[Original research papers]

Plasma diamine oxidase activity decline with diarrhea severity in calves indicating systemic dysfunction related to intestinal mucosal damage

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Abstract

The aim of the present study was to investigate whether abnormalities in plasma diamine oxidase (DAO) activity reflect the degree of intestinal mucosal disorder in calves with diarrhea. A total of 50 Holstein calves were enrolled. Thirty-six of the 50 calves presented diarrhea and were sub-classified by severity based on fecal status (0: firm, 1: pasty, 2: loose, and 3: watery) and blood pH (acidemia: blood pH <7.25) as follows: Seventeen calves exhibiting watery diarrhea and/or fall into acidemia were sub-categorized into the severe group. The other nineteen calves exhibiting pasty or loose diarrhea and not fall into acidemia were sub-categorized into the moderate group. The remaining 14 calves without diarrhea were assigned to the control group. The plasma DAO activity was significantly lower (p<0.01) in the calves with severe or moderate diarrhea than in the control group. In addition, the plasma DAO activity was significantly lower (p<0.05) in the severe group than in the moderate group. The relationship between plasma DAO activity and fecal score (r=-0.55, p<0.01) in calves with diarrhea were found to have significantly and negatively correlated by Spearman's rank test in this study. Our results suggested that plasma DAO activity reflect the degree of intestinal mucosal disorder due to diarrhea.

Keywords:

calf, diamine oxidase, diarrhea, intestinal damage, nutrition

1. Introduction

Neonatal 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 (Smith, 2009). It is well known that diarrhea leads to mucosal inflammation (Laurent et al., 1999; Mosier and Oberst, 2000) and severe villous atrophy (Heath et al., 1989) in calves. As a result, calves with diarrhea frequently develop dehydration, strong ion acidosis and electrolyte abnormalities. Therefore, it is important to know the status of the intestinal villi during treatment for diarrhea. Several studies related to the treatment of calves with diarrhea have been previously reported in which indirect indicators, such as mortality, treatment period, and fecal character, were used to evaluate the therapeutic effects (Constable, 2009; Elitok et al., 2005; Ewaschuk et al., 2006). However, there are few studies in which the state of the intestinal mucosa was used as an indicator. Indeed, there is no useful biomarker for evaluating intestinal mucosal disorder in calves with diarrhea.

Diamine oxidase (DAO) is a cytoplasmic enzyme found primarily in the villus epithelial cells of the small intestine and plays an important role in the degradation of histamine in the small intestine (Kitanaka et al., 2002). DAO is localized in the mucosa, predominantly in the top villus region and DAO activity is high in the small intestine (Biegański, 1983). Plasma DAO activity levels are positively correlated with the maturity and integrity of the intestinal mucosa (Wolvekamp and de Bruin, 1994). Luk et al. (1980) revealed that with increasing mucosal damage, there was a progressive decrease in mucosal and plasma DAO activity levels fell. As a result previous studies demonstrated that blood DAO activity is a useful predictor of intestinal mucosal damage in human (Tanaka et al., 2003; Miyoshi et al., 2015) and rats (Akimoto et al., 2006).

If plasma DAO activity reflects intestinal damage in diarrheic calves, it will become a useful marker to assess diarrhea treatment. However, to our knowledge, no report has clarified the relationship between bovine intestinal damage and plasma DAO activity. The objective of this study was to investigate whether DAO is an indicator of intestinal damage in calves with diarrhea.

2. MATERIALS AND METHODS

This animal study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University (Approval#: VH18C9). A total of 50 Holstein calves aged 11.2 ± 5.4 days old were enrolled in this study. Thirty-six of the 50 calves presented diarrhea and were sub-classified by severity based on fecal status [0: firm, 1: pasty, 2: loose, and 3: watery (Hein et al., 1984)] and blood pH (Kasari and Naylor, 1986) as figure 1: Seventeen calves exhibiting watery diarrhea and/or fall into acidemia were sub-categorized into the severe group. The other nineteen calves exhibiting pasty or loose diarrhea and not fall into acidemia were sub-categorized into the moderate group. *Cryptosporidium parvum* (*C. parvum*) was detected in the feces of calves in the severe (13/17, 76.4%) and moderate (13/19, 68.4%) groups by the *C. parvum* rapid test kit (BOX-BIOK-155-10TEST, COSMO BIO Co., Ltd., Tokyo, Japan). The onset of diarrhea was unknown, but there was no treatment history included farmer's treatment in all the calves on the initial examination. The remaining 14 calves without diarrhea (*C. parvum* free calves) that were kept at the same dairy farms were assigned to the control group. All calves were given sufficient colostrum after birth and had no medical history before this study. Normally, all calves were offered two feedings of milk at the rate of 5% of body weight per feeding in the morning (a.m. 5:00 – a.m.8:00) and afternoon (p.m. 5:00 – p.m.8:00). They also had ad libitum access to hay and water. Concentrate feeding was not allowed during the study.

Single blood samples were collected by jugular venipuncture from all calves on the initial examination. Blood collections were done at least two hours after the providing milk for calves. Heparinized blood samples were analyzed for blood pH, and blood glucose (Glu) concentration using an automatic gas analyzer (i-STAT 1, Abbott Lab, Princeton, IL, U.S.A.) and i-STAT cartridge (i-STAT EC8+ Cartridge, Abbott Lab, Princeton, IL, U.S.A.), respectively. Non-heparinized blood samples were stored in plain tubes or EDTA-2K-coated vacuumed tubes, and then centrifuged for 15 min at 3000×*g* with a standardized procedure to harvest serum and plasma, respectively. In the serum biochemical analysis, total protein (TP) concentration was measured using the Biuret and Bromocresol Green methods. The serum total cholesterol (T-Cho) concentrations were measured using enzyme methods with the Discrete Method Clinical chemistry automatic analyzer (Dade Behring, Inc., Deerfield, IL, U.S.A.). The DAO activity in plasma was also measured by ELISA using a commercial DAO ELISA kit (Bovine Diamine Oxidase ELISA kit, My BioSource, San Diego, CA, U.S.A.).

Statistical analysis: Statistical analyses were performed with 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 (Kanda, 2013). Normally distributed data are reported as the mean ± standard deviation (SD), and non-normally distributed data (fecal score, blood pH and plasma DAO activity) are expressed as median and ranges. Due to the unequal number of data points, the Steel-Dwass test was employed for comparison among groups. Spearman's rank test was also used to evaluate the correlation between plasma DAO activity and fecal score. The significance level was $p<0.05$.

3. RESULT

Figure 2 shows the fecal score in calves with diarrhea. The fecal scores (range) for severe, moderate, and control groups were 3 (1 - 3), 2 (1 - 2), and 0 (0 - 0), respectively. The fecal scores were significantly higher in the severe and moderate groups than in the control group ($p<0.01$). In addition, the fecal score in the severe group was significantly higher than that in the moderate group ($p<0.05$). The blood pH in the severe, moderate, and control groups was 7.21 (6.83-7.40), 7.38 (7.27-7.45) and 7.42 (7.38-7.44), respectively. The blood pH was significantly lower in the severe group than in the moderate ($p<0.01$) and control ($p<0.01$) groups. In addition, the blood pH in the moderate group was significantly lower than that in the control group ($p<0.05$).

The serum TP, T-Cho, and blood Glu concentrations in each group are shown in Table. The concentrations of TP in the severe, moderate, and control groups were 5.9 ± 1.3, 5.7 ± 1.0, and 5.7 ± 0.5 g/d*l*, respectively. However, there was no significant difference in TP concentration among the groups. The concentration of T-Cho in the severe, moderate, and control groups was 41.7 ± 21.1, 60.9 ± 22.6, and 66.3 ± 31.0 mg/d*l*, respectively. The concentration of T-Cho in severe group tended to be lower than in the moderate ($p=0.09$) and control ($p=0.07$) groups. The concentration of Glu in the severe, moderate, and control groups was 84.6 ± 16.7, 90.0 ± 17.2, and 104.4 ± 12.4

g/d*l*, respectively. The concentration of Glu was significantly lower in the severe (p<0.01) and moderate (p<0.05) groups than in the control group. The plasma DAO activity in the severe, moderate, and control groups was 95.4 (23.5 – 196.0), 146.7 (86.8 – 246.0), and 293.2 (130.7 - 444.9) IU/m*l*, respectively (Fig.3). The plasma DAO activity was significantly lower in the severe (p<0.01) and moderate (p<0.01) groups than in the control group. In addition, the plasma DAO activity was significantly lower (p<0.05) in the severe group than in the moderate group.

The relationships between DAO activity in plasma and the fecal scores was also investigated by Spearman's rank test (Fig.4). As a result, significantly and negatively correlation was observed between plasma DAO activity and fecal score (r=-0.55, p<0.01).

4. DISCUSSION

In this study, calves were classified based on not only fecal status (Heine, 1984) but also blood pH (Kasari and Naylor, 1986). Regardless of the pathogen or mechanism, diarrhea increases the loss of electrolytes and water in the feces of calves and decreases milk intake. This process results in dehydration, strong ion acidosis, electrolyte abnormalities, increased D-lactate concentrations and a negative energy balance (NEB) (Smith, 2009). Lorenz (2004) demonstrated that calves with acidemia had increased D-lactate concentrations. Production of D-lactate results from villous atrophy, with subsequent malabsorption and fermentation of carbohydrates by intestinal bacteria (Berchtold, 2009). Unfortunately, we did not measure D-lactate concentration, but it seems that including blood pH in classification criterion is appropriate, considering the above reports.

Significant difference was observed in plasma DAO activity among groups. In addition, the relationship between plasma DAO activity and fecal score in calves with diarrhea was found to have significantly and negatively correlated in this study. Serum DAO activity is reported to reflect the integrity and maturity of the small intestinal mucosa, and has been evaluated as a potential marker of intestinal disease in a variety of disorders, including gut atrophy, ischemia, and inflammation (Akimoto et al., 2006; Miyoshi et al., 2015; Tanaka et al., 2003; Thompson et al., 1992). Moreover, DAO activity is affected by kidney injury (DiSilvestro et al., 1997). Renal failure due to severe dehydration may also cause the release of DAO from the kidneys to plasma in diarrheic calves. This was not the case in this study, however, considering that there is no difference in degree of dehydration between all groups using serum TP concentration as an index. Therefore, our results suggested that plasma DAO activity can reflect the degree of intestinal mucosal disorder associated with diarrhea, as in humans (Miyoshi et al., 2015; Tanaka et al., 2003). The decrease in the blood Glu concentration in diarrheic calves may have been caused by malabsorption of nutrients from the intestinal tract (Berchtold, 2009). These data support our explanation.

The concentration of T-Cho in severe group tended to be lower than in the moderate and control groups in this study. The reduction of not only the blood Glu concentration, but also the serum T-Cho concentration in the severe group suggested that calves fall into NEB. In addition, acidemia accelerates proteolysis in diarrheic calves (Tsukano et al., 2017). The intestines require a large amount of energy for repair and replication of the mucosal barriers. For example, amino acids, such as arginine and glutamine, are well-known energy sources for enterocytes (Wang et al., 2015). Tsukano et al. (2018) revealed that intravenous administration of nutritional solution, especially amino acids infusion, increased plasma DAO activity in calves with diarrhea. Therefore, plasma DAO activity could not only reflect intestinal damage: Based on our results and previous reports, the plasma DAO activity may have been affected by the systemic nutritional status, as in humans (Miyoshi et al., 2015).

5. Conclusions and future trends

Our results demonstrated that plasma DAO activity reflects intestinal damage and could be affected by the systemic nutritional status. Veterinary practitioner often encounter refractory diarrhea in calves. At that time, it may be difficult to judge the quality of the treatment policy by the feces characteristics alone. Our result demonstrated that the plasma DAO activity in calves is useful for evaluating the extent of intestinal mucosa disorder due to diarrhea. Veterinary practitioner can be used for clinical cases. However, plasma DAO activity can be altered by many factors such as differences in pathogens, severity of disease, and duration of disease. Further studies are needed to investigate whether these factors affect the plasma DAO activity.

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Abbreviations

DAO	:	Diamine Oxidase
ELISA	:	Enzyme-Linked ImmunoSorbent Assay
Glu	:	Glucose
NEB	:	Negative Energy Balance
SD	:	Standard Deviation
T-Cho	:	Total Cholesterol
TP	:	Total Protein

Figure legend

Figure 1.

The classification of 36 diarrheic calves based on fecal status and blood pH. Ten of 36 diarrheic calves exhibiting watery diarrhea with (n=5) or without (n=5) acidemia. Seven of 36 diarrheic calves exhibiting pasty or loose diarrhea with acidemia. In this study, these 17 calves were sub-categorized into the severe group. The other 19 calves exhibiting pasty or loose diarrhea without acidemia were sub-categorized into the moderate group.

Figure 2

The fecal score in calves with diarrhea.

The fecal scores (range) for severe and moderate groups were 3 (1 - 3) and 2 (1 - 2), respectively. Therefore median, third quartile and maximum were equal in each diarrhea group. The fecal scores (range) for control group was 0 (0 - 0). Outliers were not observed in all groups.

a-c: $p<0.01$, d-e: $p<0.05$ by the Steel- Dwass test.

Figure 3

The plasma DAO activity in calves with diarrhea.

a-c: $p<0.01$, d-e: $p<0.05$ by the Steel- Dwass test.

Figure 4.

The relationship between plasma DAO activity and fecal score ($r=-0.55$, $p<0.01$) in calves with diarrhea were found to have significantly and negatively correlated by Spearman's rank test.

Table 1. Fecal score and blood pH in calves with diarrhea.

Parameter	Control group	Moderate group	Severe group
Fecal score	0 (0-0)^{A}	2 (1-2)$^{C, D}$	3 (1-3)$^{C, E}$
Blood pH	7.42 (7.38-7.44)^{A}	7.38 (7.27-7.45)$^{B, D}$	7.21 (6.83-7.40)$^{C, F}$

Data are presented as the median and ranges

A-B, D-E: p <0.05, A-C, D-F: p <0.01 by the Steel- Dwass test

Table 2. Blood biochemical analysis and plasma DAO activity in calves with diarrhea.

Parameter	Control group	Moderate group	Severe group
TP (g/d*l*)	5.7 ± 0.5	5.7 ± 1.0	5.9 ± 1.3
T-cho (mg/d*l*)	66.3 ± 31.0	60.9 ± 22.6	41.7 ± 21.1
Glu (mg/d*l*)	104.4 ± 12.4[A]	90.0 ± 17.2[B]	84.6 ± 16.7[C]
DAO (IU/m*l*)	293.2 (130.7-444.9)[A]	146.7 (86.8-246.0)[C, D]	95.4 (23.5-196.0)[C, E]

Data are presented as the mean ± SD or median and ranges

A-B, D-E: $p < 0.05$, A-C, D-F: $p < 0.01$ by the Steel- Dwass test

Fig. 1

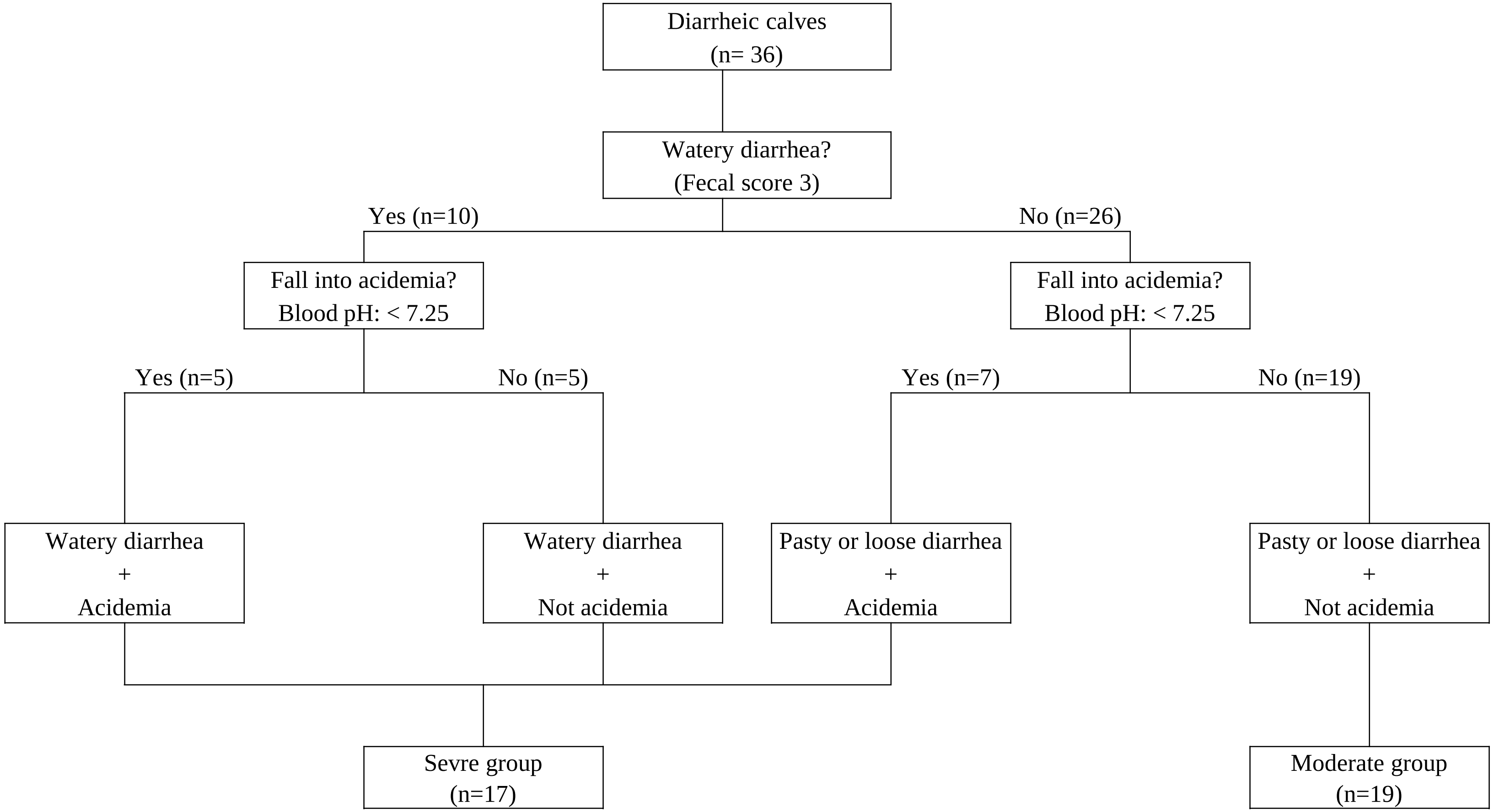

Diarrheic calves
(n= 36)
Watery diarrhea?
(Fecal score 3)
Yes (n=10)
No (n=26)
Fall into acidemia?
Blood pH: < 7.25
Fall into acidemia?
Blood pH: < 7.25
Yes (n=5)
No (n=5)
Yes (n=7)
No (n=19)
Watery diarrhea
+
Acidemia
Watery diarrhea
+
Not acidemia
Pasty or loose diarrhea
+
Acidemia
Pasty or loose diarrhea
+
Not acidemia
Sevre group
(n=17)
Moderate group
(n=19)

Fig 2

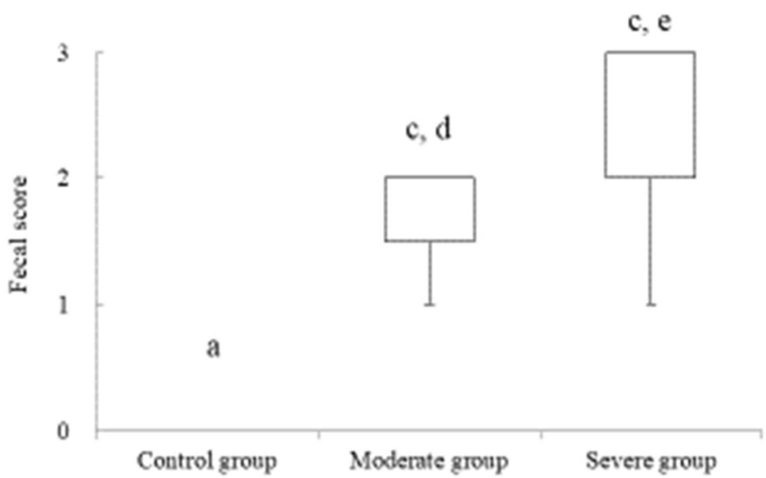

Fecal score
3
2
1
0
c, e
c, d
a
Control group
Moderate group
Severe group

Fig 3

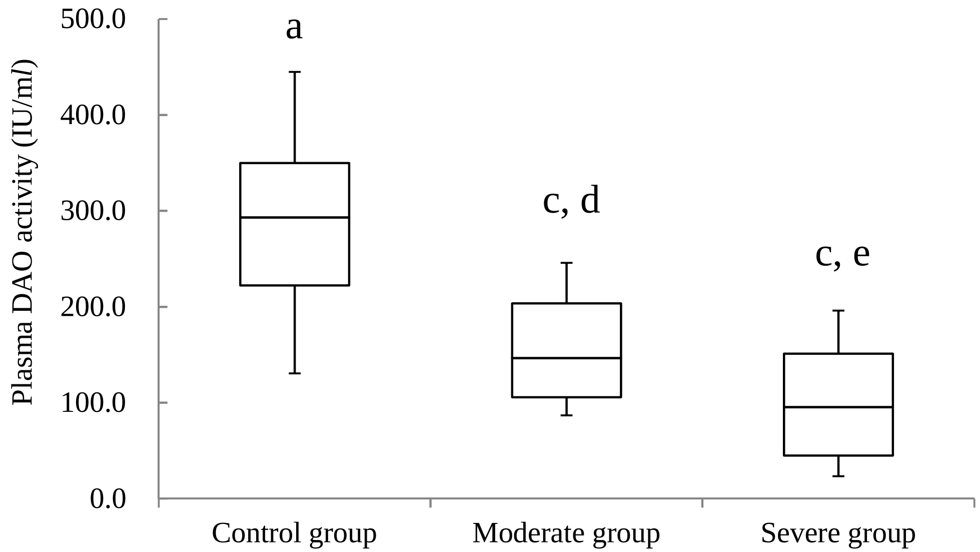
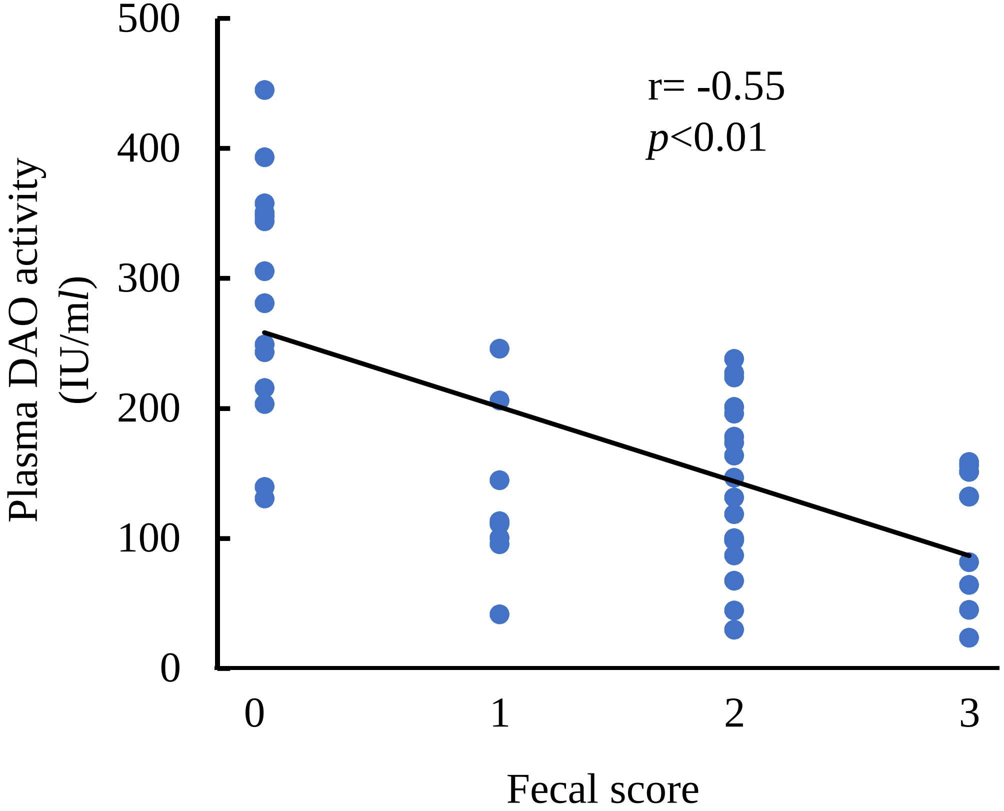

Plasma DAO activity (IU/ml)
500.0
400.0
300.0
200.0
100.0
0.0
a
c, d
c, e
Control group
Moderate group
Severe group

Fig. 4



500
400
300
200
100
0
Plasma DAO activity
(IU/ml)
r= -0.55
p<0.01
0
1
2
3
Fecal score