

Water and electrolyte management in calves before and after excessive lactose administration¹⁾

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Summary

The aim of the study was to assess the dynamics of changes in ANP, AVP, and ALDO before and after the administration of lactose (a causative agent of diarrhea) and to evaluate the ability of the kidneys to regulate isotonicity by analyzing the concentrations of the major blood plasma electrolytes and osmotic pressure before and after lactose administration. The experiment was carried out on 8 Polish Holstein-Friesian calves of the Black-and-White variety during the second and third weeks of life. Animals were fed with a milk replacer twice a day in an amount of 10% of body weight. Monohydrate lactose in an amount of 1 g kg⁻¹ of body weight was added twice to the milk replacer.

The average concentration of atrial natriuretic peptide in the blood plasma of calves was low during the second week of life, but increased in the third week. The levels of plasma ALDO and AVP before the administration of lactose was higher in two-week-old calves than it was in three-week-old calves. The concentration of vasopressin in two- and three-week-old calves decreased in successive days of the experiment. Only in two-week-old calves a statistically significant increase in the concentration of aldosterone was noted 60 h after lactose administration. The concentrations of sodium, potassium, and chloride in blood plasma before the administration of lactose were higher in three-week-old calves than they were in two-week-old calves. The administration of lactose resulted in a significant increase in the concentrations of sodium and potassium in the blood plasma of two-week-old calves and caused a significant increase in the concentration of chloride in three-week-old calves.

During the first weeks of life, the activity of factors regulating renal functions, in conjunction with relatively stable blood levels of the main electrolytes and the osmotic pressure of blood plasma, indicates a functional maturity of kidneys in healthy calves and in calves manifesting light symptoms of diarrhea. These results also suggest that the mechanisms involved in the regulation of water and electrolyte homeostasis in these calves are efficient.

Keywords: ANP, ALDO, AVP, electrolyte

The processes of adaptation, growth, and maturation are accompanied by the intensification of illnesses and considerable mortality, especially as a result of water-electrolyte imbalance. The loss of water through the digestive tract is a major cause of calf mortality (7). One of the causes of diarrhea is an excessive administration of milk replacer (22). The accumulation of undigested lactose in the gastrointestinal tract is an important factor promoting the fecal loss of water and electrolytes. If the content of lactose in milk replacer exceeds 55%, the risk of diarrhea is significantly increased because of the osmotic properties of lactose.

The hormone system is involved in maintaining water and electrolyte balance. Three major hormones,

atrial natriuretic peptide (ANP), arginine vasopressin (AVP), and aldosterone (ALDO), play an important role in body fluid homeostasis (4, 13, 25). Atrial natriuretic peptide has an important role in the regulation of sodium and water balance, blood volume, and blood pressure (4). Arginine vasopressin plays a central role in water balance and in maintaining plasma osmolality (13). Aldosterone plays a major role in sodium and potassium metabolism (25).

In the neonatal period, adaptations to the extrauterine environment affect the whole body, including the excretory system. High neonatal mortality, among others, is associated with disturbed water and electrolyte homeostasis, regulated mainly by the kidneys (5). The development of the kidneys, initiated in the prenatal period, continues for some time after birth,

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similarly to the development of systems that control them. The completion of the development of the regulatory systems and kidneys is species-specific (18, 28, 30). Our previous studies show that the adaptation of the excretory system to extrauterine life in calves is most intense in the first week of life. However, many important issues concerning the functioning of their kidneys, as well as the mechanisms that regulate it in the first week and during following days, have not been clarified.

Therefore, a study was undertaken with the primary objective to assess the dynamics of changes in atrial natriuretic peptide (ANP), vasopressin, and aldosterone before and after the administration of lactose (a causative agent of diarrhea) and to evaluate the ability of the kidneys to regulate isotonicity by analyzing the concentrations of the major blood plasma electrolytes and osmotic pressure before and after lactose administration.

Material and methods

The experiment was carried out on 8 male Polish Holstein-Friesian calves of the Black-and-White variety during their second week of life (from the 13th to the 16th day of life) and third week of life (from the 20th to the 23rd day of life). From the 4th day of life, the animals were fed milk replacer (Mlekovit Imupro[®], Polmass) twice a day in an amount of 10% of body weight. The milk replacer contained 23% crude protein, 16% crude fat, 0.1% crude fiber, 45% lactose, 7.5% crude ash, 1.7% lysine, 0.42% methionine, 0.9% calcium, 0.7% phosphorus. Furthermore, monohydrate lactose (Pharma Cosmetic) in an amount 1 g kg⁻¹ body weight was added twice to the milk replacer: on the 13th and 20th days of life (during the evening feeding) and on the 14th and 21st days of life (during the morning feeding). The use and handling of animals in this experiment were approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals (No. 3/2010 of 14.01.2010).

During the experimental periods, blood was drawn from the jugular vein of the calves into EDTA tubes (to measure hormones) and heparin tubes (to measure electrolytes and osmotic pressure) before the evening feeding. The samples were centrifuged (15 minutes, 4°C, 3000 rpm) and the harvested plasma was stored at -80°C until processing.

Plasma atrial natriuretic peptide concentrations were measured by the radioimmunoassay method with an iodine-125 radioimmunoassay kit (ANP, alpha 1-28, Phoenix Pharmaceuticals Inc. USA). The range of the standard curve was 10-1280 pg ml⁻¹.

Plasma vasopressin concentrations were determined with an iodine-125 radioimmunoassay kit (Vasopressin AVP [Arg8] Phoenix Pharmaceuticals Inc. USA). The range of the standard curve was 10-1280 pg ml⁻¹.

The levels of aldosterone in blood plasma were determined with an aldosterone ELISA kit (Aldosterone ELISA, Labor Diagnostika Nord, Germany). The range of the standard curve was 0-1000 pg ml⁻¹.

Blood plasma sodium and potassium levels were analyzed by inductively coupled plasma optical emission spec-

trometry (ICP-OES) with an Optima 2000 DV spectrometer (Perkin Elmer Inc.). Measurements were made along the plasma in the axial direction. The elements were quantified with calibration curves plotted from a multi-element standard solution (ICP Multi-element Standard IV, Merck). All samples and standard solutions were supplemented by yttrium as the internal standard.

Plasma osmotic pressure was determined by the cryoscopic method (Knauer osmometer), and the plasma chloride concentration was measured with a chloride meter (chlorimeter 100 cl, TridentMed).

Mean values and standard deviations were calculated. The resulting data were analyzed by an ANOVA with repeated measurements and Duncan's multiple range post hoc test (software: Statistica 10TM) in order to test the significance of differences.

Results and discussion

Changes in the levels of the blood plasma indicators observed in the experiment are shown in Fig. 1-7. They present changes in the levels of plasma parameters, as well as indicate means, standard deviations, and the significance of differences between the values before and after lactose administration.

The average concentration of atrial natriuretic peptide in the blood plasma of calves during the second week of life was low (64.69 pg ml⁻¹), but increased to 77.85 pg ml⁻¹ in the third week (Fig. 1). Although there was no statistically significant effect of lactose on the mean ANP concentration in blood plasma, an increased level of this hormone was recorded after 12 hours of lactose administration. It amounted to 71.45 pg ml⁻¹ in two-week-old calves and to 78.88 pg ml⁻¹ in three-week-old calves. Thus a similar trend of changes was noted in the level of this hormone in blood plasma. A decrease in the ANP concentration in blood plasma was observed in both groups 36 hours after lactose administration. The highest level of this hormone was also measured in both groups of animals 60 hours after lactose administration. In this experiment, high individual variability in the level of ANP was observed.

The level of plasma aldosterone before the administration of lactose was higher in two-week-old calves (64.22 pg ml⁻¹) than it was in three-week-old calves

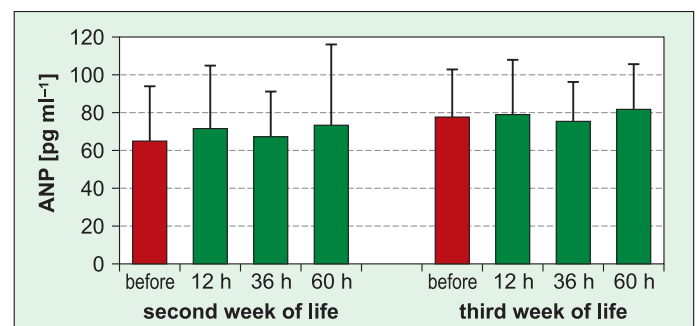


Fig. 1. Mean concentration of atrial natriuretic peptide (ANP) in the blood plasma of two- and three-week-old calves before and after lactose administration

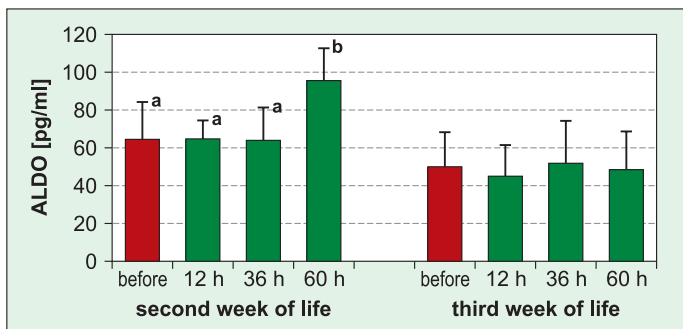


Fig. 2. Mean concentration of aldosterone (ALDO) in the blood plasma of two- and three-week-old calves before and after lactose administration

Explanation: Significant differences between the experimental periods are indicated by different letters.

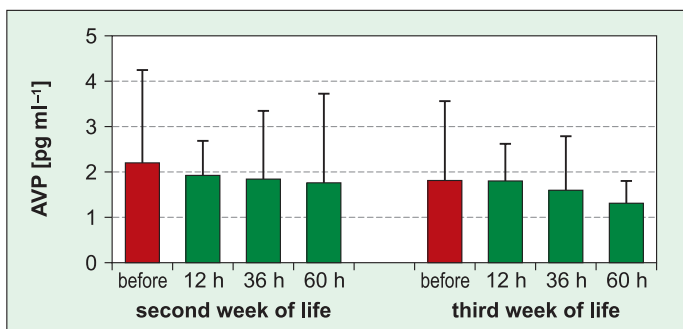


Fig. 3. Mean concentration of arginine-vasopressin (AVP) in the blood plasma of two- and three-week-old calves before and after lactose administration

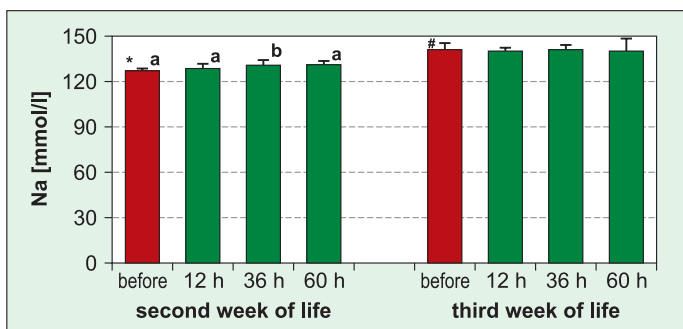


Fig. 4. Mean concentration of sodium in the blood plasma of two- and three-week-old calves before and after lactose administration

Explanation: Significant differences between the experimental periods are indicated by different letters. Significant changes in the concentration of sodium with age are indicated by different symbols (*#).

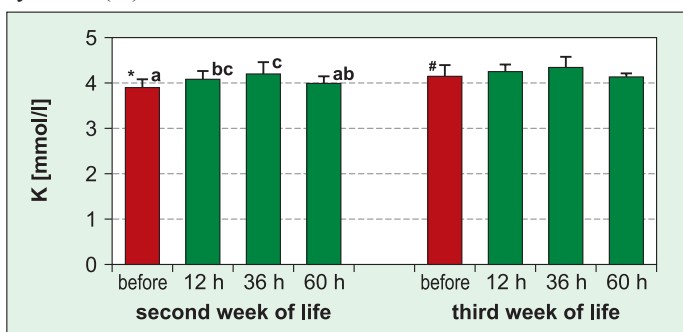


Fig. 5. Mean concentration of potassium in the blood plasma of two- and three-week-old calves before and after lactose administration

Explanation: as in Fig. 4.

(49.59 pg ml⁻¹) (Fig. 2). In two-week-old calves, there were no significant differences in the concentration of aldosterone in blood plasma 12 hours and 36 hours after lactose administration with the milk replacer. However, a statistically significant ($p \leq 0.01$) increase in the concentration of this hormone was noted 60 h after lactose administration (95.58 pg ml⁻¹).

There were no statistically significant differences in the level of aldosterone in three-week-old calves after the excessive lactose administration. It decreased after 12 hours, and then increased, reaching the highest level after 36 hours. After 60 hours, the hormone concentration decreased to a value similar to that observed prior to lactose administration. These changes, although noticeable, were not statistically significant.

The concentration of plasma vasopressin in two- and three-week-old calves decreased in successive days of the experiment (no statistical confirmation), and showed high individual variability (Fig. 3). The concentrations of this hormone in blood plasma on the 13th, 14th, 15th, and 16th days of the experiment were 2.18 pg ml⁻¹, 1.92 pg ml⁻¹, 1.82 pg ml⁻¹, and 1.75 pg ml⁻¹, respectively, whereas on the 20th, 21st, 22nd, and 23rd days of the experiment they amounted to 1.78 pg ml⁻¹, 1.79 pg ml⁻¹, 1.59 pg ml⁻¹, and 1.29 pg ml⁻¹, respectively (Fig. 3). The addition of excessive lactose to the milk replacer did not interfere with the concentration of vasopressin in blood plasma.

The average concentration of sodium in the blood plasma of calves in their second week of life (before the administration of lactose) was 126.68 mmol l⁻¹ (Fig. 4). A statistically significant increase ($p \leq 0.05$) was observed 36 hours after the administration of lactose with the milk replacer (130.70 mmol l⁻¹). It should be noted that an increased concentration of this electrolyte in blood plasma was maintained until the end of the experiment. The mean concentration of sodium in the blood plasma of calves in their third week of life, prior to lactose administration, was 140.97 mmol l⁻¹, which was significantly higher ($p \leq 0.01$) than the concentration of this electrolyte in two-week-old calves (Fig. 4). Lactose administration did not result in any significant changes in the level of this electrolyte in blood plasma.

The concentration of potassium in the blood plasma of two-week-old calves before lactose administration was 3.89 mmol l⁻¹, which was statistically significantly lower ($p \leq 0.05$) than that observed in three-week-old calves (4.15 mmol l⁻¹) (Fig. 5). After the administration of lactose with the milk replacer, both groups of calves showed a gradual increase in the concentration of this electrolyte up to the 3rd day of the experiment, and on the last day of the experiment a decrease was recorded to the value observed before lactose administration. On the 3rd day of the experiment, the level of potassium in the blood plasma of two-week-old calves was 4.19 mmol l⁻¹. These observations had statistical confirmation only in two-week-old calves ($p \leq 0.01$).

The concentration of chlorides in blood plasma was 94.52 mmol l⁻¹ in the second week of life and prior to lactose administration, but increased significantly ($p \leq 0.05$) to 96.81 mmol l⁻¹ in the third week of life (Fig. 6). The provision of excessive dietary lactose in the third week of life resulted in a statistically significant increase ($p \leq 0.05$) in the concentration of this electrolyte to 97.47 mmol l⁻¹, measured on the last day of the experiment.

The average value of the osmotic pressure of blood plasma in two-week-old calves in this study was 279.56 mOsm kg⁻¹ H₂O, and in three-week-old old calves it was 281.70 mOsm kg⁻¹ H₂O (Fig. 7). No statistically significant changes were noted in the osmotic pressure of blood plasma in the two groups, either before or after the administration of lactose in the milk replacer.

The renin-angiotensin-aldosterone system, together with ANP and vasopressin, plays an important role in the regulation of renal function in calves during their first weeks of life. The analysis of changes in the indicators examined in the present study suggests a high efficiency of the systems regulating renal function in calves in their first weeks of life. This is also confirmed by other authors in studies on both healthy calves and calves with diarrhea symptoms (8, 18, 26).

The blood plasma concentration of atrial natriuretic peptide in calves examined here was higher than that observed in adults (1). Individual differences in the concentration of ANP in blood was also observed in calves by Takemura et al. (29) and in human newborns by Ito et al. (14) and by Gemelli (10). Takemura et al. (29) observed the highest concentrations of this hormone after birth (62.7 pmol l⁻¹) and the lowest in the second week of life (11.3 pmol l⁻¹). According to these authors, there is a relationship between blood levels of hormones acting natriuretically and diuretically and the concentration of antagonistic hormones.

In the present study, the blood plasma concentration of aldosterone was lower in three-week-old calves than it was in two-week-olds. In addition, the results indicate that aldosterone is not the only (main) factor regulating the proper concentration of sodium in blood plasma (21). Riad et al. (23) observed a lower level of this hormone, which in ten-day-old calves was 28.9 pg ml⁻¹. In other studies, the authors demonstrated that plasma aldosterone in calves during their first week of life is lower than it is in their mothers during gestation (19, 26). Itoh et al. (15) report in their study that the aldosterone level was 166.3 pg ml⁻¹ at birth and increased to 173.6 pg ml⁻¹ after 14 hours, whereas on the seventh day of life it amounted to 67.0 pg ml⁻¹. Martinerie et al. (17), observed a decrease in the concentration of this hormone in the blood plasma of mice in the postnatal period.

In the current study, the lack of statistically significant changes in the concentrations of vasopressin with age probably stems from large individual differences in the concentration of this hormone in calves.

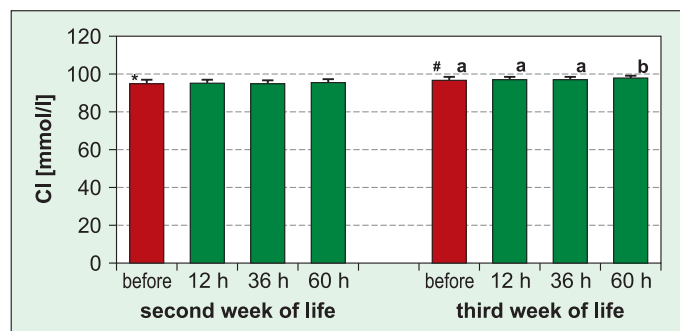


Fig. 6. Mean concentration of chloride in the blood plasma of two- and three-week-old calves before and after lactose administration

Explanation: as in Fig. 4.

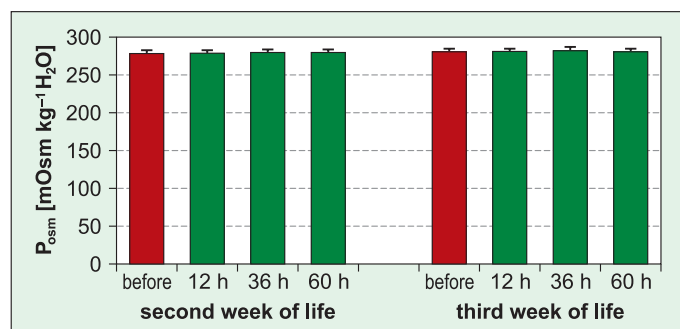


Fig. 7. Mean osmotic pressure of blood plasma in two- and three-week-old calves before and after lactose administration

Individual variation in the plasma concentration of AVP was also observed by Wong et al. (30) in foals. Amadiou-Farmakis et al. (2) demonstrated a reduction in the vasopressin level in calves with age.

The results presented here show a significant role of aldosterone, vasopressin, and ANP in maintaining a stable concentration of the major plasma electrolytes and their osmotic pressure in calves during the post-natal period.

The administration of lactose with the milk replacer caused loose stools with a delicate sour smell and small green slimy smudges, which might indicate a change in the pace of passage of digestive tract contents. This effect was manifested on average 24 hours after lactose administration in all calves.

An excessive loss of water and electrolytes via the gastrointestinal tract causes the dehydration of the body, which stimulates the endocrine system regulating the water and electrolyte balance of the organism. In the blood plasma of dehydrated rats, an increase in the vasopressin concentration and a decrease in the concentration of ANP were observed (3). A decrease in the concentration of ANP in blood during dehydration is designed to prevent further loss of water and electrolytes in urine. In the experimental calves, there was no statistically significant change in the concentration of ANP after the administration of an additional lactose dose with the milk replacer. This indicates that the mechanisms controlling water and electrolytes in calves were working properly during this period. The absence of change in plasma ANP levels was also

observed during mild dehydration in children with gastroenteritis (16).

In both age groups of calves, there was no change in the concentration of arginine-vasopressin in blood plasma after the provision of excessive lactose in the diet. It is known that the stimuli for VP release are an increased molality of plasma and a decreased volume of circulating blood. In the present experiment, in two- and three-week-old calves no change was observed in the molality of blood plasma. A study by Hayashi et al. (12) on chronically dehydrated rats showed an increase in AVP gene transcription in response to decreases in plasma volume, but not to increases in plasma osmolality. A study by Safwate et al. (26) showed that in calves suffering from diarrhea, plasma levels of arginine-vasopressin and aldosterone were 10-15 times as high as those observed in controls.

In the blood of two-week-old calves, the aldosterone concentration remained at a very even level prior to the administration of excessive dietary lactose, as well as 12 and 36 hours afterwards. A statistically significant increase in the concentration of ALDO, observed 60 hours after lactose administration, was most likely a response to the significant increase in the concentration of potassium in blood at 12 and 36 hours after the administration of lactose with the milk replacer. An increased potassium level was also observed in two-week-old diarrheic calves by Seifi et al. (27). According to Roussel (24), an increased concentration of this electrolyte in the blood of calves during diarrhea is caused by the transport of this element from intracellular to extracellular fluid in response to acidosis. However, it should be noted that the physiological movement of potassium between aqueous spaces takes place during the short-term regulation of the concentration of this element. This relatively rapid process does not always protect against excessive levels of potassium in the extracellular space. Potassium distribution is controlled mainly by hormones. Potassium absorption by the cells is stimulated by aldosterone, insulin, adrenaline, and alkalosis (9). It is known that an increase in the potassium level in blood plasma stimulates a release of aldosterone from the glomerulosa cells of the adrenal cortex. When aldosterone reaches the kidney, it activates the principal cells of the cortical collecting tubule (CCT) to secrete potassium (20). A decrease observed 60 hours after excessive lactose administration in the concentration of potassium in the blood plasma of two-week-old calves was most likely due to an increased urinary excretion of this element in response to an increased concentration of aldosterone at that time. This significant increase in blood levels of ALDO in calves appeared to be necessary due to the occurrence of physiological aldosterone resistance in neonatal animals (17). In humans and mice, low renal mineralocorticoid receptor (MR) expression was found at birth, which was followed by a significant increase in the postnatal period (17). This could explain the lack

of a statistically significant increase in ALDO in the blood of three-week-old calves 60 hours after lactose administration, despite a similar trend of changes in the concentration of potassium observed in the blood of two-week-old animals. In three-week-old calves after lactose administration, there was no change in the concentrations of sodium, potassium, aldosterone, or ANP.

It is difficult to unambiguously explain the increase in the concentration of sodium in the blood plasma of two-week-old calves after excessive lactose administration, considering that the aldosterone concentration was stable in the first three days of the experiment, and the ANP level was elevated after lactose administration. The increase in the concentration of this electrolyte may have been due to increased levels of glucose (derived from the breakdown of lactose) in the gastrointestinal tract, which created an additional source of sodium absorption from the gastrointestinal tract (through the sodium-glucose cotransporter).

It seems that indirectly obtained results suggest a high maturity of the proximal tubule and loop of Henle in calves compared with newborns of some other mammalian species. For instance, it is known that in an immature kidney of human neonates and infants, proximal tubular transport is less efficient than it is in adults. Therefore, sodium resorption in the distal parts of the nephron triggered by aldosterone plays a very important role in the proper balance of salt in human neonates and infants (6, 17).

Seifi et al. (27) observed a significantly lower chlorides concentration in the blood serum of two-week-old diarrheic calves compared with healthy animals, whereas Guzelbektes et al. (11) observed a decrease in the concentration of this electrolyte only in severely dehydrated calves. In the current experiment, there was no significant change in the concentration of chlorides during the excessive supply of lactose in the diet. A statistically significant increase in the concentration of blood chlorides in three-week-old calves in the final day of the experiment was the result of age rather than of lactose.

Despite changes in the concentration of blood potassium after the administration of lactose with the milk replacer, plasma osmolality was stable in calves of both age groups. This may indicate a hormonal efficiency in the regulation of water and electrolyte balance in calves with diarrhea induced by an excessive amount of lactose in the diet.

In conclusion, in calves, during the first weeks of life, the activity of factors regulating renal functions, in conjunction with relatively stable blood levels of the main electrolytes (sodium, potassium and chlorides) and the osmotic pressure of blood plasma, indicates the functional maturity of the kidneys in healthy calves and in calves manifesting slight symptoms of diarrhea. These results also suggest that the mechanisms involved in the regulation of water and electrolyte homeostasis in these calves are efficient.

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