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TECHNICAL NOTE

I. Lorenz · I. Hartmann · A. Gentile

Determination of D-lactate in calf serum samples – an automated enzymatic assay

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Abstract Since recent research has shown that D-lactataemia is obviously not an unusual problem in calves, determination of this metabolite may have considerable clinical significance. In analogy to the routinely used L-lactate assay, a stereospecific enzymatic assay of D-lactate was automated on a Hitachi Automatic Analyzer 911. As the method described does not require deproteinisation, and amounts of reagents are small, the determination is inexpensive and not labour-intensive. The method proved to be both accurate and reliable, with a limit of linearity of 16 mmol/l. A reference range was established using serum samples from 150 clinically healthy Simmental calves of both sexes, up to 3 weeks old, from 48 dairy farms in southern Germany. An upper limit of the reference range (95th percentile) of 3.96 mmol/l was found.

Keywords Automated enzymatic assay · Calves · D-lactate · Reference values

Introduction

As only small amounts of D-lactate are produced by the methylglyoxylase pathway in mammals, elevated levels of this isomer of lactic acid must originate from bacterial production in the gastrointestinal tract and subsequent absorption into the blood. In humans, D-lactic acidosis is reported in the so-called 'short bowel syndrome' following resection of large portions of the small intestine or jejunoileal bypass surgery. Here, formation of D-lactic acid by bacteria occurs when undigested carbohydrates are transported into the large intestine (Uribarri et al. 1998).

I. Lorenz (✉) · I. Hartmann
Second Medical Animal Clinic, University of Munich,
Veterinärstraße 13, 80539 Munich, Germany

A. Gentile
Dipartimento Clinico Veterinario,
University of Bologna, Bologna, Italy

Ludvigsen et al. (1983) developed an automated assay for the determination of D-lactate in human serum samples, which, however, is not used routinely, probably because of the rare occurrence of the 'short bowel syndrome'.

In cattle the formation of D-lactic acid was first reported as a consequence of acute ruminal acidosis due to grain overload (Dunlop and Hammond 1965), which has been the object of many scientific studies as it is easily reproducible.

In the past two decades ruminal acidosis of milk-fed calves has received an increasing amount of scientific attention (Van Weeren-Keverling Buisman et al. 1990; Baur 1993; Gentile 1995; Gentile et al. 1998, 2002). Events leading indirectly to a low pH in the reticulorumen of young calves include forced feeding (by tubing or drenching), dysfunction of the reticular groove reflex, and reflux of abomasal contents. Fermentation of carbohydrates contained in the liquids by bacteria in the rumen produces both isomers of lactic acid, among other compounds. Because mammalian organisms have no specific mechanism for the metabolisation of D-lactate, accumulation of this isomer in blood following absorption from the rumen may give rise to metabolic acidosis (Gentile et al. 2002).

During the past few years, elevated levels of D-lactate have also been reported in the serum of diarrhoeic calves (Grude et al. 1999; Omole et al. 2001; Lorenz 2002), as well as in calves with metabolic acidosis without diarrhoea and dehydration (Schelcher et al. 1998).

As D-lactate is obviously not an unusual problem in calves, determination of this metabolite may have considerable clinical significance. The object of this paper was to describe an inexpensive assay of D-lactate in calf serum.

Materials and methods

In analogy to the routinely used L-lactate assay, the stereospecific enzymatic assay of D-lactate described is based on the oxidation of

D-lactate to pyruvate by D-lactate dehydrogenase under simultaneous conversion of NAD^+ to NADH. Equilibrium of the reaction, which normally is on the side of lactate, has to be forced to NADH production through elimination of pyruvate by transamination using alanine aminotransferase. Production of NADH is proportional to the content of D-lactate in the sample, and is measured by fluorimetry (340 nm).

For automation of the assay on a Hitachi Automatic Analyzer 911 the following chemicals (R-Biopharm GmbH, Darmstadt, Germany) were used:

Reagent 1 is a mixture of L-glutamate (99 mmol/l) in glycylglycine buffer (pH∼10), and NAD^+ (21.1 mmol/l) in distilled water, in a ratio of 2:1. This solution is stable for at least 48 h at a maximum of 8 °C.

Reagent 2 contains 172.6 U/l D-lactate dehydrogenase and 11.8 U/l alanine aminotransferase, and is stable for at least 3 weeks at a maximum of 8 °C. Volumes required for measurement are 7 µl of sample, 250 µl of reagent 1, and 50 µl of reagent 2.

A D-lactate standard (R-Biopharm GmbH, Darmstadt) was used for calibration.

Within-run and between-day imprecision was determined by calculating the coefficients of variation (CV) for repeated measurement ($n = 20$) of pooled calf serum with low, medium and high D-lactate concentrations.

The range of linearity was assayed by stepwise addition of D-lactate lithium salt (Sigma, L1000) to calf serum samples. Recovery was determined by adding sodium D-lactate (Fluka, 71716) to pooled calf serum in amounts aimed at producing concentrations between 3 and 16 mmol/l.

To test their stability the calf serum samples ($n = 25$) were assayed immediately and after they had been frozen at −24 °C for 2–4 weeks.

A reference range was established using samples from 150 clinically healthy Simmental calves of both sexes, up to 3 weeks old, from 48 dairy farms in southern Germany. The upper limit of the reference range was determined by the 95th percentile interval.

Results

Within-run imprecision (CV%) at D-lactate concentrations of 0.56, 6.34 and 12.4 mmol/l was 4.11%, 1.11% and 0.91%, respectively. Between-day imprecision at concentrations of 3.83, 7.59 and 10.89 mmol/l was 4.78%, 3.34% and 2.75%.

The assay was linear up to a D-lactate concentration of 16 mmol/l. Mean recovery was 98.8%, with a standard deviation of 1.9%.

Correlation ($r = 0.997$) between values of samples, which were measured both immediately and after storage at −24°C, is depicted in Figure 1.

The D-lactate values of 150 clinically healthy calves ranged from 0 to 7.1 mmol/l, with a median of 0.21 mmol/l (Fig. 2). The four apparently unusually high values in Figure 2 were tested by the method established by Lumsden and Mullen (1978), but could not be eliminated as outliers.

The upper limit of the 95th percentile was 3.96 mmol/l.

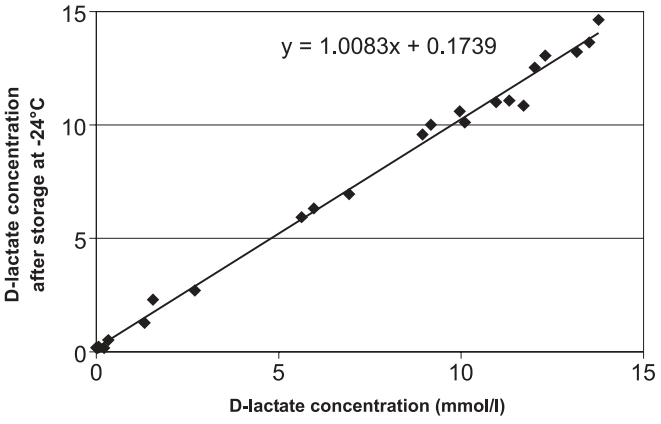

Fig. 1 D-lactate concentration in calf serum samples measured immediately and after storage at −24°C

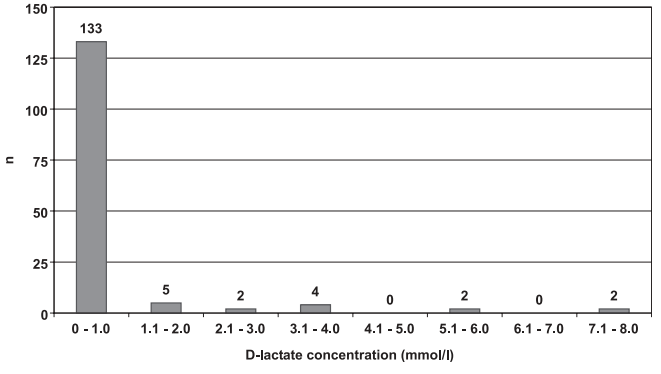

Fig. 2 Serum D-lactate concentrations of 150 clinically healthy Simmental calves up to three weeks old

Discussion

As mentioned above, recent publications (Schelcher et al. 1998; Grude et al. 1999; Omole et al. 2001; Lorenz 2002) point to a substantial significance of D-lactic acidosis in milk-fed or suckling calves. The fact that this has so far only been demonstrated in this species may be due to physiological aspects unique to calves, or to the fact that because of the lack of a routine assay, determinations of D-lactate levels have been performed only sporadically in other species.

The objective of the development of the automated assay described here was to provide the possibility of measuring D-lactate in high numbers of serum samples. As the method described does not require deproteinisation, and amounts of reagents are small, the determination is cheap and not labour-intensive. The method proved to be both accurate and reliable, and the D-lactate level of the vast majority of calf serum samples can be assumed to be below the limit of linearity of 16 mmol/l. Thus the method described is suitable for both scientific and commercial laboratories.

Marcillaud et al. (1999) set the upper limit of the reference range for D-lactate in Charolais calves younger than 3 weeks at 0.45 mmol/l. This reference range was established using an enzymatic assay following deproteinisation. The upper limit of the reference range reported here is 3.96 mmol/l. The difference may be due to the difference in husbandry. Even though Marcillaud et al. (1999) do not describe the husbandry conditions of the calves used in their study, it can be assumed on the

basis of the breed that they were from cow-calf herds on pasture. The calves enrolled in this study were bucket fed. It is conceivable that the consumption of single large volumes of milk provides bacteria in the gastrointestinal tract with more fermentable lactose, either by reflux of abomasal contents into the reticulorumen, or by rapid passage of incompletely curdled milk into the intestinal tract, without obvious detrimental effect on the condition of the calves.

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