ACTIVITY OF LDH ISOENZYMES IN DIARRHOEIC CALVES

PRZEMYSŁAW SOBIECH AND ZYGMUNT KULETA

Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-957 Olsztyn, Poland
e-mail: psobiech@uwm.edu.pl

Received for publication March 06, 2006.

Abstract

The aim of the study was to determine the level of homeostatic disturbances during the course of diarrhoea in calves. In the study there were examined 20 H-F calves divided into two groups: 10 clinically healthy and 10 with symptoms of diarrhoea. Acid-base balance parameters, electrolyte concentration, and AST, ALP and LDH activity were measured in the blood of all calves. LDH was also separated into its isoenzymatic fractions. Decompensated metabolic acidosis was found in all calves having diarrhoea as well as decreased level of Na⁺ and Cl⁻ and an increased concentration of K⁺. There was also an increase in the activity of AST and ALP. An increase in LDH₁ activity together with a decrease in LDH₅ activity was found during separation of LDH isoenzymes.

Key words: calves, diarrhoea, LDH isoenzymes.

Diarrhoeic and respiratory affections constitute about 80% of pathologic cases in calves and are the major cause of economic losses. Symptoms of the diarrhoeic syndrome usually occur in calves aged up to 6 weeks. The susceptibility of calves to the illness is high and depends on factors such as: age and season of the year, exposure to infectious factors, or the quality of colostrum intaken (15). Three periods have been identified when calves are prone to the illness: postnatal (1-4 d), weaning and transfer to a calf house. Usually, the disease spans ca. one week and the convalescence period – from 3 to 5 d. Causes of diarrhoea are of dietetic, bacterial, fungal, viral, toxic, parasitic, allergic, and stress-bearing origins. Diarrhoea is most often induced by the dietetic, bacterial and viral factors (15).

In young calves, the disease is usually of a bacterial (E. coli) or viral (rota- and corona viruses) origin, and after 2-3 weeks, invasion of cryptosporidia may also occur. The virus-borne diarrhoea, complicated with e.g. salmonellosis, observed in the first 2-3 weeks of life of calves and accompanied by dietetic and technological faults, may be the cause of numerous cases of death (5, 8, 10).

Clinical symptoms observed in the diarrhoeic calves are manifested by loose stools, a lack of appetite and abdominalgia. Persistent diarrhoea results in dehydration, severe weakness and gradual loss of a sucking reflex. A considerable loss of fluids causes hypovolaemia and circulation disorders, and leads thus to a decreased internal body temperature, whereas disturbances in the acid-base balance and electrolytic balance are likely to induce neural symptoms with convulsions leading to death (6). The clinical symptoms are accompanied by a variety of metabolic disorders manifested by changes in enzymatic and biochemical indices (14).

This study was aimed at determining the range and extent of disorders in the systemic homeostasis of calves in chronic inflammation of the gastrointestinal tract by means of assaying selected enzymatic parameters and indices of water-electrolyte and acid-base balance.

Material and Methods

Studies were carried out on 20 H-F calves, aged 4-10 d, of both sexes, kept indoors and originating from a dairy farm located in the Warmia region. The animals were housed and treated in accordance with the rules approved by the local Ethical Commission (conforming to principles of Laboratory Animal Care, NIH publication No. 86-23, revised in 1985).

The animals were divided into two groups: I – the control group constituted by 10 healthy calves, and II – the experimental group that included 10 calves with symptoms of chronic inflammation of the gastrointestinal tract. Both groups were subjected to clinical examinations as well as to biochemical analyses carried out on blood samples collected from the jugular vein.

To determine the acid-base balance, the analyses of the following parameters were performed in whole blood samples: pH, partial pressure of carbon dioxide (pCO₂), partial pressure of oxygen (pO₂), concentration of bicarbonate ions (HCO₃⁻), excess or deficiency of bases (BE), degree of haemoglobin saturation with oxygen (O₂SAT) and total content of carbon dioxide (ctCO₂). These parameters were assayed with a Corning 248 analyser.
The assessment of the electrolytic balance involved the determination of serum concentrations of sodium (Na⁺), potassium (K⁺) and chlorides (Cl⁻), which was carried out with the ion-selective method using an Easy Lyte Plus apparatus. An analysis of enzymatic indices included the determination of the activities of AST (aspartate aminotransferase), ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) that were carried out in samples of serum with the kinetic method. In addition, isoenzymatic fractions were separated from LDH with the use of high voltage electrophoresis in agar gel in the Paragon system by Beckman.

Results of laboratory tests were expressed in the SI system units and subjected to a statistical analysis with the Newman-Keuls test using Statistica 6.0 software.

Results

Over the experimental period, the control calves did not demonstrate clinically any pathological symptoms, whereas all calves from the experimental group showed impairment or loss of appetite, roughness, dryness, and decreased elasticity of skin, and - a predominating symptom – diarrhoea. All the calves defecated with loose stools yellow or gray-yellow in colour, and their internal temperature remained at a level of physiological norms or was slightly lower (37.6 – 38.9°C).

In the healthy calves, the indices of the acid-base balance were within norms considered as physiological for that species (Table 1). In the calves with diarrhoeic symptoms, a statistically significant decrease in pH, concentration of HCO₃⁻, pO₂, O₂SAT and BE number that attained negative values was observed (Table 1). In the case of the parameters of the electrolytic balance, a significant decline in the contents of sodium and chlorides with a simultaneous increase in potassium level was observed in calves from the experimental group (Table 1).

The analysis of the enzymatic indices in the calves with diarrhoea symptoms demonstrated a statistically significant increase in the activities of AST and ALP, whereas the total activity of LDH remained at a similar level in both groups of the animals (Table 2). The electrophoretic separation of LDH into isoenzymatic fractions enabled separating 5 isoenzymes in control and experimental groups. In the healthy calves, the highest isoenzyme activity was reported for the rapidly-moving fractions (LDH₁ and LDH₂), whereas the calves with diarrhoea symptoms were characterized by a statistically significant increase in the activity of the LDH₁ fraction and a decrease in the activity of isoenzyme LDH₅ (Table 2).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>pCO₂ kPa</th>
<th>ctCO₂ mmol/l</th>
<th>HCO₃⁻ mmol/l</th>
<th>pO₂ kPa</th>
<th>O₂ SAT %</th>
<th>BE mmol/l</th>
<th>Na⁺ mmol/l</th>
<th>K⁺ mmol/l</th>
<th>Cl⁻ mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy calves</td>
<td>7.36</td>
<td>8.27</td>
<td>32.05</td>
<td>34.27</td>
<td>5.25</td>
<td>48.81</td>
<td>7.23</td>
<td>143.11</td>
<td>4.32</td>
<td>106.21</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.34</td>
<td>3.11</td>
<td>1.54</td>
<td>0.78</td>
<td>3.65</td>
<td>0.87</td>
<td>1.15</td>
<td>0.34</td>
<td>0.53</td>
</tr>
<tr>
<td>Calves with diarrhoea</td>
<td>7.28A</td>
<td>7.42</td>
<td>29.59</td>
<td>27.41A</td>
<td>3.97A</td>
<td>29.33A</td>
<td>-1.17A</td>
<td>131.28A</td>
<td>5.41A</td>
<td>97.56A</td>
</tr>
<tr>
<td>SD</td>
<td>0.01</td>
<td>0.43</td>
<td>2.32</td>
<td>1.15</td>
<td>0.32</td>
<td>2.78</td>
<td>1.01</td>
<td>1.23</td>
<td>0.22</td>
<td>0.45</td>
</tr>
</tbody>
</table>

A – P ≤ 0.01

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>AST U/l</th>
<th>ALP U/l</th>
<th>LDH U/l</th>
<th>LDH¹ %</th>
<th>LDH₂ %</th>
<th>LDH₃ %</th>
<th>LDH₄ %</th>
<th>LDH₅ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy calves</td>
<td>63.3</td>
<td>124.6</td>
<td>473.9</td>
<td>43.41</td>
<td>29.71</td>
<td>13.85</td>
<td>4.87</td>
<td>8.16</td>
</tr>
<tr>
<td>SD</td>
<td>3.87</td>
<td>13.44</td>
<td>32.15</td>
<td>3.35</td>
<td>2.34</td>
<td>1.24</td>
<td>0.52</td>
<td>1.11</td>
</tr>
<tr>
<td>Calves with diarrhoea</td>
<td>105.3A</td>
<td>934.9A</td>
<td>498.6</td>
<td>57.84A</td>
<td>26.62</td>
<td>10.31</td>
<td>3.51</td>
<td>1.72A</td>
</tr>
<tr>
<td>SD</td>
<td>10.12</td>
<td>73.15</td>
<td>37.28</td>
<td>4.02</td>
<td>2.67</td>
<td>1.53</td>
<td>0.41</td>
<td>0.34</td>
</tr>
</tbody>
</table>

A – P ≤ 0.01
In the healthy calves, the indices of the acid-base balance remained within physiological norms typical of the species (6). The calves suffering from diarrhoea were characterized by disturbances in the acid-base balance manifested by decreased values of pH, bicarbonate level, partial pressure of oxygen, haemoglobin saturation with nitrogen, and reduced base reserves. These changes indicate the incidence of the state of uncompensated metabolic acidosis. In the chronic inflammation of the gastrointestinal tract, the loss of bicarbonates and electrolytes (mainly Na and K ions) with faeces is considered as the main cause of the mentioned disturbances (19). In addition, in the states of the metabolic acidosis, a progressive decline in the levels of bicarbonates may result from their binding to hydrogen ions in the extracellular hydrous space (3).

The status of the acid-base balance is closely linked with the level of electrolytes. A significant decrease in the concentrations of chloride and sodium ions in the blood of calves with diarrhoeic symptoms is connected with their loss with faeces, whereas an increase in the level of potassium ion has a direct relationship with disturbances in the acid-base balance. An increased pH value in serum results in a decreased concentration of potassium ions. In contrast, its decrease leads to an increased concentration of these ions. The mechanism of these interactions consists in the exchange of hydrogen and potassium ions between the extracellular and cellular spaces. This exchange may be evoked by the primary increase in the concentration of hydrogen ions (pH drop) in the cellular liquid, and results in their shift to cells. In accordance with the rule of electric inertness of systemic fluids, the potassium ions shift in an opposite direction (from a cell to extracellular fluid), thus causing an increase in their concentration in plasma (7, 12).

The average activity of enzymes determined in the healthy calves oscillated within physiological norms (Table 2) (11), whereas the calves with chronic inflammation of the gastrointestinal tract demonstrated substantially increased activities of AST and ALP. The elevated activities of these enzymes are likely to occur in a number of cases, e.g. in pathological states of the liver and kidneys or digestive dysfunctions (4). In the case discussed, it may be concluded that the increase in AST activity was linked with excessive load of the liver in the diarrhoeic states, whereas the high, multi-fold increase in ALP activity could have been due to the damage to the intestinal mucosa, progressive inflammatory process, and release of the intestinal fraction of that enzyme to blood circulation (21).

The electrophoretic separation of LDH into isoenzymatic fractions enabled obtaining 5 isoenzymes in both groups of the animals. In human and veterinary medicine, the isoenzymatic separation of LDH is used in the diagnosis of pathological condition of the heart, liver or skeletal muscles (2, 9, 16). Studies carried out on ruminants have indicated an increased serum activity in LDH₁ fraction in the case of hepatocellular damage and that of LDH₅ fraction in the case of myopathy (16, 20). In the serum of the healthy calves, the highest isoenzyme activity was reported for rapidly-moving fractions of LDH₁ and LDH₅ (Table 2). There are great discrepancies in reference data in the isoenzymatic profiles of LDH obtained in the serum of healthy calves and cows. In calves, Salplachta and Necas (13) demonstrated the highest activity for LDH₁ isoenzyme, whereas Avallone (1) demonstrated the highest activity for LDH₁ fraction, similar to that reported in the study. In comparing the isoenzymatic profile of LDH in the serum of adult cows (17) and calves, it may be stated that the latter is characterized by an increase in the activity in LDH₁ fraction – typical of the skeletal muscles – and a decrease in the activity of LDH₁ fraction specific for the liver.

Calves from the experimental group displayed an increase in the activity of LDH₁ fraction, which may point to destruction of hepatic cells in chronic inflammation of the gastrointestinal tract. This increase was accompanied by a reduced activity of LDH₅ fraction, yet it seems not to have any diagnostic significance in the case discussed as that change results from the shift of the isoenzymatic activity towards the LDH₁ fraction. Similar results were obtained while investigating changes in the isoenzymatic profile of LDH in the serum of diarrhoea-suffering goat kids (18).

In summary, it may be concluded that in calves with symptoms of diarrhoea there were observed numerous cases of disturbances in systemic homeostasis manifested by the incidence of uncompensated metabolic acidosis accompanied by diminished concentrations of sodium and chlorides and elevated total activity of AST and ALP. In addition, tangible changes were noted in the isoenzymatic profile of LDH in the serum consisting of an increased activity of LDH₁ fraction and reduced activity of LDH₅ isoenzyme.

References