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Original article

The influence of selenium deficiency on chosen biochemical parameters and histopathological changes in muscles of goat kids

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Abstract

The research was conducted on 40 young alpine goats (kids) divided into two groups. First group consisted of 20 kids demonstrating clinical signs of muscular dystrophy. Second group was a control and consisted of 20 animals that received intramuscular injection (2ml per animal) of vitamin E and selenium preparation containing in 1ml 50 mg of tocopherol acetate, 0.5mg of sodium selenite and solvent on 2nd day of life. The kids were clinically examined and blood for laboratory analyses was sampled three times from day 5 of their life in 10 day intervals. In addition, six 24 days old kids demonstrating clinical signs of muscular dystrophy and six control kids were subjected to biceps femoris biopsy.

Serum total protein, glucose, triglycerides, cholesterol as well as AST, CK and LDH were determined in all the animals. In addition, the activity of glutathione peroxidase (GSH-Px) was determined in whole blood and serum concentrations of selenium and vitamin E were determined in 6 kids from each group. Total lactate dehydrogenase activity and its separation into isoenzymatic fractions were determined in the collected biopsy material. The muscle samples collected were additionally subjected to histopathological examination consisting of HE staining and HBFP staining to detect necrotic muscle fibers.

Symptoms of muscular dystrophy began to appear in the first group between 17 and 23 days of age and included tremors of the limbs, poor posture, stilt gait and increased time of laying. The control animals did not show any symptoms of the disease during the experiment. Hypoproteinemia, hypoglycemia, cholesterol reduction and elevated triglycerides level associated with lipolysis of adipose tissue have been found in the sick kids. A significant decrease in selenium, vitamin E and activity of glutathione peroxidase levels was observed in the kids with symptoms of muscular dystrophy. The activity of AST, CK and LDH was significantly higher in the animals with symptoms of the disease as well. Five isoenzymes were obtained in the electrophoretic separation of lactate dehydrogenase into isoenzymatic fractions in the muscle tissue. LDH_4 and LDH_5 isoenzymes were dominating, and a significant increase in LDH_5 fraction of the sick animals was also observed. Histopathological examination of muscle samples from sick animals revealed changes characteristic for the presence of Zenker necrosis.

Key words: nutritional muscular dystrophy, selenium deficiency, LDH isoenzymes, goat kids

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Introduction

One of the main diseases caused by vitamin E and selenium deficiency in ruminants is nutritional muscular dystrophy, also called white muscle disease (WMD), which causes hyaline degeneration of skeletal muscles in various parts of the body. The disease is diagnosed on the basis of characteristic clinical symptoms, and a decrease in the serum concentrations of selenium and vitamin E. An increase in the serum activity of enzymes associated with muscle tissue such as: creatine kinase (CK), lactate dehydrogenase (LDH) or aspartate aminotransferase (AST) is also observed in the course of WMD. Literature data (Radostris et al. 2006, Delesalle et al. 2017) indicates that the most characteristic enzyme for muscle tissue is creatine kinase, whose activity during WMD can rise very intensely (depending on the disease progression). The elevated activity of AST and LDH is not so pronounced, but with a simultaneous increase in CK activity indicates muscle damage (Pavlata et al. 2001). Increased LDH activity is valuable in diagnosis of WMD (Żarczyńska et al 2017), because according to literature (Martinez-Moreno et al. 1999) its level is much higher than in cases of liver damage or gastrointestinal diseases.

Lactate dehydrogenase, due to its molecular structure, occurs in organs and tissues in the form of five isoenzymes- LDH_1 to LDH_5, referred to as fractions (Yasuda et al. 1990, Jurisic et al. 2015) The first reports on the use of LDH isoenzymes in the diagnosis of livestock diseases appeared in 1960s., when Boyd (1964) observed an increase in LDH_5 activity in sheep with acute white muscle disease. However, there are significant species differences in the activity of the individual isoenzymes.

The highest total LDH activity in ruminants is found in the myocardium and skeletal muscles, lower in the renal cortex, liver, brain and spleen (Keller 1974, Salplachta and Necas 2000). LDH_1 is a dominant fraction in the sheep's myocardium, kidneys, and liver - about 80% of the total enzyme activity. LDH_1 and LDH_3 have the highest activity in the lung tissue - approximately 30% of the total LDH activity each. LDH_5 predominates in skeletal muscles - about 70% of the total LDH activity (Beatty and Doxey 1983).

Due to scant literature data that describes research on the course of muscular dystrophy in goats, the aim of this study was to determine changes in selected biochemical parameters (with particular emphasis on isoenzymatic separation of LDH in the muscle tissue), and the histopathology changes associated with this disease.

Materials and Methods

The study was conducted on 40 Alpine kids of both sexes, from a goat farm in Warmia, Poland, where cases of WMD were regularly found. The experiment was performed during the indoor system of breeding. This project was approved by the Local Ethics Committee. Kids were divided into two groups:

- first (experimental) - 20 animals with clinical signs of nutritional muscular dystrophy
- second (control) - 20 healthy animals, that on the 2nd day of life received a single, prophylactic intramuscular injection of vitamin E and Se preparation (Eurovet Animal Health BV, Netherlands) – 2 ml per animal (50 mg of tocopherol acetate, 0.5 mg of sodium selenite and solvent in 1 ml).

The kids of both groups were fed their dams milk. Dams received in a daily ratio 1 kg of meadow hay, 0.5 kg of oats, 2 kg of fodder beets and 1 kg of carrots and had a constant access to water. Before the start of experiment (2 day of life) fecal samples for parasitological examinations (modified McMaster technique, Whitlock 1948) were collected directly from the rectum of all kids. During the experiment, all animals were subjected to clinical examinations, and the sampled material - to several laboratory tests. Blood was collected from the external jugular vein, three times at ten-day intervals, in the morning, starting from the fifth day of kid's life.

Blood for biochemical tests was collected from all animals to polyethylene tubes with clot activator. After blood centrifugation, serum was stored in single test tubes at –18°C until further analysis. The following parameters were determined in serum: concentration of total protein, glucose, triglycerides, cholesterol, as well as the activity of aspartate aminotranspherase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH). In addition, glutathione peroxidase (GSH-Px) was determined in whole blood.

Total protein was estimated by the biuret test, triglycerides – by the enzymatic method with glycerol phosphate oxidase, cholesterol – by the colorimetric method with esterase and cholesterol oxidase, CK and AST activity by the kinetic method of the International Federation of Clinical Chemistry (IFCC), LDH activity – by the kinetic method of the German Society of Clinical Chemistry (GSCC). These 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 cumenehydroperoxide and a phosphate buffer with the Epoll-20 biochemistry analyser (Poll Ltd., Poland) and Ransel kits (Randox Laboratories Ltd, UK). Lactate dehydro-

genase was separated into isoenzymatic fractions in the Pretty Interlab electrophoresis system with Inerlab kits (Interlab, Italy).

Selenium and vitamin E levels were estimated in the serum of six kids from both groups by hydride generation-flame atomic absorption spectrophotometry using the Unicam 939 Solar spectrometer (Unicam, UK). Vitamin E concentration was determined by high-performance liquid chromatography (HPLC) with a Hewlet Packard HP-1050 chromatograph and ClinRep kits (Recipe, Germany).

On day 24, samples of the biceps femoris were collected for histopathological analysis from six animals in each group (the same kids in which selenium and vitamin levels were estimated). The biopsy site was shaved, disinfected, and anesthetized by infiltration with 3 ml of 5% polocainum hydrochloricum (Biowet Drwalew, Poland). The size of muscle samples obtained by scalpel incision were 0.7 x 0.7 cm x 0.5 cm. In order to determine the total LDH activity and perform its isoenzymatic separation, the collected material was weighed and transferred to a homogenizer. After adding 1 ml of glycerol and 9 ml of 0.9 % NaCl, the material was homogenized for 3 min. at 200 rpm in 0°C using the Unipan type 319 (Unipan, Poland). Then the sample was centrifugated at 5000 rpm for 10 mins. The supernatant was diluted and the total LDH activity calculated per gram of tissue was determined, and the isoenzymatic fractions were separated using the Pretty Interlab electrophoresis system with Inerlab kits (Interlab, Italy).

For histopathological examination muscle sections were immersed in saline solution (Natrium chlorate 0.9%, Baxter, Poland) for 10 minutes, neutralized with 10% formalin (Chempur, Poland) and embedded in paraffin. Microtome sections were stained with hematoxylin and eosin (HE) and hematoxylin-basic fuchsin-picric acid (HBFP) to expose necrotic muscle fibers.

The significance of differences between sampling dates was determined for control and experimental groups at $p \leq 0.01$. The differences between the groups were determined by Manno-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

Experimental kids were free of invasion of gastrointestinal parasites and pulmonary nematodes. During first days of life, the animals had normal appetite and displayed no clinical signs of disease. Afterwards - between 15 and 23 days of age, all animals in this group began to develop symptoms of WMD. Initially, symptoms were non-specific and manifested by apathy, decreased appetite and reluctance to move. Gradually, more severe symptoms can be observed: tremors in the muscles of hind limbs, incorrect posture with widely spaced limbs and bending the spine upwards, stiff gait, and in some cases tachypnoea and coughing. Some sick animals were not able to stand and their temperature was rising up to 40.5^{0}C. In few animals, the symptoms of the disease increased with age, while others revealed only movement disorders without any general symptoms.

Control kids were in a good condition throughout the experiment, no symptoms of disease were observed, and faecal analysis excluded the presence of intestinal parasites and pulmonary nematodes.

The serum concentration of total protein in both groups was similar during the entire experiment. Only in the experimental group a slight drop in this parameter was noted in subsequent samplings, but these changes were not statistically significant ($p \leq 0.01$; Table 1). The serum glucose content in the experimental group was decreasing during the experiment, and value of this parameter in the last sampling was statistically significantly lower ($p \leq 0.01$) from the initial value (first sampling) and from the value obtained in the control group (Table 1). The serum glucose content in the control group remained at the similar level throughout the experiment.

Although the serum triglyceride level in the experimental group was the highest in the last sampling, and significantly exceeded the value of this parameter in the control group, a significant reduction ($p \leq 0.01$) of this parameter in experimental kids was noted in the second sampling compared to the base values. In the control kids, serum triglyceride levels dropped successively throughout the experiment, but these changes were not statistically significant (Table 1).

The serum cholesterol level was significantly lower ($p \leq 0.01$) in the experimental group in the last sampling compared to the control group. The value of this parameter gradually decreased in these animals during the experiment, while in the control group it remained at similar level (Table 1).

The serum selenium concentration in the experimental group was decreasing during the experiment, and reached the lowest value in the last sampling. It was statistically significantly lower ($p \leq 0.01$) in the last two samplings than that found in the control group. The highest concentration of this element in the control group was noted during the second sampling (15th day of life), while in the end of the experiment there was a slight decrease in this concentration. A statistically

Table 1. Concentration of total protein, glucose, triglycerides and cholesterol in serum of goat kids (mean±SD).

examination	TP (g/l)		GLU (mmol/l)		TG (mmol/l)		CHOL (mmol/l)	
	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+
I	53.4±10.31	54.7±9.82	5.87±1.12	5.92±1,33	1.22±0.53	1.15±0.43	2.15±0.41	2.22±0.48
II	53.6±9,52	56.6±7.13	5.07±0.77	5.64±0.91	0.71^{A}±0.43	0.98±0.55	1.91±0.38	2.29±0.26
III	52.3±5.34	55.8±5.43	4.15^{XA}±0.66	5.41±0.77	1.25^{X}±0.63	0.68±0.52	1.33^{X}±0.29	2.19±0.31

Se- – experimental group).
Se+ – control group.
A – statistically significant difference at p≤0.01 between examinations.
X – statistically significant difference at p≤0.01 between groups.

Table 2. Concentration of selenium, vitamin E and activity of creatine kinase, lactate dehydrogenase, aspartate aminotranspherase in serum and activity of glutathione peroxidase in blood (mean±SD).

examin.	Se (µg/l)		Vit. E (µg/ml)		GSH-Px (U/gHb)		CK (U/l)		LDH (U/l)		AST (U/l)	
	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+
I	20.3 ±1.15	24.5^{A} ±2.12	3.18± 1.55	3.45 ±1.89	30.3 ±3.15	34.1^{A} ±4.18	133.8 ±17.35	142.7 ±15.61	487.7 ±44.44	601.4 ±33.19	66.2 ±12.33	71.8 ±11.15
II	$18,1^{X}$ ± 1,44	43,9 ± 5,15	2.48^{X} ±2.97	3.32 ±1.89	19.5^{XA} ±3.11	156.4 ±18.13	$252,4^{XA}$ ± 89,33	132.5 ±19.55	702.3 ±55.23	587.2 ±43.12	94.5 ±15.21	78.3 ±14.43
III	17.8^{X} ±1.52	39.7 ±4.11	2.21^{X} ±1.43	3.21 ±1.43	17.3^{XA} ±1.67	178.3 ±19.21	836.9^{XA} ±221.13	153.9 ±24.44	1032.4^{XA} ±76.13	590.3 ±44.18	201.3^{XA} ±33.12	82.4 ±16.32

Se- - experimental group
Se+ - control group
A – statistically significant difference at p≤0.01 between examinations
X - statistically significant difference at p≤0.01 between groups

Table 3. Activity of lactate dehydrogenase and its isoenzymes in the muscle tissues (mean±SD).

tissue	LDH (U/g tis.)		LDH_1 (%)		LDH_2 (%)		LDH_3 (%)		LDH_4 (%)		LDH_5 (%)	
	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+	Se-	Se+
muscle	8994.4^{X} ±1994.15	5333.2 ±1532.11	0.6 ±0.03	0.8 ±0.08	1.4 ±0.12	1.8 ±0.18	9.6 ±3.2	11.3 ±2.58	20.5^{X} ± 4.15	34.2 ± 6.13	67.9^{X} ±12.33	51.9 ±10.13

Se- – experimental group.
Se+ – control group.
X – statistically significant difference at p≤0.01 between groups.

significantly lower (p≤0.01) serum concentration of selenium was also observed in this group of animals between the first and other samplings (Table 2).

The serum vitamin E content in the experimental kids decreased and in the last two samplings it was statistically significantly lower (p≤0.01) than that found in the control group. The vitamin E content in the control kids remained at an even level throughout the experiment (Table 2).

The GSH-Px activity in blood of animals from both groups was similar in the first sampling, and then significantly decreased (p≤0.01) in the experimental group. The highest activity of this enzyme in the blood of animals from the control group was recorded in the last sampling, while the values determined in the second and third sampling were statistically significantly higher (p≤0.01) both than the initial value in this group and values in the experimental group (Table 2).

Serum AST activity in kids from the experimental group increased during the experiment and reached the highest, statistically significant value (p≤0.01) in the last sampling (it was significantly higher than the initial value as well as values obtained in the control group). The activity of this enzyme in the control group, remained at an even level (Table 2).

Serum CK activity in kids from the experimental group was similar to AST activity, but in this case statistically significantly higher values (p≤0.01) between the

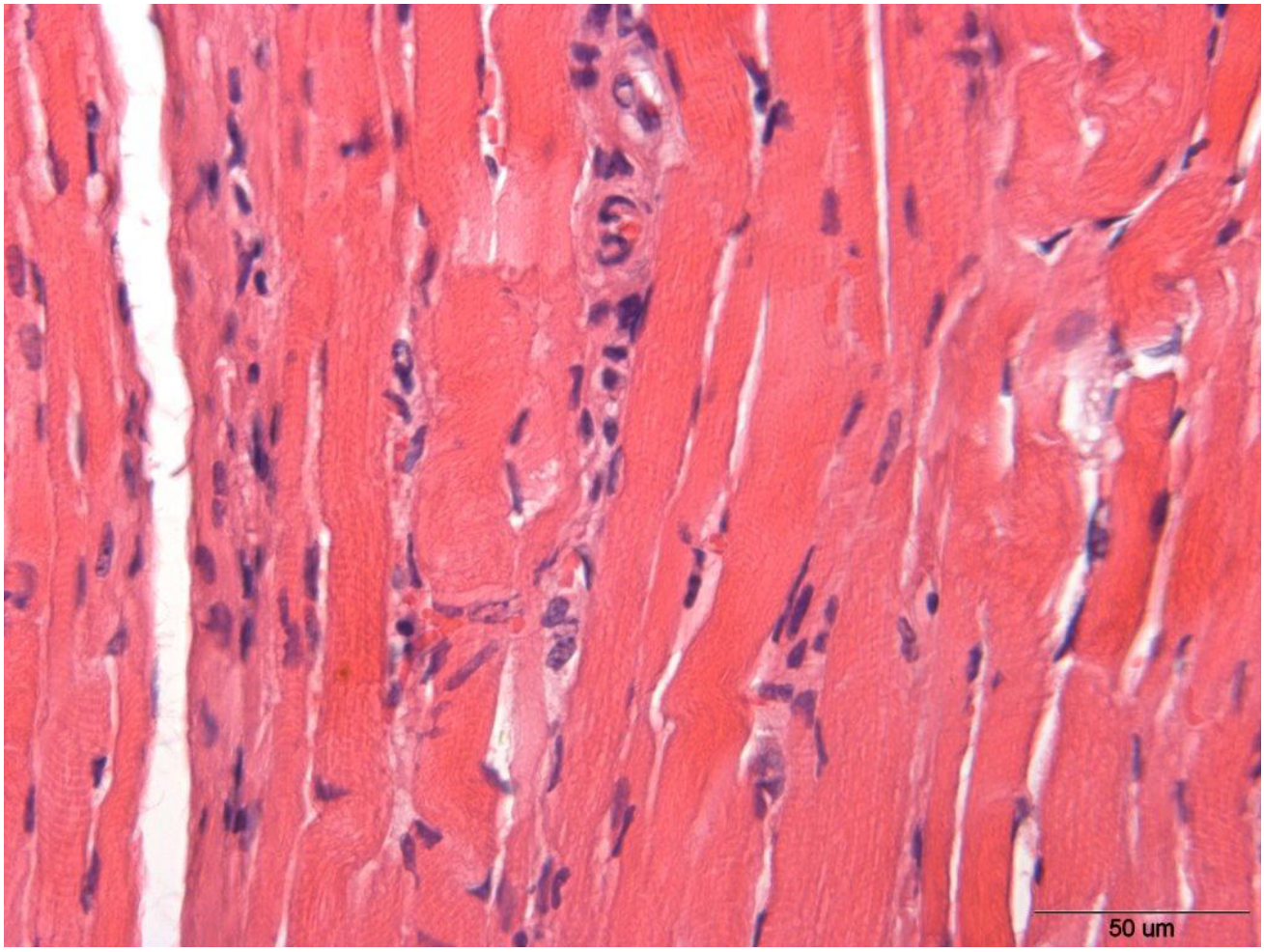

Fig. 1. Pathological changes in the biceps femoris muscle tissue of a goat kid with symptoms of white muscle disease (WMD). Segmental loss of cross striation, sarcoplasm degeneration, stimulation of satellite cells. Hematoxylin and eosin (HE) staining; x480.

groups and between the samplings were observed during the second and third sampling. The activity of this enzyme in serum of animals from the control group remained stable throughout the experiment (Table 2).

The total serum LDH activity in the experimental kids was increasing successively during the experiment. It was the highest in the last sampling and was statistically significant ($p \leq 0.01$) in relation to the initial value and to that determined in the control group (Table 2). Serum LDH activity in the control group was similar in all samplings.

Total LDH muscle activity was statistically significantly higher ($p \leq 0.01$) in kids from the experimental group (Table 3). Five isoenzymatic LDH fractions were obtained during the isoenzymatic separation of muscle tissue homogenates in both groups. The LDH_1 fraction showed the lowest activity and the LDH_5 the highest. Statistically significantly higher ($p \leq 0.01$) activity of LDH_4 and LDH_5 fractions was observed in the muscle tissue of experimental kids (Table 3).

Histopathological examinations of muscle samples in the experimental animals stained using HE method showed the presence of numerous muscle fibers with necrosis, decomposition of the sarcoplasm and loss of the cross striation. These changes were accompanied by various degrees of phagocytic infiltration and stimulation of myogenic cells (Fig. 1, Fig. 2). Changes characterized by focal necrosis of muscle fibers and segmental positive reaction in muscle filaments were found with HBFP staining (Fig. 3, Fig. 4). Normal muscle fibers with preserved cross striation and numerous myogenic cells were observed in muscle samples of the control group stained with HE (Fig. 5), while the HBFP reaction was negative and no necrotic fibers were found in the control animals (Fig. 6).

Discussion

Serum total protein concentration was slightly lower in kids suffering from WMD compared to the control kids. This difference was especially noticeable at the end of the experiment. Lowering protein concen-

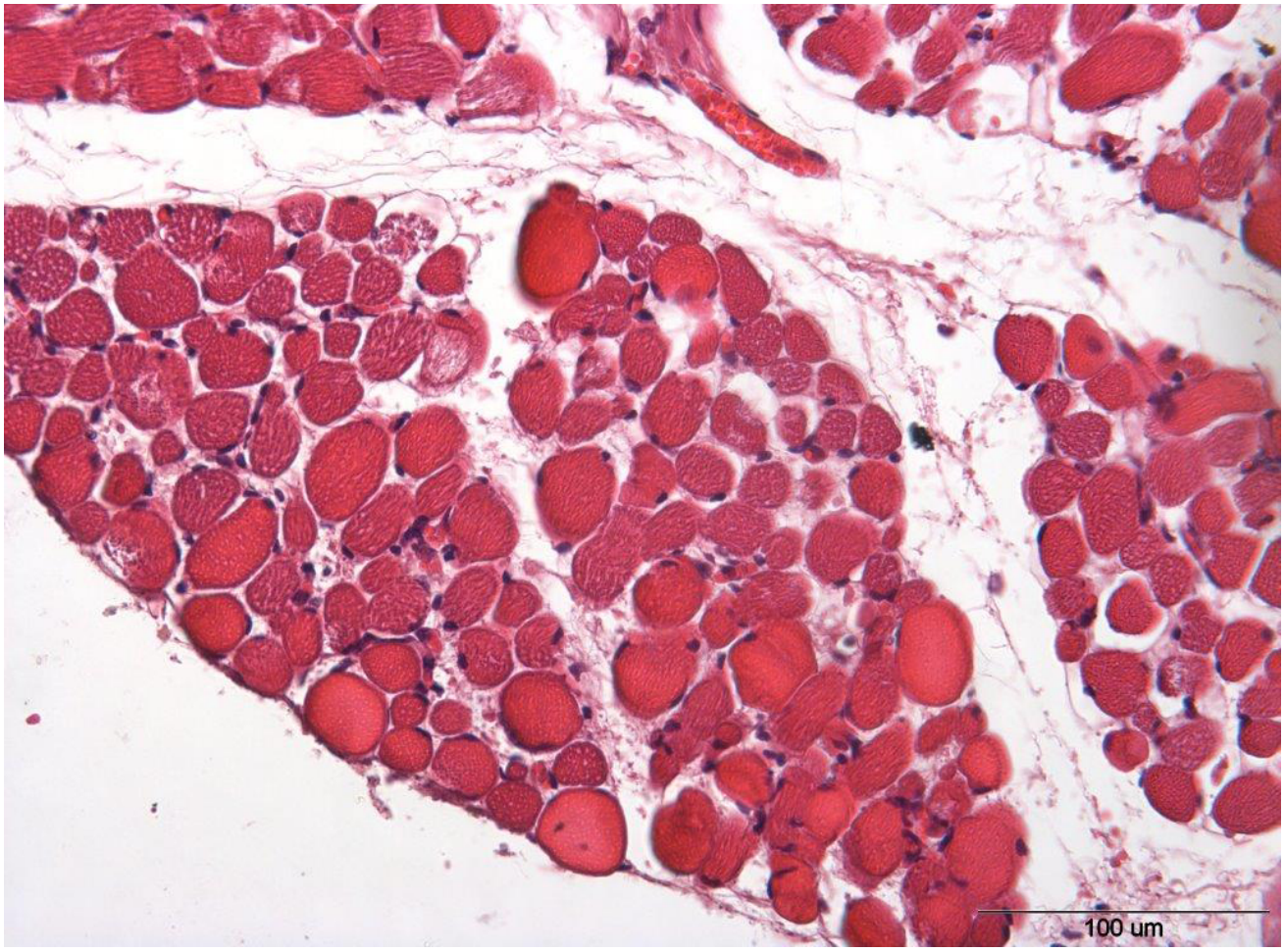

Fig. 2. Pathological changes in the biceps femoris muscle tissue of a goat kid with symptoms of white muscle disease. Giant muscle fibers with sarcoplasm hyalinisation. HE staining, x 240.

tration in the experimental group may be associated with poorer feed intake due to worsening symptoms of the disease. Similar results were observed by Abutarbush and Radostits (2003) in research on congenital nutritional muscular dystrophy. They found statistically lower total protein content and weakened immune status in sick calves, which were associated with impaired immunoglobulin uptake due to impaired sucking reflex.

The glucose level was the highest in the first sampling in both groups, then gradually decreased and during the last sampling it was statistically significantly lower in the experimental group. The highest glucose level in all kids at the beginning of the experiment is associated with intake of milk with a high lactose content - average 4.1% (Park et al. 2007), while the subsequent decrease is due to the intensive growth of animals and increasing energy demand. Similar results of glucose level in correlation with the age were also described by other authors (Tacchini et al. 2006). A statistically significant difference in glucose concentration between the groups at the end of the experiment should be explained by reduced appetite and poorer feed intake of kids with muscular dystrophy. Some authors (Mueller et al. 2003) mentioned the possible direct effect of selenium supplementation on glucose metabolism. Sheng et al. (2005) in studies conducted on mice with the type two diabetes confirmed that supplementation with sodium selenate induces glucose metabolism. Mueller et al. (2003) demonstrated an increase in glucose level in mice after selenium supplementation, but the dose used in that study was very high (approx. 2 mg/kg body weight daily) and close to the doses causing acute poisoning in most animals. In our research, the direct effect of selenium supplementation on increase in the glucose level in the control group should be excluded.

The level of triglycerides in the experimental group decreased significantly in the second sampling, and then increased at the end of the experiment, while the control animals had a non-significant decrease of this parameter in subsequent samplings. The decrease in this parameter in the first group during the second sampling should be explained by the reduced feed

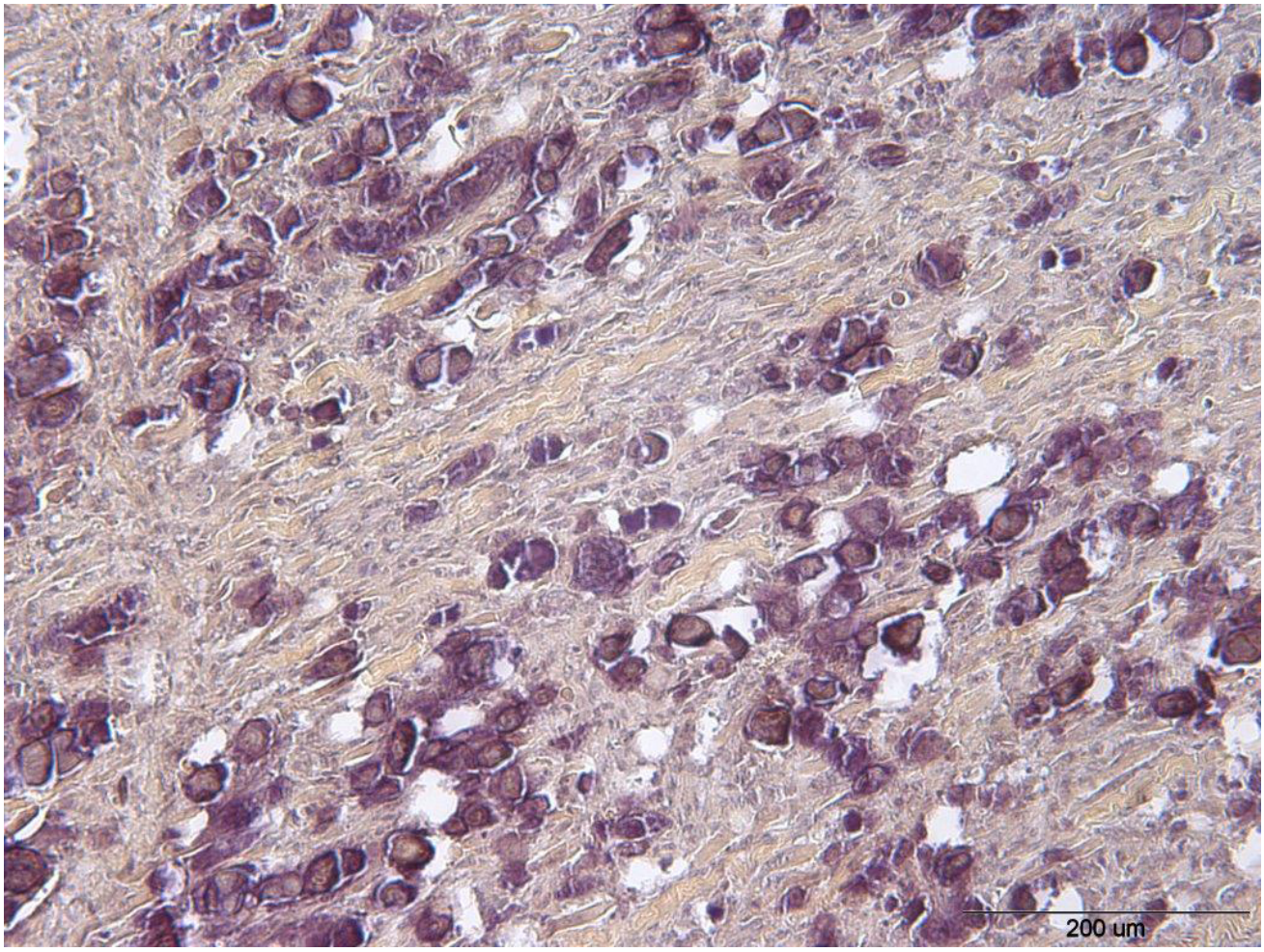

Fig.3. Extensive focal necrosis of muscle fibers in goat kid with WMD. Hematoxylin-basic fuchsin-picric acid (HBFP) staining; x120.

intake associated with the worsening of symptoms of muscular dystrophy, and the significant increase during the last sampling may be due to the lipolysis of fatty tissue associated with white muscle disease. However, a gradual decrease in serum triglyceride level of the control (healthy) kids is reported by other authors (Brzezińska et al. 2005) and is a physiological process associated with animal growth.

Throughout analysis of the cholesterol level in both groups, a decrease in animals with WMD in the course of the entire experiment and slight fluctuations in the control group were found. However, its level in healthy animals was significantly higher in the last sampling compared to the experimental group. The decrease in cholesterol in kids with symptoms of WMD may indicate disease-related disorders of cholesterol synthesis associated with liver dysfunction. Studies on rats indicate the key role of selenium and vitamin E in maintaining the integrity of the liver tissue, which is manifested by stimulation of regenerative processes and an increase in serum cholesterol level in individuals supplemented with selenium and vitamin E (Zhang et al. 1996). A decrease in the cholesterol level in the control kids were small and was correlated to the age of the animals.

The average serum selenium content in the experimental kids was definitely lower than in the control group. The content of this element slightly decreased with age and the intensification of the muscular dystrophy symptoms. Literature data are quite divergent in determining the normal level of Se in ruminant serum or plasma. According to Pugh (2002), the selenium level in healthy kids should be around 100 µg/l of serum but Pavlata et al. (2005), on the other hand, claim that 65 µg/l of serum is completely sufficient.

Many authors (Bickhardt et al. 1999, Ramirez-Bribiesca et al. 2005) point out the role of Se deficiency in the development of muscular dystrophy in goats. The Se level in the experimental group determined in our study was very low and indicated severe hyposelenaemia. The serum selenium level was statistically higher in the last two samplings in the control kids. Se supplementation in the form of intramuscular injection of sodium selenite increased serum selenium

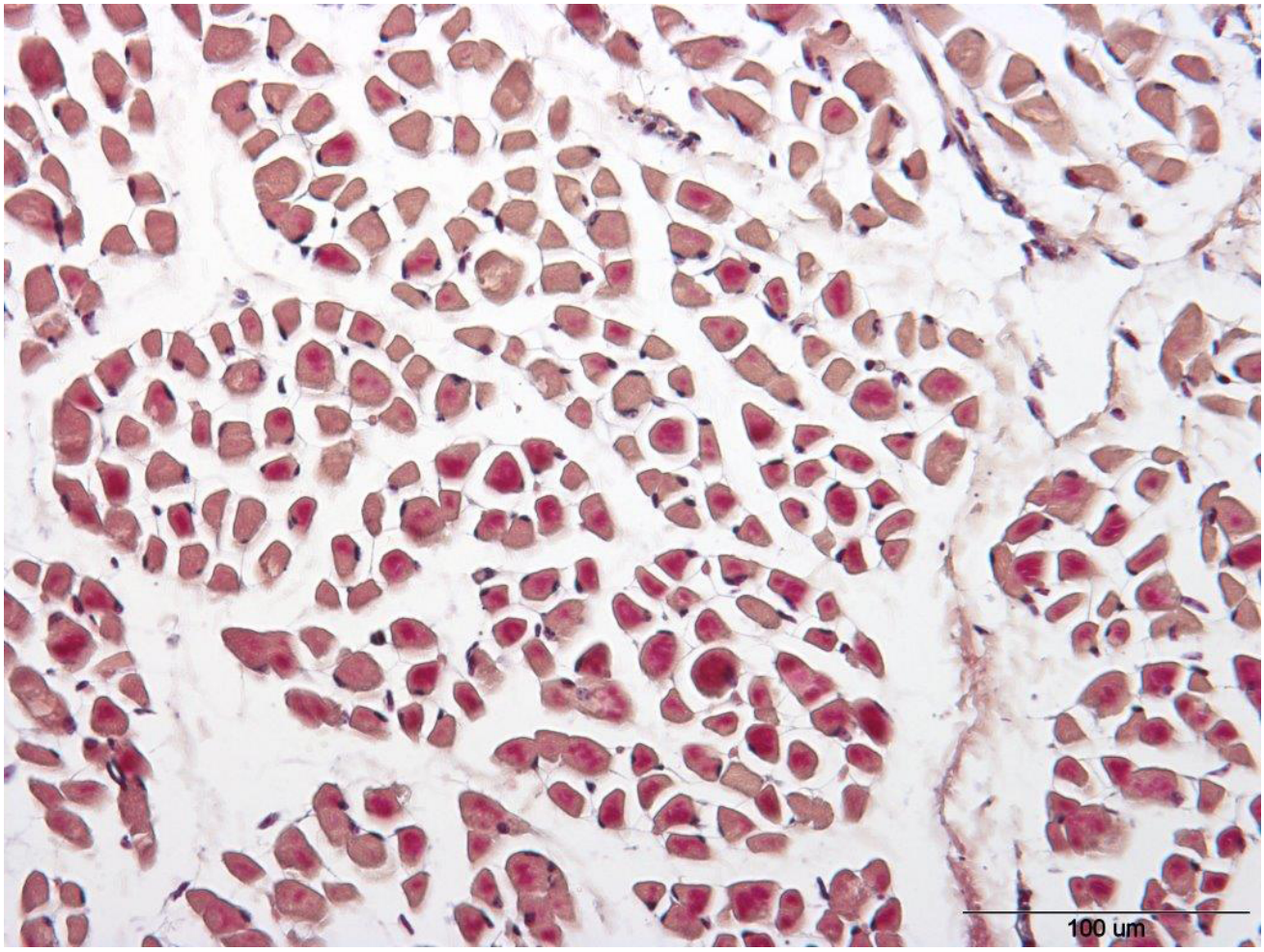

Fig. 4. Segmental positive reaction in muscle filaments of goat kid with WMD. HBFP staining; x240.

concentration in these kids. The highest selenium level was recorded in the second sampling, i.e. two weeks after supplementation. In the next sampling the level of this element slightly decreased. The highest Se level - approx. 43.9 µg/l was not very high and according to the literature cited above, it was within the values considered as physiological for this species. The dynamics of changes in serum Se demonstrated in this study does not differ from those observed in other studies (Weiss et al. 1983, Sobiech and Kuleta 2002).

Serum vitamin E levels in the control kids were statistically higher than in the experimental kids in the last two samplings. The content of a-tocopherol decreased with the age and the severity of symptoms in kids that were not supplemented with vitamin E and selenium. A similar tendency was observed in healthy kids - the highest serum vitamin E level was observed in the first sampling (3 days after supplementation), in the subsequent period there was a slight decrease in its content, but it remained at a significantly higher level than in the experimental group. The serum vitamin E content in young ruminants depends on many factors. One of the most important ones is its very low placental permeability (Gabryszuk and Klewiec 2002), and therefore the main source of this vitamin for young organisms is colostrum, and later milk. Many studies (Pavlata et al. 2004, Yang et al. 2004) showed that milk from vitamin E deficient dams was not a sufficient source of this dietary component for their offspring. Another important factor affecting the serum tocopherol level is its mechanism of transport in the body (Traber et al. 1990, Yang et al. 2004). The serum level of vitamin E is also influenced by the liver (it is its storage in the body) (Schaefer et al. 1995).

Analysing determined vitamin E values in kids with WMD symptoms, it should be stated that in all samplings a deficiency of this component was observed. Literature data on the correct level of a-tocopherol in ruminant serum varies a lot. Gabryszuk and Klewiec (2002) indicate that the level of vitamin E needed for the proper functioning of the sheep's body is about 4 µg/ml. The physiological content of vitamin E in goat's serum is between 3.5 µg/ml and about 15-20 µg/ml (Yang et al. 2004). The role of vitamin E

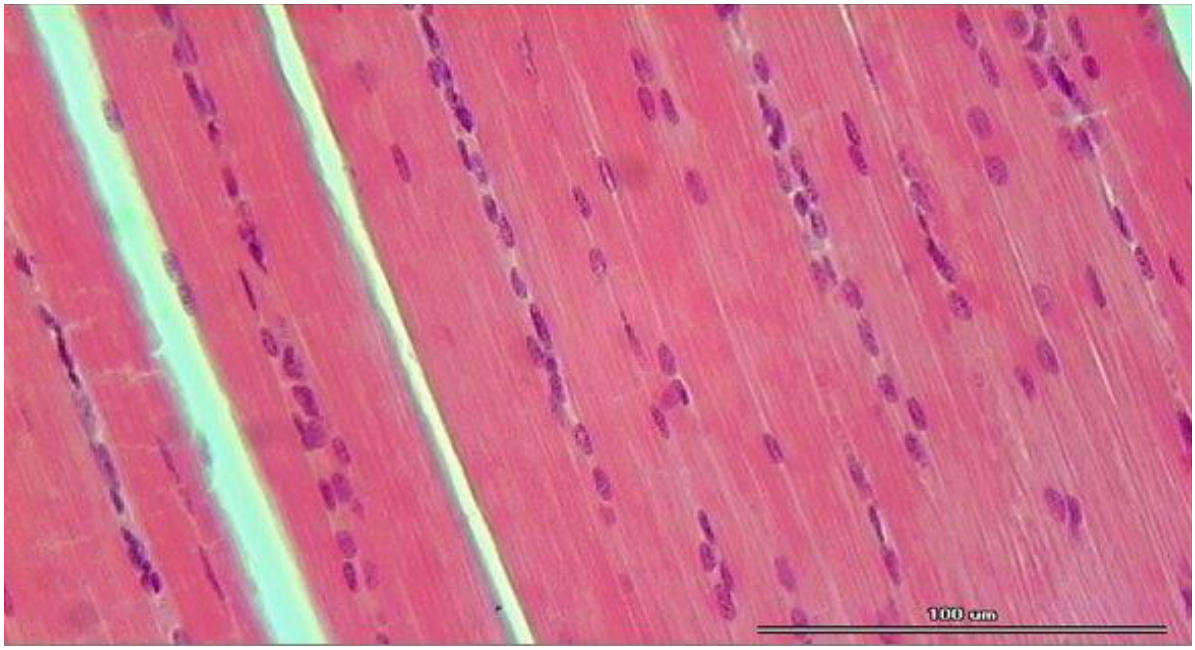

Fig 5. Normal structure of the muscle tissue in the control goat kid. HE staining; x240.

deficiency in the development of nutritional muscular dystrophy has been undeniably proven (Bickhardt et al. 1999, Kozat et al. 2007) and it was found, that this deficiency was usually associated with inadequate selenium level. A positive correlation between a decrease in vitamin E serum content and the intensification of nutritional muscular dystrophy symptoms was noted in our research, which confirms the thesis that a-tocopherol deficiency is one of the main causes of this disease.

Higher serum vitamin E levels in healthy animals were associated with supplementation with Se and a-tocopherol. Studies by other authors (Hidiroglou et al. 1990) showed that after parenteral administration, natural forms of tocopherols (D-α-tocopherol) were more absorbable than synthetic forms (i.e. DL-α-tocopherol). A commercial preparation containing tocopherol acetate was used in our research, due to its universality and availability on the market. In the discussed experiment, the highest serum vitamin E concentration was observed in the first sampling (3 days after supplemetation), next, its content began to gradually decrease in subsequent samplings, but in the control group remained at a higher level than in the experimental animals.

The activity of glutathione peroxidase (GSH-Px) in experimental kids decreased with the appearance of muscular dystrophy symptoms. Peroxidase activity level, below which symptoms of nutritional muscular dystrophy occur, have not yet to be established for goats, but it could be assumed that they are similar to those considered typical for sheep i. e. 25 U/gHb (Hudman et al. 1988). The results of our study confirmed this thesis, because GSH-Px activity in kids with WMD symptoms decreased to 17.3 U/gHb. Determination of the activity of this enzyme is considered an indirect way of assessing the Se level in the body. Research conducted by Sanchez et al. (2007) and Beytut et al. (2002) showed that GSH-Px activity better reflects Se level in the body in cases of deficiency than in the cases of normal supply with this element.

The control group showed a steady increase in glutathione peroxidase activity on days 15 and 25 after selenium supplementation. These results correspond with data obtained by other authors (Sanchez et al. 2007), who revealed an increase in glutathione peroxidase activity at the same time after selenium compounds administration. Analysing the correlation between serum Se content and peroxidase activity in this group of kids, despite a decrease in Se level, a further increase in GSH-Px activity was noted (last sampling). These data confirm the fact stated by other researchers (Milad et al. 2001), that the increase in peroxidase activity is associated with an increase in the Se serum content only until the body reaches a sufficient level of this trace element, because later such correlation no longer takes place.

Serum AST in the experimental kids increased in the course of the experiment, and was highest in the last sampling, when symptoms of WMD were clearly visible. The mean aspartate aminotransferase activity in the last sampling (201 U/l) exceeded the physiological norms for kids (Anbarasu et al. 2004) and was similar to the values obtained by other authors in goats with myopathy (Bickhardt et al. 1999). Some authors (Andres et al. 1996, Bickhardt et al. 1999) pointed

Fig. 6. Negative HBFP stain response (no necrotic fibers) in the muscle tissue of the control goat kid. HBFP staining; x240.

to a small, but statistically significant and diagnostically useful increase in AST activity in small ruminants with WMD. These authors mentioned the correlation between the increase in AST activity and the worsening of disease symptoms, which was also found in our research. The serum activity of aspartate aminotransferase in the control goats slightly increased during the experiment, but remained within the norms recognized as physiological for this species.

The enzyme with the highest affinity for muscle tissue is creatine kinase, considered as the most sensitive diagnostic indicator of diseases causing muscle damage (Kohli et al. 2005). In our study, there was a clear increase in serum activity of creatine kinase in the last two studies in kids that showed clinical signs of nutritional muscular dystrophy, and this increase was highest at the end of the experiment. The kinase activity increased significantly in the second sampling, which indicates the increasing muscles degeneration, and in the third sampling the activity of this enzyme was almost eight times higher than the values from the beginning of the experiment, and the control group. The mean serum CK activity in these kids in the third sampling reaching 836U/l was quite high, but lower than that in similar cases reported by other authors (Pavlata et al. 2001). Bickhardt et al. (1999) based the diagnosis of clinical form of nutritional muscular dystrophy in goats on a serum creatine kinase activity above 300 U / L, i.e. values similar to those obtained in our research. Serum CK activity in the control animals remained at physiological level throughout the experiment.

The total serum lactate dehydrogenase activity in the experimental group increased during the course of the experiment and in the last sampling was significantly higher than that found in the control animals. Literature data indicate a much greater increase in the activity of this enzyme in muscle dystrophies (El-Neweehy et al 2001, Ludvikova et al. 2007) than in liver diseases or gastric disorders (Mona et al. 2010, Marutsova and Binev 2018), which indicates a greater affinity of LDH for muscle tissue than for other organs. The increase in total lactate dehydrogenase activity in the experimental kids indicates the presence of dystrophic changes in muscle tissue and corresponds to the observed clinical signs. Serum LDH activity in control group remained within the reference values for the species at a similar level throughout the experiment and did not differ from results obtained in previous own studies (Sobiech et al. 2005).

The total LDH activity in homogenates obtained from samples of the biceps femoris was significantly

higher in the animals with symptoms of the disease than in the control group. The obtained LDH activity was very high and indicated a significant damage to the muscle tissue. Literature data (Salplachta and Necas 2000) indicates, that the activity of LDH in tissues can be about 100-150 times higher than that in serum. The highest activity of this enzyme in ruminants was recorded in the muscle tissue, which indicates quite high specificity of LDH. The results of muscle LDH activity correspond to the total serum activity of this enzyme (higher muscle LDH activity was accompanied by a significant increase in serum activity), indicating the usefulness of serum LDH activity in diagnosing muscular dystrophies.

In the LDH isoenzymatic separation in the muscle tissue, all five LDH fractions were determined in both groups. There was a significant increase in the activity of the LDH_5 isoenzyme, which was accompanied by a statistically significant decrease in the activity of the LDH_4 fraction in the experimental group (compared to the control group). The activity of LDH_1 and LDH_2 isoenzymes in samples of all tested animals was similar and remained at a very low level. The obtained results of LDH isoenzymatic separation clearly indicate the affinity of cathode fractions (LDH_4 and LDH_5) for muscle tissue in ruminants, which was previously demonstrated by other authors (Salplachta and Necas 2000). A pronounced increase in LDH_5 muscle activity in homogenates of the biceps femoris in kids with white muscle disease indicates damage to the examined tissue. The simultaneous decrease in LDH_4 activity associated with this increase has no diagnostic significance, as it is associated with the dominance of the muscle fraction – LDH_5 at this time. The LDH isoenzymatic separation in the muscle tissue of control animals was similar to that observed in ruminants by other authors (Salplachta and Necas 2000).

Morphological changes were found in numerous muscle fibers in histopathological preparations from the biceps femoris in the kids with symptoms of muscular dystrophy. Dead fibers with a homogeneous hyaline cytoplasm or those that decayed into different size segments or granularity were observed, and the preserved fragments did not show the transverse striation. The morphological changes found in muscles of the experimental kids indicate the presence of NMD, which is dominated by muscle waxy necrosis (also called Zenker necrosis), described for the first time in 1864 by Zenker in a patient who died of typhoid fever (Helliwell 1999), and characterized by the presence of fibers with a hyaline sarcoplasm. Similar changes in muscles have been described in ruminants by Beytut et al. (2002).

Degenerative changes in muscles are accompanied by regeneration processes that start very quickly after muscle fiber damage. Many authors (Helliwell 1999, Yavuz 2017) showed that muscle fiber regeneration can occur as soon as 1-2 days after damage. These studies demonstrate that the process of fiber renewal must be preceded by the removal of the dead tissue by infiltrating phagocytic cells. Along with this infiltration, myogenic cells appear. The presence of myogenic cells and as well as the formation of young muscle fibers were found in our research. According to some authors (Beytut et al. 2002, Yavuz 2017), both necrotic and regenerative processes are characteristic of nutritional muscular dystrophy. Significant intensification of these processes in muscles of the experimental kids contributed to the formation of characteristic clinical symptoms and denoted to the disease progression in these animals.

In summary it can be concluded, that data presented in this study indicates, that during the course of WMD in goats hypoglycaemia, hypotrigliceridemia and hypocholesterelemia is observed. Disease is caused by significant deficiency of selenium and vitamin E, and simultaneously an increase in serum AST, CK, LDH, and a reduction in blood GSH–Px activities are observed. In the muscular tissue of sick animals elevation of total LDH activity and an increase in LDH_4 and LDH_5 activities were found. The observed structural changes in muscles indicated the presence of Zenker necrosis, which is characteristic of nutritional muscular dystrophy.

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