J. Elem. s. 307–315 DOI: 10.5601/jelem.2013.18.2.10

DISTURBANCES IN THE ACID-BASE AND ELECTROLYTE BALANCE AND CHANGES IN SERUM MINERAL CONCENTRATIONS IN CALVES DIAGNOSED WITH NUTRITIONAL MUSCULAR DYSTROPHY

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Abstract

Selenium is an essential nutrient, which is crucial for proper body function. Its role is complemented by vitamin E. Nutritional muscular dystrophy (NMD) is one of the main disorders caused by a selenium deficiency. NMD most often affects calves at the age of 4 to 6 weeks. The study was performed on 40 Holstein-Friesian (HF) calves divided into two groups of 20 animals each. Control group calves were administered an IM injection of selenium and vitamin E on the second day of life. The experimental group comprised calves with symptoms of NMD. Samples of the biceps femoris muscle were collected from six animals in each group for histopathological analyses to confirm changes in muscle parameters. Blood samples were obtained from all animals on three different dates. The following blood parameters were determined in laboratory analyses: pH, pCO_2, pO_2, HCO_3^-, BE, O_2SAT, the concentrations of Na^+, K^+, Cl^-, and Ca and P levels. A drop in pH, an increase in the partial pressure of carbon dioxide, a decrease in the partial pressure of oxygen, a significant decrease in bicarbonate ion concentrations and hemoglobin oxygen saturation as well as a base deficit were reported in the group of calves demonstrating symptoms of NMD. The above changes point to the development of uncompensated metabolic acidosis due to increased levels of pyruvic acid and lactic acid produced as a result of anaerobic processes that accompany muscle fiber degeneration. Minor fluctuations in sodium and chloride levels were observed throughout the experiment, but their concentrations remained within the norm in animal groups. Potassium levels were significantly hi-

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gher in the experimental group than in the control group. The serum concentrations of inorganic phosphorus and calcium were within the reference range in both groups. In calves, NMD leads to disruptions in the acid-base equilibrium and the electrolyte balance, which are manifested by uncompensated metabolic acidosis and hyperkalemia. Significant changes in calcium and phosphorus levels are not observed in the blood serum of calves affected by NMD.

Key words: NMD, calves, acid-base balance, electrolytes, serum mineral concentrations.

ZABURZENIA RÓWNOWAGI KWASOWO-ZASADOWEJ I ELEKTROLITOWEJ ORAZ ZMIANY STĘŻENIA WSKAŹNIKÓW MINERALNYCH SUROWICY W PRZEBIEGU POKARMOWEJ DYSTROFII MIĘŚNI CIELĄT

Abstrakt

Selen jest biopierwiastkiem odgrywającym istotną rolę w prawidłowym funkcjonowaniu ustroju. W organizmie jego działanie jest ściśle powiązane z witaminą E. Jednym z podstawowych zaburzeń powodowanych przez niedobór selenu jest pokarmowa dystrofia mięśni (PDM). Najczęściej na PDM zapadają cielęta w wieku 4-6 tygodni. Badania przeprowadzono na 40 cielętach rasy h-f podzielonych na dwie grupy po 20 zwierząt każda. Cielętom z grupy kontrolnej w 2. dniu życia podano domięśniowo preparat zawierający witaminę E i selen. Grupę doświadczalną, stanowiły cielęta wykazujące objawy pokarmowej dystrofii mięśni. Od 6 cieląt z każdej grupy pobrano bioptaty z mięśnia dwugłowego uda w celu wykonania badania histopatologicznego potwierdzającego zmiany w mięśniach. Od wszystkich cieląt pobrano trzykrotnie krew do badań laboratoryjnych. Oznaczono następujące parametry: pH, pCO_2, pO_2, HCO_3^-, BE, O_2SAT, stężenie Na^+, K^+, Cl^- oraz stężenie Ca i P. W grupie cieląt z objawami pokarmowej dystrofii mięśni wystąpił spadek pH, wzrost ciśnienia parcjalnego dwutlenku węgla, spadek ciśnienia parcjalnego tlenu, statystycznie istotny spadek stężenia jonów wodorowęglanowych i wysycenia hemoglobiny tlenem oraz pojawił się niedobór zasad. Zaobserwowane zmiany mogą sugerować rozwój kwasicy metabolicznej niewyrównanej związanej z wyższym poziomem kwasu pirogronowego i mlekowego powstającego w wyniku procesów beztlenowych towarzyszących rozpadowi włókien mięśniowych. Stężenia sodu i chlorków nieznacznie wahały się w trakcie trwania doświadczenia i pozostawały w granicach wartości referencyjnych, zarówno u cieląt grupy badanej, jak i kontrolnej. Poziom potasu w grupie doświadczalnej był istotnie wyższy w porównaniu z cielętami z grupy kontrolnej. Stężenie wapnia i fosforu nieorganicznego w surowicy cieląt z obu grup utrzymywało się w granicach norm referencyjnych. Pokarmowa dystrofia mięśni cieląt przebiega z zaburzeniami równowagi kwasowo-zasadowej i gospodarki elektrolitowej objawiającymi się kwasicą metaboliczną niewyrównaną i hiperkaliemią. W przebiegu pokarmowej dystrofii mięśni cieląt nie obserwuje się istotnych zmian w stężeniu wapnia i fosforu w surowicy.

Słowa kluczowe: PDM, cielęta, równowaga kwasowo-zasadowa, elektrolity, wskaźniki mineralne.

INTRODUCTION

Selenium is an essential trace element, which is necessary for proper body function. The element participates in various metabolic pathways in the body, and its role is complemented by vitamin E and sulfur-containing amino acids. The first references to the biological role of selenium, a component of glutathione peroxidase, were made in the 1970s. Since then, more than 30 selenium-containing proteins, mostly enzymes, have been identified (GHANY-HEFNAWY, TORTORA-PEREZ 2009). To date, scientists have been successful in describing the detailed biological function of only 10 selenoproteins from the above group. Low levels of selenium and/or vitamin E are the cause of nutritional muscular dystrophy (NMD, *dystrophia musculorum enzootia*). This condition, also known as white muscle disease, induces hyaline degeneration of skeletal muscle cells in various parts of the body, including the diaphragm and the heart muscle. NMD most often affects calves at the age of 4 to 6 weeks.

The acid-base balance is a condition where the optimal concentrations of hydrogen ions are maintained in intercellular and extracellular spaces as a result of various homeostatic control mechanisms in the body. Disturbances in that balance can be a primary source of disease, but they generally accompany other bodily dysfunctions, adding to their complexity and hindering the correct diagnosis and therapy. The diagnosis and treatment of acid-base imbalances requires the determination of pH, pCO_2, HCO_3 concentrations and the partial pressure of oxygen (pO_2). The above parameters are measured in arterial blood or arterialized capillary blood to fully identify the degree of blood oxidation and alveolar gas exchange (STOPYRA et al. 2012). In medical and veterinary practice, the acid-base balance is usually determined in samples of venous blood. This procedure is burdened by error due to hemostasis, which occurs when pressure is applied to the vein, and the activity of skeletal muscles during immobilization, which can produce false acidosis results. The above method also prevents precise determination of the partial pressure of carbon dioxide. Venous blood samples are generally used in evaluations of disorders that are not accompanied by complications (POMIANOWSKI et al. 2004).

Electrolytes are substances which, when dissolved in water, conduct electrical impulses essential for regulating vital bodily functions. They are charged ions, positive cations (sodium, potassium, calcium and magnesium) and negative anions (bicarbonates, chloride and phosphorus ions, sulfates, organic acids and protein components). Electrolytes control water distribution in the body, absorption, diffusion, the acid-base equilibrium, nerve and muscle function. An electrolyte balance is required for the body to perform its complex functions during significant changes in the diet, metabolism and kidney function (ROBERTS 2005).

MATERIAL AND METHODS

The study was performed on 40 HF calves. The control group comprised 20 healthy animals which were administered a single IM injection of selenium and vitamin E comprising tocopherol acetate – 50 mg and sodium selenate – 0.5 mg at a dose of 8 ml/calf on the second day of life. The experimental group consisted of animals demonstrating symptoms of NMD. Blood samples were collected from all animals on three different dates, at seven-day intervals, starting on day 5. Blood was sampled from the external jugular vein in the morning. On day 19, samples of the biceps femoris muscle were collected from six animals in each group to confirm changes in muscle parameters. The place of incision was shaved, disinfected and anesthetized by infiltration with a lignocaine solution. Muscle samples were obtained by scalpel incision of 0.8 x 0.8 cm with a depth of 0.7 cm. Muscle sections were immersed in a saline solution for 10 minutes, neutralized with 10% formalin and embedded in paraffin. Microtome sections were stained with hematoxylin and eosin (HE).

The acid-base balance and electrolyte concentrations in venous blood were determined with ion-selective electrodes using a RapidLab 348 analyzer (Siemens). The following parameters were identified: pH, the partial pressure of carbon dioxide (pCO_2), the partial pressure of oxygen (pO_2), bicarbonate ion concentrations (HCO_3^-), base excess (BE), hemoglobin oxygen saturation (O_2SAT) and the concentrations of Na^+, K^+ and Cl^-.

The content of minerals, calcium and phosphorus was determined using a Cormay Accent 200 automatic biochemical analyzer. Serum calcium levels were determined by the Arsenazo III colorimetric method (Cormay diagnostic kit), and phosphorus concentrations were measured by the colorimetric molybdenum ion test (Cormay diagnostic kit).

The results of laboratory tests were presented in SI units and processed in the Statistica 9.0 application. The significance of differences between test results and groups was determined by ANOVA at $p \leq 0.01$.

RESULTS AND DISCUSSION

Symptoms of NMD were not observed in experimental group calves (not administered the selenium and vitamin E supplement) on the first days of life. Signs of nutritional muscular dystrophy were reported in all experimental animals between day 10 and 17. The initial symptoms were non-specific, and they were manifested by apathy, lethargic behavior, decreased appetite and reluctance to move. They were followed by tremor in hind limbs, incorrect posture with widely spread limbs, a hunched-up spine, stilted gait and, in some cases, an elevated respiratory rate and cough. Several animals remained recumbent and their body temperature exceeded 40°C. Clinical symptoms of NMD were not observed in control group calves.

Numerous muscle fibers with hyaline degeneration of the sarcoplasm, granular degeneration of the sarcoplasm and striatal atrophy were observed in all biceps femoris samples stained by HE (Figure 1). Similar morphological changes in the muscles of ruminants affected by NMD were reported by HAFNER et al. (1996), BEYTUT et al. (2002), SOBIECH (2009) and TUNCA et al. (2009). HE-stained muscle samples from healthy calves showed correctly formed muscle fibers with clear striation.

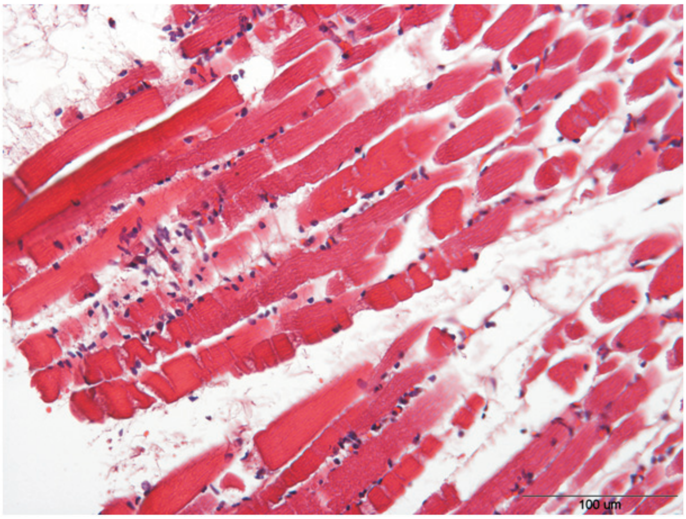

Fig. 1. Muscle of calf with symptoms of NMD. Muscle fibers with sarcoplasma degeneration (long arrow) and disappearance of cross-striation (short arrow) HE staining, magnification x 240

An analysis of the acid-base equilibrium in control and experimental animals did not reveal significant differences in test I (Table 1). The examined parameters were within the reference range. Numerous authors (LISBOA et al. 2002, TAVERNE 2008) have observed symptoms of mixed metabolic and respiratory acidosis in calves in the first hours of life. The described condition is intensified by the type of birth (natural, assisted or C-section) and post-natal vitality. Acidosis is compensated already on the second day postpartum.

A comparison of acid-base balance parameters in experimental and control animals revealed significant differences between groups in tests II and

Table 1

Parameters of blood acid-base balance

Examination	pH		pCO_2 (mmHg)		pO_2 (mmHg)		HCO_3^- (mmol l^{-1})		BE (mmol l^{-1})		O_2SAT %	
	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.
I	7.34 ±0.04	7.35 ±0.06	52.27 ±4.50	52.05 ±5.93	29.74 ±2.14	29.04 ±2.83	30.56 ±2.16	29.37 ±3.60	2.49 ±3.33	3.01 ±2.72	47.12 ±9.42	47.57 ±11.30
II	7.26^{B} ±0.04	7.34 ±0.05	54.17 ±3.11	51.31 ±5.37	28.28^{B} ±2.82	32.29 ±3.42	28.73 ±2.74	29.72 ±4.67	-1.67^{B} ±2.48	3.61 ±1.28	40.76 ±6.90	44.35 ±11.86
III	7.24^{B} ±0.04	7.37 ±0.06	56.67^{B} ±3.52	50.25 ±6.14	26.94^{B} ±2.29	32.70 ±3.81	25.84^{BA} ±3.13	30.05 ±4.52	-2.96^{B} ±1.04	3.80 ±1.69	34.28^{BA} ±5.45	44.01 ±9.21

A – statistically significant difference at $p \leq 0.01$ between examinations
B – statistically significant difference at $p \leq 0.01$ between groups

Table 2

Concentration of electrolytes and minerals in serum

Examination	Na^+ (mmol l^{-1})		K^+ (mmol l^{-1})		Cl^- (mmol l^{-1})		Ca (mmol l^{-1})		P (mmol l^{-1})	
	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.	exper.	contr.
I	138.20 ±4.35	137.10 ±4.67	4.50 ±0.64	4.79 ±0.48	98.30 ±3.85	100.65 ±4.88	2.75^{A} ±0.20	2.87^{A} ±0.17	2.13 ±0.35	2.18 ±0.49
II	137.35 ±3.87	138.65 ±5.53	5.57^{BA} ±0.63	4.51 ±0.35	99.20 ±3.14	99.35 ±4.53	2.57 ±0.27	2.55 ±0.28	2.12 ±0.41	2.17 ±0.44
III	137.10 ±3.14	136.80 ±4.72	5.87^{BA} ±0.55	4.70 ±0.44	98.45 ±4.36	99.80 ±4.27	2.54 ±0.24	2.54 ±0.35	2.17 ±0.43	2.14 ±0.40

A – statistically significant difference at $p \leq 0.01$ between examinations
B – statistically significant difference at $p \leq 0.01$ between groups

III (Table 1). A drop in pH, an increase in the partial pressure of carbon dioxide, a decrease in the partial pressure of oxygen, a significant decrease in bicarbonate ion concentrations and hemoglobin oxygen saturation as well as a base deficit were reported in calves demonstrating symptoms of NMD. The observed changes point to the development of uncompensated metabolic acidosis due to increased levels of pyruvic acid and lactic acid produced as a result of anaerobic processes that accompany muscle fiber degeneration. Similar results were reported by KATZ et al. (2009) in a case study of a foal with NMD and by SOBIECH, KULETA (2002), who analyzed the acid-base equilibrium in goats affected by NMD.

Minor fluctuations in sodium and chloride levels were observed throughout the experiment, but their concentrations remained within the norm in both groups of animals (Table 2). Our results corroborate the findings of GRZEBUŁA (1989) who did not report changes in the concentrations of the above electrolytes in a study of foals with clinical symptoms of NMD. KOZAT et al. (2009) observed hyponatremia and hypochloremia in a foal with NMD. In the above study, changes in sodium and chloride levels were noted at advanced stages of NMD, which were accompanied by secondary pathological conditions resulting from impaired membrane permeability and the release of electrolytes from damaged muscle fibers. In our study, the absence of significant variations in sodium and chloride concentrations between control and experimental groups indicates that the examined animals were not affected by equally advanced pathological changes.

Tests II and III revealed a significant increase in potassium ion concentrations in the blood serum of experimental calves (Table 2). In this group of animals, potassium levels were significantly higher than in control. The presence of hyperkalemia can be attributed to a close correlation between the concentrations of K^+ ions and pH. An increase in the pH of blood lowers potassium ion levels, whereas a drop in pH leads to higher concentrations of K^+ ions. The above processes rely on the exchange of hydrogen and potassium ions between extracellular and intracellular spaces. This exchange can be induced by a primary increase in hydrogen ion concentrations (lower pH) in extracellular fluid which leads to the transfer of ions into the cell. In line with the law of electrical neutrality of bodily fluids, potassium ions move in the opposite direction, which leads to an increase in their plasma concentrations (HOLTENIUS 1990, DUBOSE 2000). The above phenomenon was observed in diseased cattle, in which progressing metabolic acidosis was accompanied by an increase in potassium ion levels.

The serum concentrations of inorganic phosphorus and calcium remained within the reference range in both groups of animals – Table 2 (EGLI, BLUM 1998, JEZEK et al. 2006). Minor fluctuations in calcium and phosphorus levels were observed, and significantly higher calcium concentrations in the serum of both groups noted in the first test can be attributed to the absorption of calcium from colostrum (STEINHARDT et al. 1993, SOBIECH et al. 2008). PAVLATA

(2001) and KATZ et al. (2009) did not report significant variations in serum levels of calcium and phosphorus in animals affected by NMD, and their findings are consistent with our results. Elevated calcium concentrations in the blood serum of lambs with NMD were noted by KOZAT (2009), who attributed this increase to sarcolemmal damage and the opening of calcium channels. No significant correlation between selenium supplementation and serum mineral concentrations was observed in studies of goats (ORDEN et al. 2000, HAYASHIDA et al. 2003).

CONCLUSIONS

1. Nutritional muscular dystrophy in calves involves disorders of the acid-base equilibrium and the electrolyte balance, which are manifested by uncompensated metabolic acidosis and hyperkalemia.

2. Significant changes in the serum concentrations of calcium and phosphorus are not observed in calves affected by nutritional muscular dystrophy.

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