval#: VH18C9). Fifty-eight healthy Japanese Black calves in the Miyagi region of Japan at 2 (n=8), 7 (n=10), 10 (n=12), 14 (n=7), 21 (n=10), and 180 postnatal days (n=11) were enrolled to investigate the relationship between DAO activity and postnatal days. All calves were fed dam's milk freely and managed in a nursing barn with dams until 21 days after calving. At 180 postnatal days (=6 months), calves were separated from dams and weaned. All calves had *ad libitum* access to hay and water.

In addition, 14 calves that were presently diarrheic (watery and/or muddy diarrhea) but had no other clinical signs, such as fever (>39.5°C), decreased suckle reflex or deeply sunken eyes, were enrolled as diarrheic calves (diarrhea group) to investigate the relationship between DAO activity and plasma Cu concentration. The average and range of postnatal days were 11.9 ± 1.4 (mean ± SD) days and 10–14 days, respectively. As a control group, 19 healthy calves at 10 to 15 postnatal days were extracted from the above study. The mean postnatal days in the control group was 12.6 ± 1.6 days, which was similar to that in the diarrhea group. The fecal status was assessed using a scoring system (0: firm, 1: pasty, 2: loose, and 3: watery) described previously [14]. Fecal tests were performed to identify the causative organisms for diarrhea (rotavirus, coronavirus, *Cryptosporidium parvum*, and K99 *Escherichia coli*) using the fecal strip test (Tetrastrips, Bio-X Diagnostics S.A., Rochefort, Belgium).

Twenty-ml blood samples were collected from the jugular vein using a sterile disposable plastic syringe, and immediately stored in 10-ml vacuum serum separation and heparinized blood collection tubes (Venoject II, TERUMO, Tokyo, Japan). Blood samples were then centrifuged at 3,000 × g for 15 min in order to harvest serum and/or plasma. Serum and/or plasma samples were stored at -20°C until assayed.

Serum DAO activity was measured by a bovine specific ELISA system (Bovine DAO ELISA kit, My BioSource Inc., San Diego, CA, USA). In addition, serum aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) activity in the diarrhea and the control groups were also measured using an automatic biochemical analyzer (Dade Behring, Inc., Deerfield, IL, USA). In this study, the mean concentrations of Cu in plasma were measured by particle-induced X-ray emission (PIXE) analysis at the Nishina Memorial Cyclotron Center (Takizawa, Iwate, Japan). The lower limit of detection for this assay is 10–5 μg/ml in plasma. A detailed description of the preparation, methodology, and experimental setup was reported previously [3, 22].

Data are shown as means ± standard deviation (SD). All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [15]. Changes in serum DAO activity and plasma Cu concentration by postnatal day were assessed by one-way ANOVA followed by Tukey's honestly significant difference test or Kruskal-Wallis test, followed by Steel-Dwass test after assessment of variance. The correlation between the Cu concentration and DAO at each postnatal day was evaluated using Pearson's correlation coefficient after evaluating the variance. Comparisons of serum DAO activity and plasma Cu concentration between diarrhea and control groups were assessed by Student's *t* test or Mann-Whitney *U* test after evaluating variance. The significance level was *P*<0.05.

Sequential changes in serum DAO activity and plasma Cu concentration by postnatal day are shown in Fig. 1. The serum DAO activity was the highest the 2 postnatal days reaching 388.7 ± 75.1 IU/ml, and then decreased significantly by 7 postnatal day and stabilized from 7 (180.0 ± 70.6, *P*<0.00001 vs. 2 postnatal days) to 180 postnatal days (130.6 ± 71.9, *P*<0.00001 vs. 2 postnatal days). Several studies reported that the reference range for serum DAO activity in healthy Angus steers and heifers, and Japanese Black calves (11.5 ± 0.8 postnatal days) were 127.8–157.7 IU/ml [17] and 153.9 ± 72.0 IU/ml [11], respectively. Therefore, the average value of serum DAO activity in Japanese Black calves 7 to 180 postnatal days in this study was similar to previous studies [11, 17]. Luk *et al.* [18] reported that DAO activity in rats was low at birth, increased gradually with age, reached a peak at 22 days, and then remained at normal adult levels. This is because in the first 3 postnatal weeks the crypts elongate, cell proliferation increases, the villi lengthen, and mature mucosal cells bearing sucrase, maltase, and other brush border enzymes appear. On the other hand, neonatal calf enterocytes have the unique ability to non-selectively absorb numerous macromolecules, including immunoglobulins, by pinocytosis [5]. This cessation of macromolecular absorption occurs approximately 24–36 hr postpartum and is termed gut closure [21]. This study did not clarify why calf serum DAO activity behaved differently from that of rats, but may

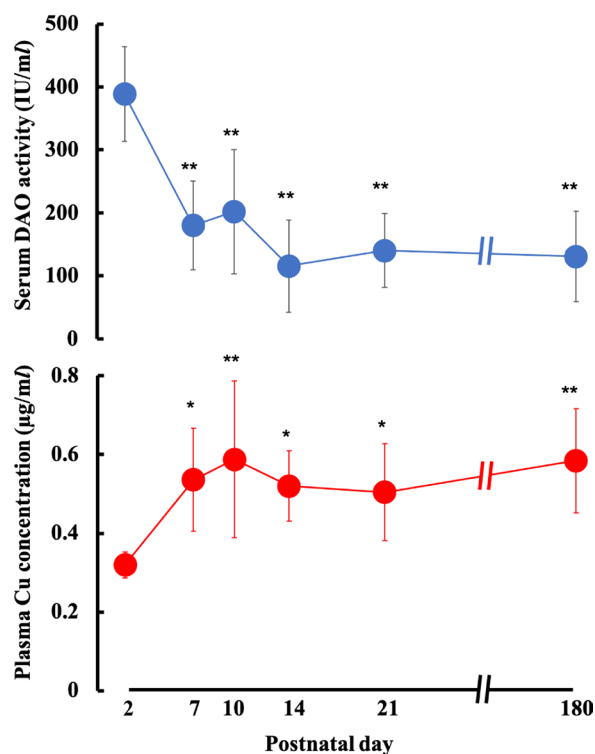


Fig. 1. Sequential changes in serum diamine oxidase (DAO) activity and plasma Cu concentration according to postnatal day. Serum DAO activity peaked at 2 postnatal days and then significantly decreased. The plasma Cu concentration was the lowest at 2 postnatal days and then increased significantly. *: *P*<0.05, **: *P*<0.01 vs. 2 postnatal days. Significant differences were assessed by one-way ANOVA followed by Tukey's HSD test or Kruskal-Wallis test, followed by the Steel-Dwass test after assessment of variance.

reflect these cow-specific intestinal changes.

The plasma Cu concentration exhibited a change opposite to the serum DAO activity by the postnatal day. The plasma Cu concentration was the lowest at 2 postnatal days ($0.32 \pm 0.033 \mu\text{g/ml}$), and then it significantly increased and stabilized after 7 postnatal days (0.54 ± 0.13 , $P < 0.05$). Therefore, no significant correlation was found between serum DAO activity and plasma Cu concentration at all postnatal days. The increase in plasma Cu concentration in this study was also considered to accompany growth.

Since serum DAO activity and plasma Cu concentration stabilized 7 postnatal day, the comparison between serum DAO activity and plasma Cu concentration in control and diarrhea group was evaluated at 10 to 15 postnatal days. The median fecal scores in the diarrhea and control groups were 2 (Min–Max: 2–3) and 0 (Min–Max: 0–0), respectively. As a result of the fecal strip test, diarrhea-inducing organisms were not detected in 10 calves and rotavirus was detected in 4. The serum DAO activity of rotavirus infected ($n=4$) or not infected calves ($n=10$) was 66.15 ± 10.62 and 67.02 ± 16.14 IU/ml, respectively, but no significant difference was observed ($P=0.923$). Rotavirus preferentially targets the mature villous enterocytes and spares the crypts, generally causing moderate villous damage [9]. In serum DAO activity, however, no significant difference was observed between rotavirus-positive and -negative calves. Therefore, damage to the intestinal mucosa was comparable to that of calves in which rotavirus was not detected. Acres *et al.* [1] reported that rotavirus was detected in healthy and diarrheic calves. Since diarrheic calves in this study had few pathogens, it was unclear that serum DAO activity altered by differences in pathogens or type of diarrhea such as osmotic or malabsorptive diarrhea. However, pathogen detection could not be always correlating with severity of intestinal damage. From this point of view, measurement of serum DAO activity might be useful for veterinary practitioners due to offering additional information related to intestinal mucosal states regardless of the type of diarrhea and pathogens.

In this study, the serum DAO activity in the diarrhea group (66.78 ± 14.37 IU/ml) was significantly lower than that in the control group (170.33 ± 97.83 IU/ml, $P < 0.0001$). The plasma Cu concentrations in diarrheic and control groups were 0.54 ± 0.16 and $0.56 \pm 0.17 \mu\text{g/ml}$, respectively. Therefore, no significant difference was observed between groups ($P=0.741$). Plasma Cu concentrations in both groups were over $0.50 \mu\text{g/ml}$ [17], which is the standard value for Cu in cattle. In this study suggested that the presence of diarrhea did not reduce calf plasma Cu concentration to deficiency. Cu deficiency in ruminants is a primary deficiency in which Cu intake is insufficient or as a secondary deficiency, whereby other factors in the diet interfere with the absorption or metabolism of Cu [12]. Javad *et al.* [23] reported that the plasma concentrations of Cu in calves with spontaneous diarrhea hardly changed. We hypothesize that the Cu deficiency in calves is more general under chronic conditions. This suggestion may be supported by the previous study evaluated blood Cu concentration as biomarkers of poor neonatal growth [13].

As the postnatal change in DAO stabilizes 7 postnatal days and there was few Cu deficiency in spontaneous diarrheic calves, the serum DAO activity of diarrheic calves after 7 postnatal days may reflect injury of the intestinal mucosa.

While, liver function in particular affects the alteration of blood DAO activity [4]. In this study, it was necessary to examine liver function. The serum AST activity in diarrheic and control groups were 46.3 ± 10.3 and 59.5 ± 9.5 IU/l, respectively. Although, significant difference was observed between groups ($P < 0.001$), these values remained within normal range [11]. The serum activity of GGT in diarrheic and control groups were 109.4 ± 44.0 and 167.8 ± 115.0 IU/l, respectively, but no significant difference was observed ($P=0.18$). Therefore, the effects of liver function on the serum DAO activity examined in this study were minimal.

In conclusion, we demonstrated that serum DAO activities and plasma Cu levels in calves were stable after 7 postnatal days. In addition, it seems that presence of acute diarrhea does not affect plasma Cu concentration in calves under field condition. Therefore, our results indicated that serum DAO activity in spontaneous diarrheic calves older than 7 postnatal days could be used as a marker of intestinal damage without consideration of postnatal days and plasma Cu levels, due to calves with diarrhea showed no significant decrease in plasma Cu concentration.

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