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SERUM BIOCHEMICAL AND HISTOPATHOLOGICAL EXAMINATIONS OF SOME TISSUES OF LAMBS WITH MUSCULAR DYSTROPHY IN VAN

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Abstract

White muscle disease (WMD), is an important disease also known as 'muscular dystrophy' in the lamb and calf. White muscle disease is the result of degeneration of skeletal and cardiac muscles in lambs. Lambs mostly affected with the congenital form either born death or die a few day after birth. The disease is a manifestation of lack of selenium, vitamin E or both. Van and surrounding villiages were visited and lambs with WMD examined. The lambs with 3-10 days of age were used as research materials. Necropsy and gross examinations was performed to all lambs. The blood samples were analyzed for Vitamin E amount, Creatine kinase (CK), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) activities. The level of Vitamin E was decreased, but the other parameters significantly increased. In heart, chest and gluteal muscle lesions in lambs were found. Tissue samples were histopathologically examined. Muscular dystrophic calcification in necrotic areas as well as hyaline degeneration and Zenker necrosis were determined. In the calcified region mononuclear cell infiltration mainly macrophages, were observed. In conclusion in lamb with white muscle disease; the activities of enzymes related to muscle health were raised drastically. In gluteal, chest and especially in heart muscle the hyalin and Zenker degeneration were noted.

Key words*: Enzymes, Muscular dystrophy, lamb, tissue, Vitamin E.*

INTRODUCTION

White muscle disease (WMD) also known as "subacute enzootic muscular dystrophy" or "stiff–lamb disease" can occur in newborn lambs, but is more commonly seen in lambs up to 3 months of age. It is seen in some areas in young sheep grazing stubble or rank, dry feed or in young sheep being maintained on hay and grain rations. This condition is associated with a vitamin E deficiency.

Deficiencies of either or both selenium and vitamin E can cause weaners couring, reduced wool production, reduced ewe fertility, reduced immune response, and white muscle disease. Selenium deficiency is more common in high rainfall areas while vitamin E deficiency occurs when sheep are on dry feed for long periods. Both of them can be provided as a supplement (McDowell, 2004).

Vitamins are essential for health of all kind living organisms. Fat soluble vitamin are vitamin E, A, D and K. Vitamin E (α tocopherol) is important as a biological antioxidant for oxidant and also required for normal cell differentiation and function (Mert, 1996). Deprivation of vitamin E causes different disturbances such as disorder of reproduction, muscle function, cardiovascular system, brain and liver. But cardiac disease is really severe problems in ruminants especially in new born animals (McDowell, 2004). Although WMD was once thought to be responsive solely to selenium it is now known to also be responsive to vitamin E. Muscle dystrophy in lamb, calf and kid is generally interacted with the Se deficiency. Skeletal muscles are the most affected tissue and it is common, but the heart lession can be seen less but it is severe. It affects cardiac function, ECG pattern changes and sudden death occurs. During the Vitamin E deficiency, usually associated with a lack of green feed, myocardial changes such as hyalinization of fiber, basophilic development and Zenker necrosis could occur (Kozat et al., 2007; Deger et al., 2008). Microscopically will show severe changes in the muscle. In other cases the muscle takes on a pale 'fish-flesh' appearance (Van Metre, 2001). Treatment is accomplished by the use of both vitamin E and selenium because the condition may be caused by a

deficiency of selenium, vitamin E or both. Since the two elements compliment each other both are used in treatment. Selenium is more important in selenium deficient areas and vitamin E in selenium sufficient areas or diets (Kennedy, 2013).

MATERIALS AND METHODS

In this study, 17 lambs with 3-10 days of age from different flocks with WMD and 10 healthy lambs, raised in Van and surrounding villages, in Se deficient areas were used as research materials.

Blood samples and postmortem tissue samples of gluteal and heart muscle were taken. Blood sera were separated and analyzed for creatine kinase, aspartate aminotransferase and lactate dehydrogenase by autoanalyzer. In addition serum vitamin E levels were spectrophotometrically measured (Martinek 1964).

Tissue samples were both evaluated grossly and histopathologically. Tissues were fixed in 10% formalin solution. embedded in paraffin wax, and then stained with hematoxylin– eosin stain, examined by light microscope.

Mann Whitney-U test was used for statistical analysis and significances between groupswere calculated.

RESULTS

The serum analysis of all lambs were shown in table 1. The individual findings were also shown in table 2.

In the pathological examination 17 lambs were used. Gross Zenker necrosis was observed in heart muscle, in gluteal and chest muscle and sometimes in two different tissues (Figure 1-3) Histopathological examination of both groups were also done.

In the WMD the heart and gluteal muscles showed similar apperances such as swollen fibers, homogeneus pink and pycnotic nuclei, hyperemic vessels and hemorrhages were observed.

Furthermore hyaline degeneration and Zenker necrosis, necrotic areas with dystrophic calcification and mononuclear cell infiltration mostly with macrophages in calcified areas were clearly seen.

Table1. Some serum biochemical parameters of WMD and healthy lambs.

Parameters	n	Healthy Lambs	n	WMD Lambs	P
Vitamin E (µg/mL)	9	1.93±0.14	8	0.518±0.060	p≤0.01
Creatine Kinase (IU/L)	9	62.10±17.5	7	2804,5±67.69	p≤0.001
AST (IU/L)	9	106.14±6.36	7	675,65 ±32.53	p≤0.001
LDH (IU/L)	9	448.7±43.80	7	987.67±54.51	p≤0.001

Table2. Individual reports for gross examination of the lambs with WMD.

Number	Ages (Days)	Locations of Lesion-1	Location of Lesion-2	Severity of Lesion
1	5	Heart Muscle		Mild
2	10	Gluteal Muscle	Heart Muscle	Medium
3	3	Heart Muscle		Medium
4	3	Heart Muscle		Medium
5	3	Heart Muscle	Gluteal Muscle	Severe
6	10	Heart Muscle		Mild
7	3	Gluteal Muscle	Chest muscle	Severe
8	5	Heart Muscle		Severe
9	3	Heart Muscle		Severe
10	5	Heart Muscle		Severe
11	3	Heart Muscle		Medium
12	8	Heart Muscle	Gluteal Muscle	Severe
13	9	Gluteal Muscle	Chest muscle	Medium
14	3	Heart Muscle		Severe
15	3	Heart Muscle		Severe
16	5	Heart Muscle		Mild
17	3	Heart Muscle		Medium
18	3	Heart Muscle		Mild
19	9	Gluteal Muscle	Chest muscle	Severe
20	5	Heart Muscle		Severe
21	5	Heart Muscle	Gluteal Muscle	Severe
22	3	Heart Muscle		Severe

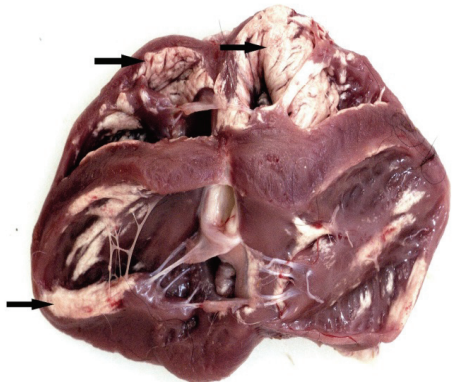

Figure 1. Zenker necrosis in heart muscle (black arrow)

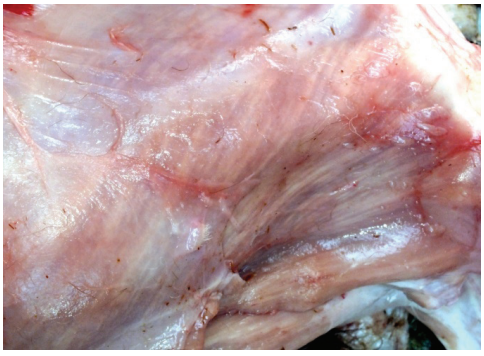

Figure 2. Zenker necrosis in gluteal muscle

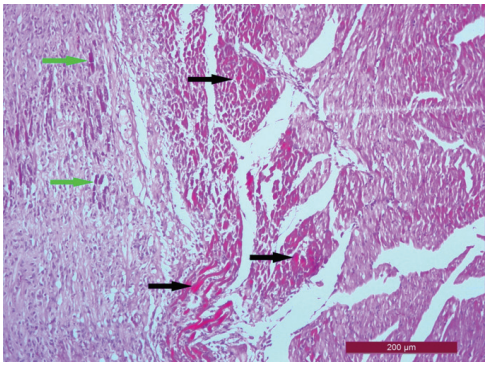

Figure 3. Dystrophic calcification (green arrow) and Zenker necrosis (black arrow) at heart muscle, and mononuclear cell infiltration. H&E Bar: 200µm.

DISCUSSIONS

Absence of Se and vitamin E cause White Muscle Disease in lambs. As known Calcium (Ca) ions is necessary for the contraction of muscle. In order to have normal muscle contraction Ca ions must go in and out of cell at necessary amounts. Excess amount of Ca are toxic for mitochondria. The peroxidation of membrane can be result of Ca infiltration to sarcoplasm and formation of mitochondrial damages in myocytes. Immidiately the energy of cells is exhausted, degeneration of myofibrils begins and intracellular Ca accumulation occurs. After these events intracellular enzymes go out to extracellular space and to blood. Troponin and AST levels increase. This events is important for the clinicopathological perspective. As result degenerative cells pass to necrosis step named Zenker necrosis. If the vitamin E levels decrease the following process is the immunodeficiency status which in secondary infection can easily occurs, immunosupression and bacterial infection can be easily seen (Hulland, 1985).

As shown in Table 1 the serum level of AST was significantly raised in lambs with WMD, $p \leq 0.001$.

Adenosin phosphate (ATP) is the universal energy currency for most of the energy requiring processes in biological systems (Lehninger, 1982). Tissues, e.g. skeletal and cardiac muscle, brain, photoreceptor cells, spermatozoa, all depend on the immediate availability of vast amounts of energy. Heart cells, which in general depend on anoxidative metabolism, energy derived from glycolysis can also contribute to the maintenance of high energy phosphate levels and contractility if oxidative phosphorylation of these cells is inhibited (Doorey and Barry, 1983).

CK activity is the greatest in striated muscle, heart tissue, and brain. The determination of CK activity is a proven tool in the investigation of skeletal muscle disease (muscular dystrophy) and is also useful in the diagnosis of myocardial infarction (MI) and cerebrovascular accidents. Increased levels of CK also can be found in viral myositis, polymyositis, and hypothyroidism (Mert, 1996). In the presented study the serum CK activity was significantly

increased ($p \leq 0.001$) there was massive degenerations in muscles.

Plasma creatine kinase (P-CK)_activities were significantly increased after physical exercise in healthy turkeys and in turkeys with genetic muscular dystrophy. Moderate exercise did not significantly affect P-CK activity in lambs. Increases in P-CK activity during expression of nutritional muscular dystrophy were readily distinguished from exercise effects; activity exceeded 160,000 mU/ml in lambs during expression of that condition. The extent of muscle damage after intramuscular administrations of some veterinary drug formulations was estimated from the total creatine kinase activity released in plasma during the 72 hours following the injection (Tripp and Schmitz 1982)

Lactate dehydrogenase (LDH) is involved in the final step of anaerobic glycolysis, catalyzes the conversion of lactate to pyruvate, consists of a system of five isoenzymes. It is not tissue-specific, being found in a variety of tissues, including liver, heart and skeletal muscle. The enzyme is tetrameric and is composed of four subunits of two molecules, M (muscle) and H (heart). Various combinations of these two molecules result in five different isoenzymes. Increased LDH5 were reported in sheep, cattle and horses, e.g. selenium and vitamin E deficient myopathy in cattle and sheep, exertional rhabdomyolysis in horses.In this research the activity of LDH was also severely incresased ($p \leq 0.001$).

Treating the heart form of white muscle disease is usually ineffective. The skeletal muscle form of the disease can be treated with supplementry selenium and/or vitamin E. Aksakal et al. (1996) tried to understand the effects of vitamin A, vitamin E, and Se in the ethiology of WMD on sheep and feed them with different diet supplemented with vitamin A+E+Se. They concluded that vitamin A like vitamin E help to maintain the normal blood paramaters in physiological levels and can be also used as prophylactic purpose. Whanger et al. (1977) reported that Vitamin E alone was more effective in the prevention of WMD than selenium alone. Ewes may be fed vitamin E prior to lambing, a therapeutic dose two to four weeks before lambing works well. In this study the vitamin E levels were low in lamb with WMD ($p \leq 0.01$).

As conclusion, muscular dystrophy is still a problem for sheep breeders. Special care and attention must give to eliminate this nutritonal disease and clinical studies must be performed to understand the changes in tissues and specimen for an applicable treatment to sick animals.

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