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Increase in branched-chain amino acids due to acidemia in neonatal calves with diarrhoea

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ABSTRACT

The aim of this study was to investigate the relationships between acid-base status and plasma branched-chain amino acids (BCAA) concentration in calves with diarrhoea for intravenous nutrition, especially with amino acid solution in calves with diarrhoea. Thirty-four Holstein calves aged 11.0±5.9 days old were enrolled in this study. In 10 of 34 calves exhibiting clinical signs of diarrhoea, severe dehydration and acidemia were observed (severe group: pH: 7.04±0.11, base excess (BE): −17.4±4.5) based on blood gas analysis. In 7 of 34 calves exhibiting clinical signs of diarrhoea, mild dehydration and acidemia were observed (mild group: pH: 7.29±0.06, BE: 0.0±5.2). The other 17 calves did not exhibit dehydration or acidemia (pH: 7.41±0.02, BE: 11.2±3.5) based on clinical signs and blood gas analysis. The plasma concentration of BCAA was significantly higher in the severe group than in the other groups. In addition, the blood pH and plasma concentrations of BCAA ($r=-0.41$, $P<0.05$) were significantly and negatively correlated. As calves with metabolic acidosis have increased plasma BCAA concentrations due to hypermetabolic states of proteolysis, amino acid solutions containing low concentrations of BCAA may be useful to gradually correct the negative nitrogen balance.

INTRODUCTION

Neonatal diarrhoea 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.[1] Diarrhoea not only leads to overall absorption of electrolytes and water, but also decreases carbohydrates, lipids and amino acids in calves.[2] The mean faecal fat content was higher in calves with diarrhoea than in the healthy calves, and the mean caloric uptake from milk was decreased by 31 per cent in diarrhoeic calves compared with that in healthy calves. The negative energy balance continues during the diarrhoeic period.[3] This suggests that diarrhoeic calves need more energy for maintenance. In addition, diarrhoeic calves fall into metabolic acidosis.[3] Several studies have reported that metabolic acidosis activated the catabolism of protein and oxidation of branched-chain amino acids (BCAA) in the muscle.[4 5] Even a mild decrease in extracellular pH is sufficient to activate proteolysis in human beings.[6] If plasma amino acid profiles of diarrhoeic calves are similar to those in human beings with acidemia, a greater energy supply may be needed for diarrhoeic calves with acidemia. However, intravenous nutrition for diarrhoeic calves with proteolysis-induced acidemia is poorly understood. The aim of this study was to investigate the relationships between acid-base status and plasma BCAA concentration in calves with diarrhoea. These data may be useful for intravenous nutrition, especially with amino acid solution in calves with diarrhoea.

MATERIALS AND METHODS

All procedures were performed in accordance with the Good for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University (approval #VH16C1) and the National Research Council.[7] Thirty-four Holstein calves aged 11.0±5.9 days old were enrolled in this study. Seventeen calves were patients at the Minami Hokkaido Agricultural Mutual Relief Association showing clinical sings of diarrhoea. *Cryptosporidium parvum* was detected in the faeces of all 17 calves by a *C parvum* rapid test kit (BOX-BIOK-155-10TEST, Cosmo Bio, Tokyo, Japan). Ten of 17 calves exhibiting clinical signs of severe dehydration and acidemia were observed (severe group) based on physical examination and blood gas analysis (i-STAT 1, Abbott Lab, Princeton, IL, USA). In calves of the severe group, veterinary practitioners detected that parenteral fluid therapy was necessary. Seven of 17 calves exhibiting clinical signs of mild dehydration and acidemia were observed (mild group) based on physical examination and blood gas analysis. Calves of the mild group were administered oral electrolyte solutions for treatment. As controls, 17 *C parvum*-free calves with no abnormal clinical

signs (control group) were kept at the dairy farms same as patients, respectively. All calves were given enough colostrum after birth, and had no medical history before this study.

Single blood samples were collected by jugular venepuncture from all calves. Some heparinised blood samples were analysed for BHBA concentrations with an automatic analyser (Precision Xceed, Abbott Lab), and for haematocrit concentration, haemoglobin concentration, blood pH and base excess (BE) using an automatic gas analyser (i-STAT 1, Abbott Lab). Non-heparinised blood samples were stored in EDTA-2K-coated vacuumed tubes and then centrifuged for 15 minutes at 3000 *g* with a standardised procedure to harvest plasma.

Analysis

Free amino acid concentrations in plasma were determined using an automated amino acid analysis system (Shimadzu Prominence and LCMS-2020, Shimadzu, Kyoto, Japan). Plasma was diluted 1:1 with 5 per cent trichloroacetic acid, swirled for 30 seconds and centrifuged for 3 minutes at 10,000 *g* at 4°C. The supernatant was diluted 10 times with 0.2 N lithium citrate buffer (pH=2.2) and was filtered through a 0.45 µm membrane filter (GHP Acrodisc Syringe Filter GF (4559), Shimadzu GLC, Tokyo, Japan). A portion of each sample (20 µl) of the filtrate was injected onto a high performance liquid chromatography (HPLC) column (Shim-pack Amino-Li type, 6.0 mm in diameter × 100 mm in length, Shimadzu). The mobile phase was a linear gradient from pH 2.2 with 0.15 N lithium citrate and 7 per cent methyl cellosolve solution to pH 10.0 with 0.3 N lithium citrate produced using a commercial mobile reagent kit (flow rate, 0.6 ml/minute; column temperature, 39°C; Amino Acid Mobile Phase Kit (Li type), Shimadzu). The postcolumn fluorescence derivatisation was conducted using an o-phthalaldehyde/N-acetylcysteine method and a commercial reagent kit (flow rate, 0.2 ml/min; reaction temperature, 39°C; Amino Acid Reaction Reagent Kit, Shimadzu) as described by Gnanou and others.[8] Individual amino acids were detected by a fluorescence detector (run time, 160 minutes; excitation on wavelength, 350 nm; and emission wavelength, 450 nm; RF-10Axs, Shimadzu). A mixed solution of amino acids was prepared as above and used as a standard (Type AN-II (011–14463) and Type B (123–02505), Wako Pure Chemical Industries, Osaka, Japan).

The authors calculated the total amino acids (TAA: threonine + valine + methionine + isoleucine + leucine + phenylalanine + histidine + lysine + arginine + tryptophan + serine + glutamic acid + glycine + alanine + tyrosine + proline + aspartic acid + asparagine + glutamine + cysteine-cysteine) and BCAA (valine + leucine + isoleucine).

Statistical analysis

Normally distributed data are reported as mean±sd, and non-normally distributed data (blood pH and BHBA) are expressed as median and ranges. Because of not equal number of data, the Steel-Dwass test employed for comparison among groups. The relationship of the blood pH concentrations with plasma concentrations of TAA, BCAA and BHBA was evaluated by Spearman's rank test. The significance level was P<0.05.

TABLE 1: Plasma concentration of free amino acid in each groups

	Severe (n=10)	Mild (n=7)	Control (n=17)
Total amino acids	3110.9±451.0	2498.1±419.3	2612.2±380.9
Valine	277.9±67.2*	211.9±41.4	219.3±70.1*
Leucine	184.3±36.1*	136.4±31.4*	141.0±30.1*
Isoleucine	129.4±33.5*	85.7±21.3*	98.9±17.9

Mean±sd, unit: nmol/ml.
*p<0.05.

RESULTS

The median blood pH in the severe, mild and control groups was 7.01 (min to max, 6.98–7.19), 7.30 (min to max, 7.21–7.34) and 7.41 (min to max, 7.37–7.41), respectively. The values of BE in the severe, mild and control groups were –17.4±4.5 mM, 0.0±5.2 mM and 11.2±3.5 mM, respectively. The median blood pH and values of BE were significantly lower (p<0.001) in the severe group than those of the other groups, respectively. The median blood pH and values of BE were significantly lower (p<0.001) in the mild group than those of the control group. The concentration of haematocrit in the severe, mild and control groups was 36.8±10.7 per cent, 22.6±5.0 per cent and 35.2±3.8 per cent, respectively. The concentration of haematocrit was significantly higher (p<0.05) in the severe and control groups than those of the mild group. The concentration of haemoglobin in the severe, mild and control groups was 12.2±3.5 g/dL, 7.6±1.7 g/dL and 11.6±1.2 g/dL, respectively. The value of haemoglobin was significantly higher (p<0.05) in the severe and control groups than those of the mild group.

Table 1 shows the plasma concentration of free amino acid in each groups. The values of plasma concentration of TAA in the severe, mild and control groups were 3110.9±451.0 mM, 2498.1±419.3 mM and 2612.2±380.9 mM, respectively. The values of plasma TAA were slightly higher in the severe group than those of the other groups. The blood pH and plasma concentrations of TAA (r=–0.40, p<0.05) were significantly and negatively correlated.

The values of plasma concentration of valine in the severe, mild and control groups were 277.9±67.2 mM, 211.9±41.4 mM and 219.3±70.1 mM, respectively. The values of plasma valine were significantly higher (p<0.05) in the severe group than those of the control group. The values of plasma concentration of leucine in the severe, mild and control groups were 184.3±36.1 mM, 136.4±31.4 mM and 141.0±30.1 mM, respectively. The values of plasma leucine were significantly higher

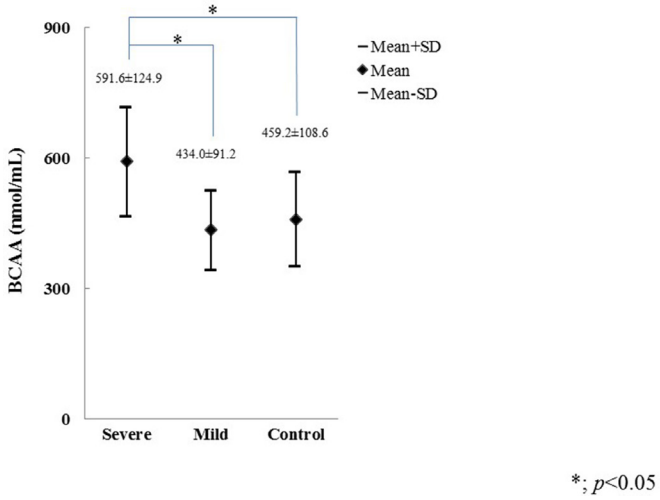

FIG 1: Severe acidemia affected the plasma branched-chain amino acids (BCAA) concentrations in neonatal calves with diarrhoea. Graphs depicting the value of plasma BCAA concentration in the severe, mild and control groups. Levels of significance indicated;
*p<0.05 by Steel-Dwass test.

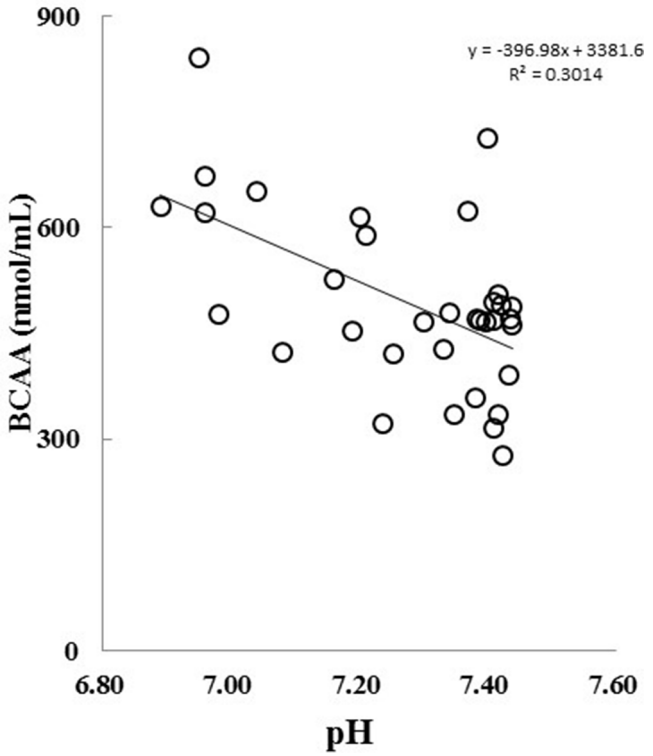

FIG 2: Increase in branched-chain amino acids (BCAA) due to acidemia in neonatal calves with diarrhoea. Relationships between the plasma BCAA concentration and blood pH in diarrhoeic calves.

(p<0.05) in the severe group than those of the other groups, respectively. The values of plasma concentration of isoleucine in the severe, mild and control groups were 129.4±33.5 mM, 85.7±21.3 mM and 98.9±17.9 mM, respectively. The values of plasma isoleucine were significantly higher (p<0.05) in the severe group than those of the mild group. Therefore the values of plasma concentration of BCAA in the severe, mild and control groups were 591.6±124.9 mM, 434.0±91.2 mM and 459.2±108.6 mM, respectively. The plasma concentration of BCAA was significantly higher (p<0.05) in the severe group than in the other groups (Fig 1). In addition, the blood pH and plasma concentrations of BCAA (r=−0.41, P<0.05) were significantly and negatively correlated (Fig 2).

The median of plasma BHBA in the severe, mild and control groups was 0.2 (min to max, 0.1–0.3), 0.1 (min to max, 0.1–0.1) and 0.0 (min to max, 0.0–0.1), respectively. The median of plasma BHBA was significantly higher in the severe group than those of the mild (p<0.05) and control (p<0.001) groups, respectively. The median of plasma BHBA was significantly higher (p<0.001) in the mild group than those of the control group. Therefore, the blood pH and BHBA (r=−0.72, p<0.001) were significantly and negatively correlated.

DISCUSSION

C parvum damages the small intestinal villi, which results in failure to absorb electrolytes and water (malabsorptive diarrhoea). Several investigators reported that amino acid transporters are present in the bovine small intestine.[9 10] Damage to the intestinal epithelium could reduce nutrient absorption and animal growth. As a result of diarrhoea, calves fell into dehydration, had increased D-lactate concentrations and a negative energy balance from anorexia and malabsorption of nutrients.[11] The largest pools of free amino acids are within the skeletal musculature, and intracellular amino acid levels in the muscle tissue partly reflect the changes in the extracellular level.[12] Negative energy balance could affect plasma TAA levels.

The relationship between acid-base status and plasma concentrations of amino acids in calves with diarrhoea was found to be significantly and negatively correlated in TAA and BCAA. The major pathway responsible for muscle protein degradation in catabolic conditions is the adenosine triphosphate (ATP)-dependent ubiquitin-proteasome system (UPS). The UPS may be upregulated in skeletal muscle by a number of factors, including metabolic acidosis.[13] In addition, BCAA is first transaminated and then irreversibly catabolised by the branched-chain ketoacid dehydrogenase complex (BCKDH),[14 15] the rate-limiting reaction for BCAA degradation, at least in skeletal muscle.[15] Metabolic acidosis also increases the activity of the BCKDH.[4] The study's results suggest that increases of plasma TAA and BCAA concentrations in diarrhoeic calves with severe acidemia resulted from negative energy balance and acceleration of proteolysis, especially in the muscle. Even if calves fell into a negative energy balance and reduced the quantity of TAAs in the body, plasma TAA and BCAA increased due to severe acidemia. The authors' suggestion was supported by beta-hydroxybutyrate (BHBA) concentrations in this study. They demonstrated that the relationship between acid-base status and plasma concentrations BHBA in

calves with diarrhoea was found to be significantly and negatively correlated. The results suggest that the degree of energy exhaustion depended on blood pH in diarrhoeic calves.

From a haematocrit and haemoglobin concentration of view, severe dehydration was observed in the severe group. Dehydration may have also contributed to catabolism in diarrhoeic calves. However, the newest study demonstrated that because of a concomitant reduction in metabolic rate, there were no differences in instantaneous leucine and lipid oxidation in water-deprived mice.[16] This suggests initial dehydration may not seriously affect proteolysis and lipolysis. Nevertheless it was unclear whether severe dehydration affects catabolism. Therefore, the authors' suggestion has limitations, and additional studies need to focus on dissecting whether plasma amino acid changes are associated with dehydration.

Because of hypermetabolic states, the body temperature could affect plasma amino acid profiles. The median of the body temperature in the severe, mild and control groups was 38.9 (min to max, 38.4–40.2), 38.9 (min to max, 38.5–39.2) and 38.7 (min to max, 38.4–39.1), respectively. There were no significant differences among groups. Therefore, the relationship of the body temperature with plasma concentrations of TAA (r=0.001) and BCAA (r=0.05) was not significantly correlated. In this study, it seems that the body temperature is not involved in plasma amino acid concentrations.

The authors demonstrated that blood pH and plasma concentrations of TAA and BCAA in calves with diarrhoea were significantly and negatively correlated. In particular, the plasma BCAA concentration dependent on blood pH was indicative of catabolism of protein in the muscle. The results were similar to those in human beings with acute acidosis.[6] These short-term effects of acid-base balance on protein metabolism seem to translate to long-term effects on skeletal muscle mass. Therefore, it is important for inhibition of proteolysis to correct acidosis in diarrhoeic calves. Furthermore, supplying amino acids to diarrhoeic calves with metabolic acidosis was important. As calves with metabolic acidosis have increased plasma BCAA concentrations due to hypermetabolic states of proteolysis, amino acid solutions containing low concentrations of BCAA may be useful to gradually correct the negative nitrogen balance. Future studies that examine the prescription of parenteral nutrition infusion solution with an amino acid ratio suitable for calf diarrhoea are needed.

Contributors All listed authors fulfil all three of the ICMJE guidelines for authorship. Substantial contributions to conception and design; all authors. Acquisition of data; KT and KS. Analysis of amino acids; HI. Interpretation of data; all authors. Drafting the article: all authors. Approval of the version to be published; all authors.

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