

ASSESSMENT OF TREATMENT EFFICIENCY OF NEONATAL CALF DIARRHOEA AS PER QUANTITATIVE CHANGES OF BLOOD CALCIUM, UREA, ALBUMINS AND LEUKOCYTES

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Abstract. The aim of the research is to evaluate the clinical significance of the total amount of calcium, urea, total proteins and blood leukocytes in the blood serum of diarrhoeic calves and to determine the efficiency of rehydration, anti-inflammatory and detoxifying therapy.

The experiment was carried out on 60 calves from birth to 10 days of age. All calves with symptoms of neonatal diarrhoea in 1-4 days after birth were divided into three groups according to the selected treatment method. The first group (I) was treated by applying rehydration therapy with electrolyte solutions *per os** and injections of ketoprofen (100 mg/ml), injecting 3 mg of ketoprofen for 1 kg body weight i.m. once per day three days in a row. The second group (II) was treated by applying rehydration therapy *per os* with electrolyte solutions once per day three days in a row. The third group (III) was treated by applying rehydration therapy with electrolyte solutions *per os*, i.e. injections in a jugular vein using a preparation stabilizing metabolism of mineral substances and carbohydrates* injecting 250 ml i.v. once per day.

The impact of rehydration, anti-inflammatory and detoxifying therapy on calves' blood indicators reports statistically reliable variations in leukocyte, calcium, albumin and urea concentrations ($P < 0.05$). Calves' recovery can be judged based on variations of these indicators.

Ten days after the treatment the blood of calves which were treated by applying rehydration therapy *per os* with electrolyte solutions and anti-inflammatory therapy in combination with anti-inflammatory non-steroid preparations showed a statistically reliable decrease of the amount of leukocytes. At the same time on the fifth and tenth day after the treatment the blood serum of the calves of this group contained statistically reliably ($P < 0.05$) higher calcium concentration and statistically reliably ($P < 0.05$) lower urea concentration. Due to the application of this therapy the recovery of calves' digestion function was the fastest (based on the fecal consistency). This kind of diarrhoea treatment therapy is applied in practice.

Keywords: calves, diarrhoea, calcium, leukocyte, albumin

Introduction. Neonatal calf mortality is one of the most common animal health concerns for dairy industry. Diarrhoea has been recognized as an important condition of animals because of high morbidity and mortality and has been ranked as one of the main six causes of all deaths from infectious diseases (Dhama *et al.*, 2009). Diarrhoea and respiratory disease problems are the most important infirmities of neonatal calves, causing the greatest economic loss in this age group in both dairy and beef herds (Callan and Gary 2002). Diarrhoea in calves during the early age has mixed etiology and, irrespective of etiological agent the physiological conditions, age, breed, sex, stress, nutritional status and treatment, always affects mortality (Kumar *et al.*, 2012). According to Larson and Tyler (2005) and Maillard (2006), the most frequent cause of diarrhoea in calves is low quality colostrum. The quality of the latter depends on a number of factors, e.g. sanitary and zoo-hygienic conditions, feeding and breeding of in-calf cows, their morbidity of various diseases (Gulliksen. *et al.*, 2007). Lassen and Järvis, (2009) ascertained that eimeria and cryptosporidium are widely spread among livestock of various age groups in the Lithuanian farms. These parasites frequently cause diarrhoea.

There are six main known infectious agents of diarrhoea in neonatal calves. They include enterotoxigenic *Escherichia coli* (ETEC), rotavirus,

coronavirus, cryptosporidium parvum (*C. parvum*) type 2, and salmonella enterica (*S. enterica*) subtype enterica serovars. The number of coliform bacteria in the intestines of diarrheic calves frequently increases and functional intestinal disorders, morphological damages and endotoxemia occur (Jiminez *et al.*, 2007, Berchtold and Constable 2008, Foster *et al.*, 2009). Among the infectious agents, Rotavirus, Coronavirus, *Cryptosporidium*, and *Escherichia coli* collectively are responsible for 75–95 % of infection in neonatal calves worldwide, and especially rotavirus accounts for about 27–36 % (Gumusova *et al.*, 2007; Uhde *et al.*, 2008; Dhama *et al.*, 2009). Rotaviruses are important enteric pathogens of humans and animals worldwide (Tate *et al.*, 2009; Martella *et al.*, 2010;). Rotavirus-associated enteritis is a common problem in calves. The affected young calves may die as a result of severe dehydration or secondary bacterial infections (; Dhama *et al.*, 2009).

Calves mostly die of dehydration, enterotoxaemia and arrhythmia, which develop as a result of low potassium levels (Razzaque *et al.*, 2009). Calves that have diarrhoea are in a state of negative energy balance (Geof, . Smith, 2009).

As a result of dehydration, calves can lose weight and die, thus increasing rearing costs (Timmerman *et al.*, 2005). According to Gottardo and Mattiello (2002), in order to compensate the loss of liquids and avoid

hypovolemic shock, a calf should receive liquids as per ratio 70 ml/kg its body weight.

According to the available literary data, electrolyte solutions are the most efficient treatment of diarrhoea in calves, however electrolytes do not affect the cause of diarrhoea (McDonough 1994). Based on the data of researches made by Roussel et al., (1993), after intravenous administration of 6 mg/kg ketoprofen twice every four hours the symptoms of diarrhoea in calves lessened. Good treatment results were also achieved by Berchtold Constable (2008) who treated calves with B-group and fat-soluble vitamins (Russell, Roussel2007).

Despite the fact that the results of laboratory tests are used to evaluate the success of the treatment, the opinion regarding the significance of biochemical blood parameters in treatment of calves is not unanimous. The treatment time of sick calves or treatment effect was noted for any leukocyte or biochemical variable of biological significance (Cobb et al., 2014).

The laboratory tests provide aid in making a diagnosis, determining a prognosis, formulating a treatment plan, and monitoring the response. The serum chemistry profile is readily available to the veterinarian surgeons.

The treatment of diarrheic calves is required at the earliest with the attention to restore all physiological parameters. However, it is not always possible to perform routine blood profile at the door step of owner prior to start the treatment. Under such circumstances, the information of haematology may be of great value in the investigations of animal diseases and restoration of normalcy (Mahima et al., 2013). Moreover, the often ignored imbalance of minerals and dehydration are the major cause of calves' mortality (Malik et al., 2012).

The aim of the research is to evaluate the clinical significance of the total amount of calcium, urea, total proteins and blood leukocytes in the blood serum of diarrheic calves and to determine the efficiency of rehydration, anti-inflammatory and detoxifying therapy.

Methods and materials. The research was implemented from the 1st of November 2009 to the 1st of November 2013 in an agricultural company with 750 milk cattle heads including 450 milkers. Each month there were 40–50 calving cows. There was no calving seasonality. The farm applied cold rearing conditions. Dry cows were divided into two groups according to the calving period and different feeding rations were applied. The body condition score of calving cows was 3.5–3.75 (on a five point scale). After the birth calves were separated from cows. Antiseptic treatment with 5 % iodine solution was applied to the umbilical cord and 30 minutes later the calves were given 2 litres of their mothers' colostrum. The calves were reared in individual cabins in outdoor conditions until they reached 2 weeks of age.

The experiment was carried out on 60 calves from birth to 10 days of age. All calves with symptoms of neonatal diarrhoea 1–4 days after birth were divided into three groups according to the selected treatment method (Table 1). The health condition of the calves was

observed for 10 days after diagnosis of neonatal diarrhoea. The fecal consistency and colour as well as the dehydration level were evaluated every day, in the morning after feeding.

Table 1. **Studied groups**

Number of the group	Applied treatment	Number of cattle heads (n)
I	Rehydration therapy <i>per os</i> and ketoprofen (100 mg/ml)	20
II	Rehydration therapy <i>per os</i>	20
III	Rehydration therapy <i>per os</i> and a preparation stabilizing metabolism of mineral substances and carbohydrates	20

The first group (I) was treated by applying rehydration therapy with electrolyte solutions *per os** and injections of ketoprofen (100 mg/ml) to reduce the cortisol concentration, injecting 3 mg of ketoprofen for 1 kg body weight i.m. once per day three days in a row. The second group (II) was treated by applying rehydration therapy *per os* with electrolyte solutions once per day three days in a row. The third group (III) was treated by applying rehydration therapy with electrolyte solutions *per os*, i.e. injections in a jugular vein using a preparation stabilizing metabolism of mineral substances and carbohydrates** injecting 250 ml i.v. once per day.

Blood samples were collected into vacuum test-tubes without and with a preservative before the treatment and five and ten days after the treatment (BD Vacutainer, England). Biochemical and morphological blood tests were performed at the Clinical Research Laboratory of the Faculty of Non-infectious Diseases of the Veterinary Academy of the Lithuanian University of Health Sciences. The concentration of the total content of albumins (Alb)), urea (U) and calcium (Ca) in the blood serum was measured by a computerized analyzer Hitachi 705 (Hitachi, Japan) using DiaSys (Diagnostic Systems GmbH, Germany). The morphological blood test was performed from preserved blood by a blood morphological composition analyzer Abacus using the EDTA preservative to measure the total amount of leukocytes.

Fecal scoring. Fecal scoring of fecal fluidity, consistency, odour, and days scoured was conducted daily in the morning (8 AM). Fecal scores based on a four-point scale were recorded using the procedure of Larson et al. (1977). The scoring was as follows: for fecal fluidity, 1 = normal, 2 = soft, 3 = runny, or 4 = watery. A scour day was recorded if the fecal fluidity was 3 or 4; calves were considered recovered with the fecal consistency equalling to 1 or 2 points.

The test data were processed using the SPSS statistical package (SPSS for Windows 15.0, SPSS Inc., Chicago, IL, USA, 2006). The data were considered reliable from the statistical point of view when $p < 0.05$.

*Proteins – 14.0 g; fat – 7.0 g; natural fibre – 4.0 g;

dextrose – 36,0 g; calcium – 0.67 g; phosphorus – 0.3 g; magnesium – 0.4 g; potassium – 0.4 g; natrium – 2.5 g; chlorine – 3.0 g; vitamin A – 5000 TV; vitamin D – 500 TV; vitamin E – 5.0 mg; vitamin K – 0.25 mg; vitamin B1 – 1.48 mg; vitamin B2 – 0.96 mg; niacin – 5.0 mg; vitamin B6 – 1.0 mg; vitamin B12 – 0.05 mg; calcium pantothenate – 1.96 mg; folic acid – 0.03 mg; biotin – 0.01 mg; choline chloride – 10.0 mg; selenium – 0.05 mg; manganese – 4.32 mg; zinc – 4.86 mg; copper – 1.12 mg; betaine – 200 mg.

**Carnitine hydrochloride (equivalent to l-Carnitine - 500.0 mg) - 613.3 mg; Thiocctic acid - 20.0 mg; Pyridoxine hydrochloride - 15.0 mg; Cyanocobalamin - 3.0 mg; D,L-acetylmethionine - 2.0 g; l-Arginine - 240.0 mg; l-Ornithine hydrochloride (equivalent to l-Ornithine - 120.0 mg) - 153.2 mg; l-Citrulline - 120.0 mg; l-Lysine hydrochloride (equivalent to l-Lysine - 50.0 mg) - 62.5 mg; Glycine - 150.0 mg; Aspartic acid - 150.0 mg; Glutamic acid - 150.0 mg; Fructose - 5.0 g; Sorbitol - 8.0 g; Excipients up to 100.0 ml.

Results and discussion. 95 % of the examined calves clinically recovered (no diarrhoea symptoms) during 10 days after treatment. The fecal consistency evaluation in a four point scale using the procedure suggested by Larson et al. (1977) showed that the digestion function was more quickly restored in those calves which were treated with rehydration and anti-inflammatory therapy (Fig.1). The average duration of the treatment was three days to the disappearance of clinical symptoms.

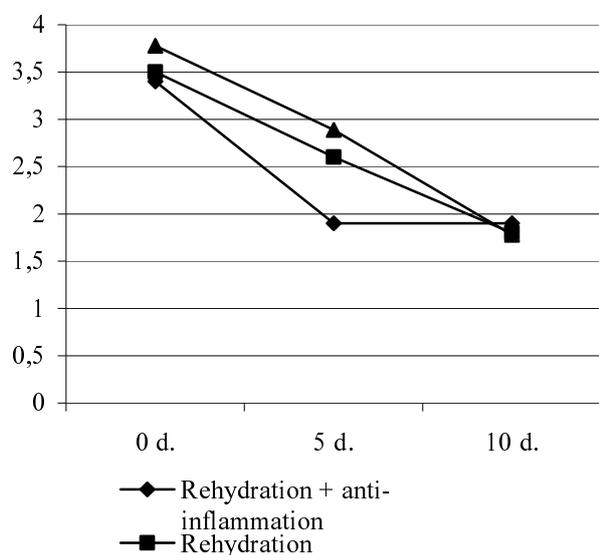


Fig. 1. Fecal scoring

From the diagnostic point of view, blood is an important object of clinical researches since various blood parameters change in case of illness (Ginsburg and Haga, 2006). The study showed that during the research period the concentrations of leukocytes, calcium, albumins and urea statistically reliably varied among the examined

groups. Calves with diarrhoea lose large amounts of fluid and electrolytes with attendant dehydration and acidosis that requires rehydration therapy (Groutides and Michell, 1990).

The data in Fig. 2 show that the levels of leukocytes changed evenly during almost the whole research period. Five days after the treatment (5 d.), no significant changes in the levels of leukocytes were observed. After ten days of the treatment (10 d.), a statistically reliable decrease of the number of leukocytes ($P < 0.05$) was recorded in all the groups. During the same period, a statistically reliable decrease in the amount of leukocytes was recorded in the group which was treated applying rehydration and anti-inflammatory cure (group I), ($P < 0.05$), (Fig.1). Diarrhoea is one of the main causes of calf mortality and morbidity and also of economic loss in the cattle industry. It is known that calves are affected by diarrhoea more severely in the early stage of lactation period (Malik et al., 2012). It is assumed that at this time calves are suffering from certain unhealthy conditions caused mainly by immaturity of the immune system.

The leukocyte concentration in calves' blood is higher than in adult cattle blood. The study also recorded that the leukocyte concentration in calves' blood varies more than the leukocyte concentration in adult cattle blood (Kraft, 1999). Various types of leukocytes have a different life span. Blood performs only the transportation function to the inflamed area (Kraft et al., 1999). The leukocyte concentration in blood increases mostly due to inflammatory processes or stress. The leukocyte concentration increases from birth to the tenth week of age.

Leukocytosis can be described as a consequence of dehydration and endotoxemia (Kasari et Naylor, 1986). Ten days after the treatment, the observed decrease of the levels of leukocytes in all the groups can be described as the decrease of dehydration and endotoxemia, as all the groups were treated by applying rehydration therapy.

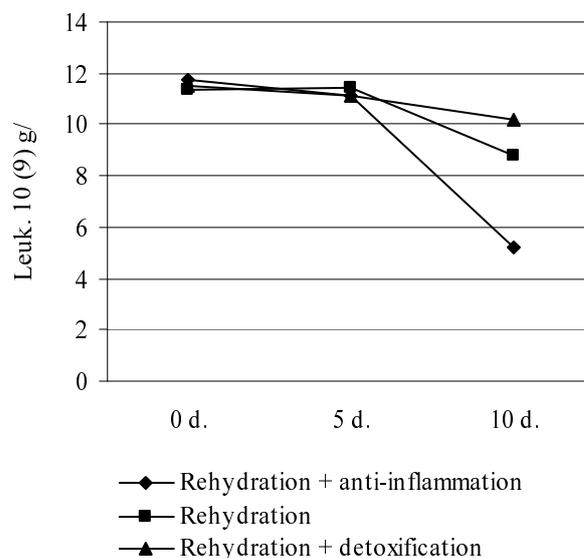


Fig. 2. Levels of leukocytes in the examined calves' blood

The study indicates that on the fifth and tenth days after the treatment, the calcium concentration was statistically reliably ($p < 0.05$) higher in the blood serum of those calves which were treated by applying rehydration and anti-inflammatory therapy (Fig. 3). However during the research period, a trend of the calcium concentration increase in the blood serum in all the groups of the examined calves was observed. The major amount of calcium (about 90 %) is deposited in bones and teeth. Calcium plays an important role enzyme and hormone activation, blood coagulation, transmission of nerve impulse and muscle contractions. 55 % of calcium which appears in blood serum has the ionized form and is a biological active part of calcium. Ionized calcium largely depends on the blood pH. If the pH decreases, the ionized calcium levels increase. Part of calcium in the blood serum is associated with albumins (40 %) and organic acids (5 %) (Kraft, 1999). The majority of calcium is absorbed in the small intestine. The calcium concentration in the blood serum of neonatal calves is 3.35 ± 0.27 mmol/l. Six hours after birth, the calcium concentration decreases to 2.41 ± 0.18 mmol/l and in the next few days it changes marginally. Mohri et al. (2007) reports that in the first two weeks the calcium concentration decreases and later it gradually increases. However, although total calcium concentrations in serum remained within the reference range of values for adult cattle (Kaneko et al. 2008), ionized calcium levels were below the reference for this species (Kaneko et al. 2008). Neonatal calves need maternal immunological assistance in the first hours after birth, provided by colostrum intake, since they have a higher likelihood of succumbing to infections that are innocuous to adult animals, so neonates that suffer failure of passive transfer of maternal immunoglobulin may be at increased risk for disease (Barrington et al., 2002). In veal calves fed reconstituted milk replacers as the sole source of nutrition, it has been observed that high calcium intakes cause a reduction of fat digestibility (Yuangklang et al., 2004). Rat studies indicate that the inhibitory effect of calcium on fat digestibility can be explained by an increase in the amount of insoluble calcium phosphate sediment in the intestinal digesta (Brink et al., 1992).

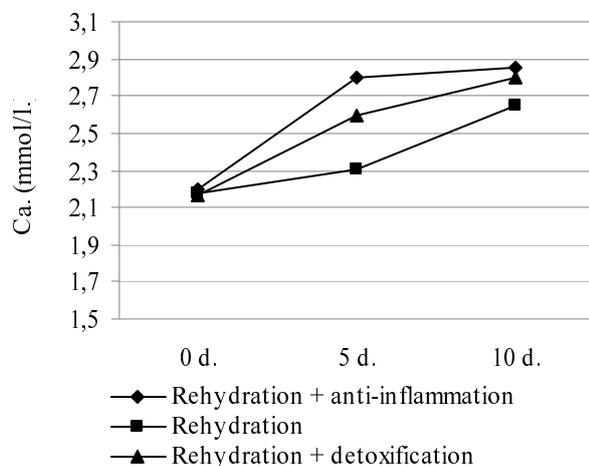


Fig 3. Calcium concentration variation in examined calves' blood

During the research period, five and ten days after the treatment, the urea concentration was statistically reliably ($p < 0.05$) lower in the blood of those calves which were treated by applying anti-inflammatory and rehydration therapy (Fig. 4). The urea concentration in blood serum depends on nutrition and kidney diseases (Kraft et al., 1999;). The increased urea concentration in blood serum indicates intensification of decomposition of proteins due to chronic diarrhoea. Colostrum intake does not affect the variation of the urea concentration (Steinhardt and Thielscher 2000). The urea concentration in the blood serum in calves suffering from diarrhoea for a long time is higher than the urea concentration in the blood serum of healthy calves (Maach et al., 1992). This can be related to dehydration processes and acid-alkali balance disorders (Maach et al., 1992).

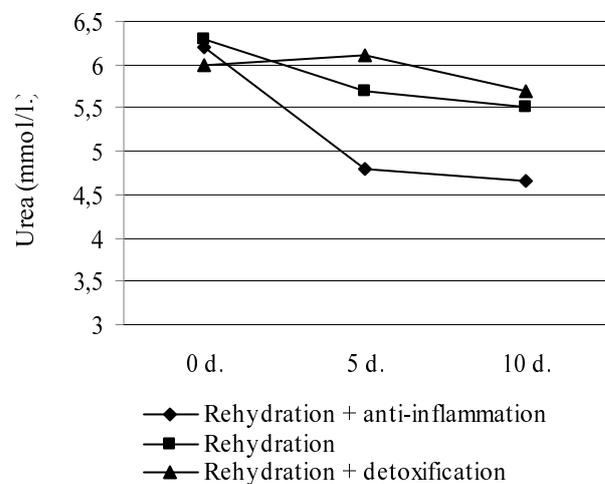


Fig. 4. Urea concentration variation in examined calves' blood

During the research period, the albumin concentration was gradually increasing and on the tenth treatment day it was statistically reliably higher compared to the albumin concentration at the beginning of the treatment ($P < 0.05$); however among the groups of the research it did not differ statistically reliably (Fig. 5). The low albumin concentration in blood indicates the disease severity, the increased concentration indicates recovery (Michael et al., 2003). The albumin concentration in blood serum may decrease due to the hepatocellular disorder, which occurs during severe inflammation and possible sepsis (Mendez et al., 2003). In case of infections (e.g. pneumonia, gastroenteritis, sepsis), the cytokine concentration in blood increases considerably, which affects the decrease of the albumin concentration (Song et al. 2004).

After colostrum intakes, the albumin concentration decreases and remains stable until calves grow up (Muri et al., 2005). The albumin concentration depends on calves' nutrition and their liver functions. Albumins are synthesized in the liver and in case of liver disorders the albumin concentration decreases (Steinhardt and Thielscher, 2000). The lack of albumins may occur due to

prolonged diarrhoea, which results in liver disorders (Jazbec, 1990). The albumin concentration depends on a calf's age (Steinhardt and Thielscher, 2000). A decline in serum albumin levels in the first two days of life had already been reported by Kurz and Willett (1991); after these moments, the mean concentration of albumin increased slowly from birth to 30 days of age in both groups, which was also reported by Ježek et al. (2006).

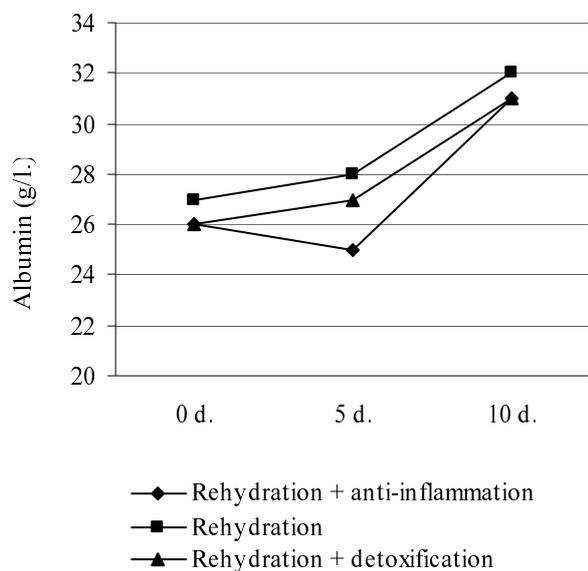


Fig. 5. Albumin concentration variation in examined calves' blood

Furthermore, the knowledge of the changes in the serum biochemical profile of calves, allied to other data obtained from the animals, may be useful for establishing the initial baseline parameters for a patient, formulating a problem or rule-out list, confirming a diagnosis, determining the prognosis, planning therapeutic options, and monitoring response to treatment (Russel and Roussel 2007).

Conclusions

The impact of rehydration, anti-inflammatory and detoxifying therapy on calves' blood indicators reports statistically reliable variations in leukocyte, calcium, albumin and urea concentrations ($P < 0.05$). Calves' recovery can be judged based on variations of these indicators.

Ten days after the treatment, the blood of calves which were treated by applying rehydration therapy *per os* with electrolyte solutions and anti-inflammatory therapy in combination with anti-inflammatory non-steroid preparations showed a statistically reliable decrease of the amount of leukocytes. At the same time, on the fifth and tenth day after the treatment, the blood serum of the calves of this group contained statistically reliably ($P < 0.05$) higher calcium concentration and statistically reliably ($P < 0.05$) lower urea concentration. Due to the application of this therapy the recovery of calves' digestion function was the fastest (based on the fecal consistency). This kind of diarrhoea treatment therapy is applied in practice.

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