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Applicability of the protein-lipid profile and activity of lactate dehydrogenase isoenzymes for diagnosing nutritional muscular dystrophy in calves

Katarzyna Żarczyńska[1], Przemysław Sobiech[1], Anna Snarska[1], Dawid Tobolski[2], Chandra Shekhar Pareek[3], Dariusz Bednarek[4]

[1]Department and Clinic of Internal Diseases, [2]Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland
[3]Division of Functional Genomics in Biological and Biomedical Science, Interdisciplinary Centre of Modern Technology, Nicolaus Copernicus University, 87-100 Torun, Poland,
[4]Department of Cattle and Sheep Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland
katarzyna.zarczynska@uwm.edu.pl

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Abstract

Introduction: In calves, hyposelenosis degenerates skeletal muscles in different parts of the body. The extent of damage to muscle cells can be diagnosed by determining the activity of creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). The aim of this study was to analyse variations in the serum levels of LDH isoenzymes in calves with nutritional muscular dystrophy (NMD), to determine the applicability of this parameter for diagnosing NMD, and to describe the influence of hyposelenosis on total protein (TP), triglyceride (TG), and cholesterol (CHOL) levels. **Material and Methods:** Two groups of calves (n = six animals per group) were used. After birth, control group calves (SC) were intramuscularly administered 10 ml of a preparation containing selenium (Se) and vitamin E, and experimental group animals (SE) that were not injected. Blood was collected after 5, 15, and 25 days, and the concentrations of Se, vitamin E, TP, TG, and CHOL and the activity of glutathione peroxidase (GSH-Px), CK, and LDH fractions were determined. **Results:** Hypocholesterolaemia and elevated TG levels were found in SE group calves whose LDH fractions revealed a significant increase in LDH_4 and LDH_5 activity and a decrease in LDH_1 activity when electrophoretically separated. **Conclusions:** Nutritional muscular dystrophy is accompanied by hypocholesterolaemia and elevated TG levels caused by muscle lipolysis. LDH_4 and LDH_5 activity parameters assist early diagnosis of NMD in calves.

Keywords: calf, selenium, nutritional muscular dystrophy, isoenzymes.

Introduction

Nutritional muscular dystrophy (NMD, white muscle disease) is a serious disease caused by selenium (Se) and/or vitamin E deficiency which leads to hyaline degeneration of skeletal muscles in various parts of the body. The disease is diagnosed based on clinical symptoms and Se concentrations in the serum or in whole blood. The activity of indicator enzymes: creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) is also determined to evaluate the extent of damage to skeletal muscle cells. An increase in CK activity, which can range from 1,000 to 50,000 U/L in various stages of the disease, is the most sensitive and specific indicator of muscle dysfunction (25). An increase in AST and LDH activity is less specific, and when not accompanied by an increase in CK activity, it is not always indicative of muscle damage because AST and LDH are released by other damaged cells, in particular hepatocytes (20). However, according to the literature, the increase in LDH activity is much higher in muscular dystrophy than in liver or gastrointestinal diseases (16). Lactate dehydrogenase is a tetrameric enzyme of the

oxidoreductase family which catalyses the reversible conversion of pyruvate to lactate in the presence of NADH. This enzyme is present in all cells and body fluids in the form of five isoenzymes: LDH_1, LDH_2, LDH_3, LDH_4, and LDH_5. The electrophoretic separation products of LDH were first used to diagnose livestock diseases in the 1960s when Boyd (5) observed an increase in LDH_5 activity in sheep with acute NMD. In cattle, the highest total LDH activity was noted in the heart muscle, liver, and kidneys where LDH_1 and LDH_2 fractions were predominant (around 35%) (29). These fractions are also predominant (25%) in the blood serum of cattle. The activity of different LDH isoenzymes can differ between breeds. Isoenzyme LDH_1 was predominant in dairy cattle, whereas LDH_5 activity increased mostly in beef cattle (27). Nagy *et al.* (17) analysed LDH activity in calves with respiratory problems and in healthy calves and reported a significant increase in total LDH activity and a clear increase in LDH_1 activity in diseased animals.

The activity of LDH isoenzymes has rarely been studied in calf diseases, in particular in skeletal muscle dysfunctions. Therefore, the aim of this study was to analyse variations in the serum levels of LDH isoenzymes in calves with NMD, and to determine the applicability of these parameters for diagnosing NMD in cattle. The effect of Se deficiency on total protein (TP), triglyceride (TG), and cholesterol (CHOL) levels in calves was also investigated.

Material and Methods

The study was conducted on a dairy cattle farm (around 100 adult animals) in the region of Warmia and Mazury in Northern Poland. Cases of NMD had been previously reported among calves on the farm, in particular in the first month of life. The experiment was performed on 12 Holstein-Friesian calves divided into two equal groups. The control group (SC) was composed of three male and three female calves which were intramuscularly administered 10 mL of a preparation containing vitamin E and Se (50 mg/mL of tocopherol acetate and 0.5 mg/mL of sodium selenate) (Eurovet Animal Health B.V., the Netherlands) on the day of birth. The experimental group (SE) was composed of two female and four male calves which did not receive the injection. The animals were kept indoors, and both groups were fed only the mother's milk. The mothers were fed a total mixed ration (TMR) (Table 1) supplemented with a vitamin and mineral premix (Table 2) and concentrate at 6 kg/animal.

Blood was sampled from all calves from the external jugular vein 5, 15, and 25 days after birth and collected into test tubes containing a clotting activator. The activator unabled serum and lithium heparin to determine the activity of glutathione peroxidase (GSH-Px). Selenium, vitamin E, TP, TG, and CHOL concentrations and the activity of CK, LDH, and LDH isoenzymes were determined in the blood serum, while glutathione peroxidase activity was determined in whole blood.

Table 1. Rations fed to the mothers of the evaluated calves (kg/animal)

Ingredient	kg/animal
Maize silage	23.9
Grass haylage	15.2
Rapeseed meal	1.3
Soybean meal, 46%	1.1
Vitamin and mineral premix	0.15
Agrocell (yeast)	0.05
Sodium bicarbonate	0.10
Limestone	0.10
Salt	0.05

Selenium levels were determined by hydride generation-flame atomic absorption spectrophotometry in a Unicam 939 Solar spectrometer (Unicam, UK). Vitamin E concentration was determined by high-performance liquid chromatography (HPLC) with a Hewlett Packard HP-1050 chromatograph and ClinRep kits (Recipe, Germany). Total protein was determined by the biuret test, TG- by the enzymatic method with glycerol phosphate oxidase, CHOL- by the colorimetric method with esterase and cholesterol oxidase, CK- by the kinetic method of the International Federation of Clinical Chemistry (IFCC), and LDH activity- by the kinetic method of the German Society of Clinical Chemistry (GSCC). The parameters were determined with the use of the Cormay ACCENT 200 automatic biochemical analyser and Cormay diagnostic kits (Cormay, Poland). The activity of GSH-Px was determined by the kinetic method with cumene hydroperoxide and a phosphate buffer with an Epoll-20 biochemistry analyser (Poll Ltd., Poland) and Ransel kits (Randox Laboratories Ltd, UK). Lactate dehydrogenase was separated into isoenzymatic fractions in the Pretty Interlab electrophoresis system with Interlab kits (Interlab, Italy) for the electrophoretic separation of LDH.

The significance of differences between sampling dates was determined for control and experimental groups at $P \leq 0.05$. The differences between the groups were determined by Mann-Whitney U test. The comparison values between 5 and 15 and 5 and 25 days were derived separately for both groups by the Wilcoxon signed-rank test.

Results

During the experiment (from birth to 25 days of age), SE group calves presented with symptoms

characteristic of NMD, including a poor sucking reflex, problems with standing up, and maintaining an upright standing position, and muscle tremors, in particular in the hind limbs. SC group calves were in good health throughout the experiment. Serum Se levels in the group administered the Se preparation (SC) peaked on the first sampling date (day 5) and statistically significantly decreased within the reference range to fall in its lower end in the last sampling. Selenium concentration was significantly higher in the SC group than in the SE group throughout the experiment (Table 3).

Table 2. Composition of the vitamin and mineral premix (quantity/kg)

Ingredient	Quantity/kg
Calcium	150 g
Phosphorus	50 g
Magnesium	70 g
Sodium	60 g
Copper	2,500 mg
Zinc	7,000 mg (zinc chelate 3,900 mg)
Manganese	4,400 mg
Iron	2,700 mg
Iodine	145 mg
Cobalt	50 mg
Selenium	20 mg (organic Se 30 mg)
Vitamin A	900,000 IU
Vitamin D3	210,000 IU
Vitamin E	6,000 mg
Vitamin B1	100 mg
Vitamin B2	100 mg
Rumen-protected niacin	1,000 mg
Pantothenic acid	250 mg
Vitamin B6	50 mg
Folic acid	15 mg
Vitamin B12	3,200 µg
Biotin	100,000 µg

In the SC group, the average concentration of vitamin E peaked on day 5 and also remained significantly higher than in the SE group on all sampling dates (Table 3). The activity of GSH-Px was similar in both groups on day 5. In the SC group, GSH-Px activity increased significantly on the second and third sampling dates (days 15 and 25, respectively) and was significantly higher than in the SE group (Table 3). Total protein levels in the blood serum of SE group calves were the highest on the first sampling date (day 5) and decreased on successive sampling dates. The observed changes were not statistically significant and remained within the reference range (Table 3). Triglyceride levels in the blood serum of SE group calves increased during the experiment and were significantly higher than in the SC group on days 15 and 25 (Table 3). In the SE group, serum CHOL concentration was significantly lower than in SC group animals on the third sampling date, but it was within the reference range (Table 3). The activity of CK in the blood serum of calves with symptoms of NMD increased significantly on the second and third sampling dates and was significantly higher than in healthy animals on all sampling dates (Table 4).

Total LDH activity remained stable throughout the experiment in the SC group, and no significant differences were observed between sampling dates. In the SE group, LDH activity was already significantly higher than in the control group on the first sampling date, continued to increase during the experiment, and peaked on day 25. Significant differences in LDH activities were observed between the SE and SC groups on all sampling dates (Table 4). The electrophoretic separation of LDH produced five isoenzymatic fractions (LDH_1 to LDH_5) in both groups. The activity of LDH_1 in calves administered the Se preparation remained stable throughout the experiment (Table 4). In the SE group, LDH_1 activity statistically significantly decreased during the experiment and was significantly lower than in the SC group on all sampling dates. The activity of LDH_2 remained stable on successive sampling dates in the control group, whereas a minor decrease in this parameter was noted in the experimental group. On all sampling dates, LDH_2 activity was significantly lower in the SE group than in the SC group. The activity of LDH_3 remained stable throughout the experiment in SC animals, whereas a statistically significant decrease in the parameter was observed in the SE group on the last sampling date. In the SE group, LDH_3 activity was significantly higher than in the SC group on the first and second sampling dates. The activity of LDH_4 remained stable throughout the experiment in healthy calves, and it increased significantly in the experimental group on the second and third sampling dates. The parameter was significantly higher in the experimental group than in the control group throughout the experiment. The activity of LDH_5 remained similar on all sampling dates in the SC group, and it increased significantly in the SE group during the experiment. The parameter was significantly higher in the SE group than in the SC group on all sampling dates (Table 4).

Table 3. The average selenium, vitamin E, total protein, triglyceride, and cholesterol levels and GSH-Px activity in the blood serum of SE group (without selenium and vitamin E supplementation) and SC group (with selenium and vitamin E supplementation) calves (mean +SD)

	Sampling date					
	Day 5		Day 15		Day 25	
Parameter	SE	SC	SE	SC	SE	SC
Selenium (µg/L)	$35.45^{B} \pm 4.17$	56.54 ± 4.58	$33.36^{B} \pm 3.52$	45.62 ± 5.36	$30.92^{B} \pm 3.58$	$38.23^{A} \pm 3.59$
Vitamin E (µg/mL)	$1.52^{B} \pm 0.33$	3.29 ± 0.46	$1.40^{B} \pm 0.34$	3.0 ± 0.51	$1.22^{B} \pm 0.26$	2.68 ± 0.45
GSH-Px (U/gHb)	41.21 ± 11.56	49.48 ± 10.25	$32.78^{B} \pm 9.12$	$139.65^{A} \pm 37.76$	$25.89^{BA} \pm 5.56$	$132.62^{A} \pm 41.45$
TP (g/L)	57.54 ± 9.1	61.43 ± 6.38	53.71 ± 6.8	58.73 ± 8.72	51.91 ± 6.68	58.86 ± 10.02
TG mmol/L	0.47 ± 0.15	0.32 ± 0.18	$0.57^{B} \pm 0.22$	0.33 ± 0.13	$0.58^{B} \pm 0.17$	0.33 ± 0.18
CHOL mmol/L	2.02 ± 0.73	2.45 ± 0.59	1.91 ± 0.65	2.57 ± 0.64	$1.79^{B} \pm 0.41$	2.71 ± 0.62

A – $P \leq 0.05$ between sampling dates (comparison of days 5 and 15 and of 5 and 25 in the SE and SC groups)
B – $P \leq 0.05$ between groups

Discussion

In the present study, serum Se levels were significantly lower in the SE group than in the SC group and decreased statistically significantly with animal age in the SC group. According to Abutarbush and Radostits (1) and Żarczyńska *et al.* (30), Se deficiency plays an important role in NMD in calves. In this study, the Se preparation administered in the form of an intramuscular injection increased serum Se levels in SC group animals. The highest Se concentration was noted on day 5. A similar increase in Se levels was reported by Abutarbush and Radostits (1) who administered Se parenterally to a calf with symptoms of NMD, and by Żarczyńska *et al.* (30) who administered Se to calves after birth. According to Grzebuła (14), the highest serum Se levels can already be observed several hours after parenteral injection. In a study by Sobiech (28), serum Se concentration in goat kids increased two weeks after supplementation.

Serum vitamin E levels were significantly lower in the SE group than in the SC group calves throughout the experiment. In calves which did not receive the injection, vitamin E as well as Se concentrations decreased with age, but not statistically significantly. A similar trend was observed in the SC group. Other authors have also observed that α-tocopherol deficiency is one of the main causes of NMD (1, 27, 30). In the present study, the administration of the Se and α-tocopherol preparation increased vitamin E levels in the SC group. Eichler *et al.* (9) demonstrated a significant increase in serum vitamin E concentration as early as 14–32 h after supplementation. In this study, serum vitamin E concentration peaked on the first sampling date (5 days after supplementation) and decreased steadily on successive sampling dates, but remained higher in SC group animals than in those of the SE group throughout the experiment. A similar trend was reported by Pavlata *et al.* (22) who observed that the administration of vitamin E preparations in a single dose or repeated doses was not sufficient to induce a significant increase in serum vitamin E levels in calves. This could be attributed to the fact that the baseline serum vitamin E concentration was very low, and α-tocopherol was used to build vitamin E reserves. To guarantee the optimal supply of vitamin E, α-tocopherol should be administered several times in combination with other vitamins, rather than the potentially toxic Se.

In the SE group, the activity of GSH-Px continued to decrease during the experiment and was significantly below the norm (20) on the last sampling date. The applicability of the GSH-Px assay for detecting and monitoring NMD was studied by Or *et al.* (18) and Pavlata *et al.* (20). In the current study, GSH-Px activity increased in the SC group on days 15 and 25. In other studies, the above parameter increased 10–12 days after Se supplementation, which could be attributed to the incorporation of Se into red blood cells during erythropoiesis and the time required for GSH-Px biosynthesis (2, 21). An analysis of the correlations between Se concentration and GSH-Px activity in SC group calves revealed GSH-Px activity continued to increase despite the decrease in Se levels. Similar results were reported by Pavlata *et al.* (20).

Serum TP levels were not statistically significantly lower in the SE group than in SC group calves throughout the experiment. In SE group animals, hypoproteinaemia was probably caused by lower feed intake and Se deficiency which impairs protein biosynthesis. Abutarbush and Radostits (1) observed a significant decrease in serum TP caused by a failure of passive immunoglobulin transfer in a calf with congenital NMD. El-Shahat and Abdel Monem (10) observed a significant increase in TP and albumin levels in the serum of lambs whose mothers were administered Se and vitamin E. The above findings indicate that Se and vitamin E enhance protein biosynthesis and transport.

Table 4. The average activity of creatine kinase, lactate dehydrogenase, and their isoenzymes in the blood serum of SE group (without selenium and vitamin E supplementation) and SC group (with selenium and vitamin E supplementation) calves (mean +SD)

	Sampling date					
	Day 5		Day 15		Day 25	
Parameter	SE	SC	SE	SC	SE	SC
CK (U/l)	$245.25^{B} \pm 35.69$	188.78 ± 35.23	$365.54^{BA} \pm 68.45$	175.89 ± 65.17	$453.25^{BA} \pm 68.27$	189.96 ± 68.81
LDH (U/l)	$5569.75^{B} \pm 356.42$	4599.56 ± 307.22	$6384.65^{B} \pm 458.21$	4649.29 ± 294.33	$7501.61^{BA} \pm 398.45$	4598.55 ± 275.26
LDH_1 (%)	$32.8^{B} \pm 2.77$	42.5 ± 2.15	$28.1^{BA} \pm 3.01$	39.8 ± 2.78	$24.2^{BA} \pm 2.45$	41.5 ± 1.84
LDH_2 (%)	$22.6^{B} \pm 3.12$	29.5 ± 3.49	$19.3^{B} \pm 2.16$	28.5 ± 1.98	$17.8^{B} \pm 3.05$	30.1 ± 3.15
LDH_3 (%)	$18.9^{B} \pm 1.02$	12.8 ± 0.61	$18.7^{B} \pm 1.94$	14.3 ± 0.65	$13.3^{A} \pm 0.56$	12.4 ± 0.78
LDH_4 (%)	$9.8^{B} \pm 0.43$	6.3 ± 0.62	$13.8^{BA} \pm 1.56$	7.1 ± 1.26	$16.4^{BA} \pm 1.78$	6.9 ± 0.55
LDH_5 (%)	$15.9^{B} \pm 0.94$	8.9 ± 1.35	$20.1^{BA} \pm 2.68$	10.3 ± 1.95	$28.3^{BA} \pm 2.36$	9.3 ± 1.25

A – $P \leq 0.05$ between sampling dates (comparison of days 5 and 15 and of 5 and 25 in the SE and SC groups)
B – $P \leq 0.05$ between groups

In this study, serum triglyceride levels in the SE group increased on successive sampling dates and were significantly higher than in SC group animals (on the second and third sampling dates). Sobiech (28) reported similar results in goat kids with NMD and attributed these findings to intensified lipolysis which accompanies the disease. During lipolysis, free fatty acids are released from adipocytes into the blood stream. In the liver, free fatty acids are re-esterified to triglyceride and are transported by very low-density lipoproteins (VLDL) from hepatocytes to the blood. In contrast, Or *et al.* (18) did not observe changes in the TG levels of lambs with white muscle disease. Douillet *et al.* (7) examined the influence of Se and vitamin E on lipid metabolism in rats with experimentally induced diabetes and observed that Se supplementation normalised triglyceride concentration in the liver. The above mechanism can be attributed to the insulin-like properties of Se. In the present study, serum triglyceride levels in healthy calves remained fairly stable throughout the experiment. Similar results were reported by other authors who did not observe correlations between TG levels and animal age (23).

Serum cholesterol concentration was significantly lower in the SE group than in SC group calves on the third sampling date. The observed changes could be indicative of disrupted cholesterol synthesis which accompanies liver dysfunction. A study of rats demonstrated that Se and vitamin E are powerful antioxidants with synergistic effects which play a key role in hepatocyte protection (13, 19). Grzebuła (14) reported an increase in cholesterol levels in foals with NMD. According to many authors, Se significantly influences thyroid hormone metabolism. Selenium deficiency decreases T3 and T4 concentrations, but Se supplementation does not restore normal hormone levels in animals. Thyroid hormone deficiency increases serum cholesterol concentration. Mild hyperlipidaemia, intensified cholesterol production (during mevalonate synthesis), decreased activity of lipoprotein lipase, intensified LDL oxidation, a decrease in the number of LDL receptors in the liver, and disrupted activity of cholesterol ester transfer protein are observed in humans with subclinical hypothyroidism (24). In this study, SC group calves were characterised by relatively high serum cholesterol concentration with a minor increasing trend throughout the experiment. A study of calves, lambs, piglets, and foals demonstrated that serum cholesterol levels are the lowest at birth, increase during nursing, and decrease after weaning (6). Hall *et al.* (15) observed that dairy cows receiving organic Se before parturition were characterised by lower serum cholesterol levels during calving and 48 h after parturition than non-supplemented cows.

In the present study, serum CK activity in SE group calves increased during the experiment, and the highest increase was noted on the last sampling date. In SC group animals, serum CK activity remained stable throughout the study. On day 25, serum CK activity was determined at 453.25 U/L in the SE group and was significantly higher than in healthy calves. In other studies, CK activity did not exceed 400 U/L in Se-deficient calves (3) and 340 U/L in dairy cows with NMD (20). Abutarbush and Radostits (1) reported CK activity of 29,000 U/L in a calf with congenital NMD on the second day of life. In an experiment conducted by Andres *et al.* (2), serum CK activity exceeded 1,500 U/L in lambs with clinical NMD. In this study, serum CK activity exceeded 450 U/L in calves with NMD, and similar results were noted in cattle by other authors (20, 30), which points to similarities in the progression of NMD symptoms.

Serum LDH activity in calves presenting symptoms of NMD increased significantly during the experiment and was significantly higher than in healthy

animals on all sampling dates. Pavlata *et al.* (20), Żarczyńska *et al.* (30), and Sobiech (28) observed a significant increase in LDH activity in various species of animals with NMD, which indicates that this enzyme is a reliable indicator for diagnosing the disease. In the control group, a minor increase in serum LDH activity was noted during the experiment, but this parameter remained within the reference ranges, which points to the absence of pathological changes in skeletal muscles. An increase in LDH activity until the 56th day of life was also reported by other authors who attributed this finding to physiological development (8).

The electrophoretic separation of LDH from the blood serum of both animal groups produced five enzyme fractions. In the SE group, LDH_1 activity decreased statistically significantly during the experiment and was significantly lower than in the control group on all sampling dates. In ruminants, this enzyme fraction is specific for the heart muscle, brain, liver, kidneys, and spleen, and its activity in the blood serum increases when the functions of these organs are disrupted (11). In a study by Asefa Asmare *et al.* (4), LDH_1 activity increased with an increase in milk yield, which could be attributed to hepatic overload. In the current study, LDH_1 activity was within the reference range in SC animals, whereas the decrease observed in the experimental group (in particular on the last sampling date) could be associated with enhanced activity of the LDH_4 and LDH_5 fractions, and it does not appear to have diagnostic significance.

The activity of LDH_2 was stable and high during the experiment in SC group calves, and it decreased gradually in SE group animals. In ruminants, LDH_2 is found specifically in gastric and intestinal mucosa and in red blood cells (4), but it is also isolated in large quantities from the lungs (17). This isoenzyme is characterised by high mobility in an electric field; therefore, its activity can increase following damage to the organs which stimulate increased activity of LDH_1. In the SC group, the observed decrease in LDH_2 activity can probably be attributed to a shift of activity towards slow moving fractions.

Some significant differences in LDH_3 activity were observed between sampling dates or groups. This isoenzyme is not tissue-specific, and it is found in the heart, liver, intestinal mucosa, pancreas, and lungs (17, 29). For this reason, changes in LDH_3 activity do not appear to have diagnostic significance.

The activity of LDH_4 was significantly higher in SE group calves on all sampling dates than in SC group animals, and it increased gradually with the progression of NMD. According to some authors, LDH_4 is not organ-specific (29), whereas other researchers have argued that it is specific to muscle tissue in ruminants (4). In the present study, the increase in LDH_4 activity correlated with the progression of NMD, which suggests that this isoenzyme is specific for muscle tissue and is released from damaged muscles.

The activity of LDH_5 increased significantly in the blood serum of SE group calves, and it remained stable in SC group animals throughout the experiment. In ruminants, this isoenzyme is highly muscle-specific (26, 29). The activity of CK and LDH_5 increases with the progression of degenerative changes in muscles in the findings of Goddard *et al.* (12). A similar correlation was observed in this study, which suggests that CK and LDH_5 activities should be assayed jointly in diagnoses of skeletal muscle dysfunctions.

In conclusion, NMD disrupts lipid metabolism in calves by decreasing cholesterol levels and increasing triglyceride levels due to adipose tissue lipolysis. The disease contributes to a significant increase in the serum activity of CK and LDH and a considerable amplification in the activity of LDH_4 and LDH_5 fractions. The results of this study indicate that LDH_4 and LDH_5 activity could be useful parameters for early diagnosis of NMD.

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References

1. Abutarbush S.M., Radostits O.M.: Congenital nutritional muscular dystrophy in a beef calf. Can Vet J 2003, 44, 738–739.
2. Andrés S., Mañé M.C., Sánchez J., Barrera B., Jiménez A.: Changes in GSHPx and muscle enzyme activities in lambs with nutritional myodegeneration following a single treatment with sodium selenite. Small Rumin Res 1996, 23, 183–186.
3. Arthur J.R.: Effects of selenium and vitamin E status on plasma creatine kinase activity in calves. J Nutr 1988, 118, 747–755.
4. Asefa Asmare A., Kovác G., Reichel P., Buleca J., Scurokova E.: Serum isoenzyme activity of lactate dehydrogenase in dairy cows at different production stages. Folia Vet 1998, 42, 77–81.
5. Boyd J.W.: Serum enzyme changes in lambs with experimentally induced acute muscular dystrophy. Res Vet Sci 1964, 5, 419–433.
6. Carroll K.K., Hamilton R.M.G., Macleod G.K.: Plasma cholesterol levels in suckling and weaned calves, lambs, pigs and colts. Lipids 1973, 8, 635–640.
7. Douillet C., Bost M., Accominotti M., Borson-Chazot F., Ciavatti M.: Effect of selenium and vitamin E supplements on tissue lipids, peroxides, and fatty acid distribution in experimental diabetes. Lipids 1998, 33, 393–399.
8. Egli C.P., Blum J.W.: Clinical, hematological, metabolic and endocrine traits during the first three months of life of suckling simmentaler calves held in a cow-calf operation. J Vet Med A 1998, 45, 99–118.
9. Eichler S.D., Morrill J.L., Velazco J.: Bioavailability of α-tocopherol fed with retinol and relative bioavailability of

D-α-tocopherol of DL-α-tocopherol acetate. J Dairy Sci 1997, 80, 393–399.
10. El-Shahat K.H., Abdel Monem U.M.: Effects of dietary supplementation with vitamin E and/or selenium on metabolic and reproductive performance of Egyptian Baladi ewes under subtropical conditions. World Appl Sci J 2011, 12, 1492–1499.
11. Georgieva D., Todorov B.: Total LDH activity and isoenzyme spectrum in experimental *Cysticercus ovis* infestation of lambs and kids. Vet Med Nauki 1987, 24, 28–35.
12. Goddard P.J., Keay G., Grigor P.N.: Lactate dehydrogenase quantification and isoenzyme distribution in physiological response to stress in red deer (*Cervus elaphus*). Res Vet Sci 1997, 63, 119–122.
13. Gonca S., Ceylan S., Yardimoğlu M., Dalçık H., Köktürk S., Filiz S., Yumbul Z.: Histopathological effects of cholesterol and protective effects of vitamin E and selenium on the morphology of liver. Turk J Med Sci 2000, 30, 551–555.
14. Grzebuła S. Studia nad pokarmową dystrofią mięśni źrebiąt. Thesis. Agricult Acad, Lublin, 1989.
15. Hall J.A., Bobe G., Vorachek W.R., Kasper K., Traber M.G., Mosher W.D., Pirelli G.J., Gamroth M.: Effect of supranutritional organic selenium supplementation on postpartum blood micronutrients, antioxidants, metabolites, and inflammation biomarkers in selenium-replete dairy cows. Biol. Trace Elem Res 2014, 161, 272–287.
16. Martínez-Moreno A., Jiménez-Luque V., Moreno T., Redondo E.S., de las Mulas J.M., Pérez J.: Liver pathology and immune response in experimental *Fasciola hepatica* infections of goats. Vet Parasitol 1999, 82, 19–33.
17. Nagy O., Tóthová Cs., Seidel H., Paulíková I., Kovác G.: The effect of respiratory diseases on serum lactate dehydrogenase and its isoenzyme patterns in calves. Pol J Vet Sci 2013, 16, 211–218.
18. Or M.E., Dodurka H.T., Kayar A.: The diagnostic importance of erythrocyte glutathione peroxidase (GSH-Px) activity and some serum parameters in lambs. Turk J Vet Anim Sci 2003, 27, 1–6.
19. Ozdil S., Bolkent S., Yanardag R., Arda-Pirincci P.: Protective effects of ascorbic acid, DL-α-tocopherol acetate, and sodium selenate on ethnol-induced liver damage of rats. Biol Trace Elem Res 2004, 97, 149–161.
20. Pavlata L., Pechová A., Illek J.: Muscular dystrophy in dairy cows following a change in housing technology. Acta Vet Brno 2001, 70, 269–275.
21. Pavlata L., Mišurová L., Pechová A., Dvořák R.: The effect of inorganic and organically bound forms of selenium on glutathione peroxidase activity in the blood of goats. Vet Med-Czech 2011, 56, 75–81.
22. Pavlata L., Podhorský A., Pechová A., Dvořák R.: Incidence of hypovitaminosis E in calves and therapeutic remedy by selenium-vitamin supplementation. Acta Vet Brno 2005, 74, 209–216.
23. Piccione G., Casella S., Pennisi P., Giannetto C., Costa A., Caola G.: Monitoring of physiological and blood parameters during perinatal and neonatal period in calves. Arg Bras Med Vet Zootec 2010, 62, 1–12.
24. Pucci E., Chiovato L., Pinchera A.: Thyroid and lipid metabolism. Int J Obes Relat Metab Disord 2000, 24, 109–112.
25. Radostits O.M., Gay C.C., Blood D.C., Hinchcliff K.W.: Veterinary Medicine. A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Saunders Ltd. New York, 2000, pp. 1515–1533.
26. Šalplachta J., Nečas J.: Lactate dehydrogenase isoenzyme pattern in tissues and serum of the calf. Acta Vet Brno 2000, 69, 267–275.
27. Sobiech P., Kuleta Z., Jałyński M.: Serum LDH isoenzyme activity in dairy and beef cows. Indian Vet J 2004, 81, 755–756
28. Sobiech P.: Hematological, biochemical and histopathological picture of nutritional muscular dystrophy (NMD) in goat kids. Monographs, UWM, Olsztyn, 2009.
29. Yasuda J., Syuto B., Too K., Ohfuji S.: Lactate dehydrogenase isoenzyme patterns in bovine liver tissue. Jap J Vet Sci 1989, 51, 733–739.
30. Żarczyńska K., Baumgartner W., Sobiech P.: Coagulology, biochemical profile, and muscle pathology in calves diagnosed with nutritional muscular dystrophy. Pol J Vet Sci 2017, 20, 387–394.