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Retinol, α-tocopherol and vitamin D_3 in White Muscle Disease[1)]

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Summary

Vitamins are essential for the health of all living organisms. Vitamins E, A, D and K are known as fat-soluble vitamins, and deprivation of vitamin E causes various disorders, especially in the reproduction and cardiovascular systems and in muscle functions. Vitamin A, on the other hand, has roles in various biological functions – like eyesight – and the growth, reproduction and differentiation of epithelial cells. Vitamin A deficiency leads to the keratinization of the epithelium, and disorders related to the metaplasies of the genital and genitourinary systems. Conversely, vitamin D is defined as a pro-hormone and is responsible for Cahomeostasis, and thus indirectly affects the bone metabolism, bone structure, and cellular and neural functions of Ca. White Muscle Disease (WMD) can occur in newborn lambs, but is more commonly seen in lambs of up to 3 months of age. In this study, 30 lambs of 3 to 50-days-old from different flocks diagnosed with White Muscle Disease (WMD) were selected as research material, while the control group consisted of 8 healthy lambs. With the aim of clarifying the cause of WMD, serum fat-soluble vitamins, retinol, α-tocopherol and vitamin D3 levels were determined in 16 lambs. Gluteal and heart musclet issue samples also were taken from 30 lambs with WMD. The vitamin levels of the samples were analysed by HPLC. The levels of serum α-tocopherol, retinols, and vitamin D3 were foundto be low in the diseased animals, but only retinol ($p < 0.001$) and α-tocopherol ($p < 0.001$) level differences were statistically relevant. Macroscopically, Zenker's necrosis was determined in the heart muscles of 17 lambs, and in the gluteal and chest muscles of 6 lambs. 7 lambs displayed necrosis in both their heart and in gluteal muscles. The samples were analyzed microscopically to reach similar findings: swollen homogeneous pink muscles, pycnotic nuclei, and hyperaemic and haemorrhagic blood vessels in gluteal, chest and heart muscles. Hyaline degeneration and Zenker's necrosis, dystrophic regions in necrotic areas, cc was detected as a severe disease in lambs at an early stage of life with advanced degeneration in different muscle tissues. Deficiency of fat-soluble vitamins was also detected in the sick animals. Control group lambs had higher levels of α-tocopherol and retinol ($p < 0.001$) compared to the sick lambs.

Keywords: α-tocopherol, retinol, vitamin D_3, White Muscle Disease, Zenker'snecrosis

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 on 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. Selenium and vitamin E deficiencies, or both, can cause weaner ill thrift, reduced wool production, reduced ewe fertility, reduced immune response, and white muscle disease. Selenium deficiency is more common in areas with high rainfall while vitamin E deficiency occurs when sheep are on dry feed for long periods. Both of them can be provided as feed supplements (6, 10, 16).

Vitamins are essential for the health of all kind living organisms. Fat soluble vitamins are vitamin E,

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A, D and K. Vitamin E (α-tocopherol) is important as a biological antioxidant for oxidant and also necessary for normal cell differentiation and functioning (4, 12). The increase of oxidative stress causes the decrease of antioxidant vitamins such as vitamin E (2). The deprivation of vitamin E causes various disturbances such as disorder of reproduction, muscle functioning, cardiovascular system, brain and liver. But heart disease is a very severe problem in ruminants, especially in newborns (3, 10). Although WMD was once thought to be responsive solely to selenium it is now known that it also reacts to vitamin E. Muscular dystrophy in lambs, calves, kids is generally related to Se deficiency. Skeletal muscles are the most affected tissue and this is common, but while the heart lesion may be observed less frequently it is severe. It affects cardiac functioning, ECG pattern changes and sudden death occurs. During vitamin E deficiency, usually associated with a lack of green food, myocardial changes such as hyalinization of the fiber, basophilic development and Zenker's necrosis may occur (5, 9). Under the microscope severe changes in the muscle will be seen. In other cases, the muscle takes on a pale appearance of "fish-flesh" (13). Treatment is accomplished by the use of vitamin E and selenium because the condition can be caused by a deficiency of selenium, vitamin E or both. Since the two elements complement each other, both are used in the treatment. Selenium is more important in selenium deficient regions and vitamin E in selenium-rich areas or diets (8).

Vitamin A is a general name for all carotenoids that show the activity of vitamin A in the biological world. All-trans retinol of β-carotene is the predominant and most stable form in nature. Ruminants derive retinol from the ingested plant carotenoids. Vitamin A has different biological functions such as vision, growth, reproduction and differentiation of epithelial cells. In case of vitamin A deficiency keratinization of the epithelium, disturbances, metaplasies of genital and genitourinary system occur.

Cholecalciferol is a form of vitamin D and has a similar structure to that of steroid hormones. Sometimes vitamin D is defined as prohormone and is responsible for the Ca homeostasis of bone metabolism, bone structure, maintain cellular and neural Ca functions (10). The classic function of vitamin D is to increase the intestinal absorption of calcium for the mineralization of bone. The active form of vitamin D, 1.25-dihydroxyvitamin D (1.25(OH)2D), acts as a steroid hormone by binding to the vitamin D receptor (VDR) that is present in many cells of the body including cardiomyocytes, vascular smooth muscle and endothelium (11).

In this study, levels and interactions of fat soluble vitamins with WMD and the pathological examinations of the affected muscle were the aim of the investigations.

Material and methods

Thirty lambs aged 3 to 50 days from different flocks with WMD and 8 healthy lambs were used as research material in the study. Their blood samples and postmortem gluteal and cardiac tissue samples were collected according to ethical guidelines. Tissue samples were evaluated macroscopically and histopathologically. Tissues were stored in 10% formalin solution, and samples were embedded in paraffin and then sliced (4 μm), stained with Hematoxylin and Eosin dyes, examined by a light microscope. In addition, serum levels of retinol, vitamin E and vitamin D were determined by HPLC (14, 15, 18). The Mann Whitney-U test technique was used for statistical analysis and meaning between groups were calculated.

Results and discussion

The results of fat soluble vitamin levels of lambs with WMD and the healthy control are presented in Table 1. The amounts of retinol of the sick group were lower than the controls and statistically significant differences were found ($p < 0.001$). The average concentration of α-tocopherol between groups was also statistically significant. Control lambs exhibited a higher level of α-tocopherol ($p < 0.001$) then the diseased lambs.

Tab. 1. Fat soluble vitamins levels of lambs with WMD and the healthy control (n = 8)

Parameters	Group	$\bar{x} \pm S_x$	p
Retinol (μg/mL)	Control	1.019 ± 0.126	0.001
	Diseased	0.769 ± 0.048	
α-Tocopherol (μg/mL)	Control	2.199 ± 0.263	0.001
	Diseased	1.762 ± 0.260	
Vitamin D_3 (μg/mL)	Control	0.021 ± 0.006	0.074
	Diseased	0.016 ± 0.006	

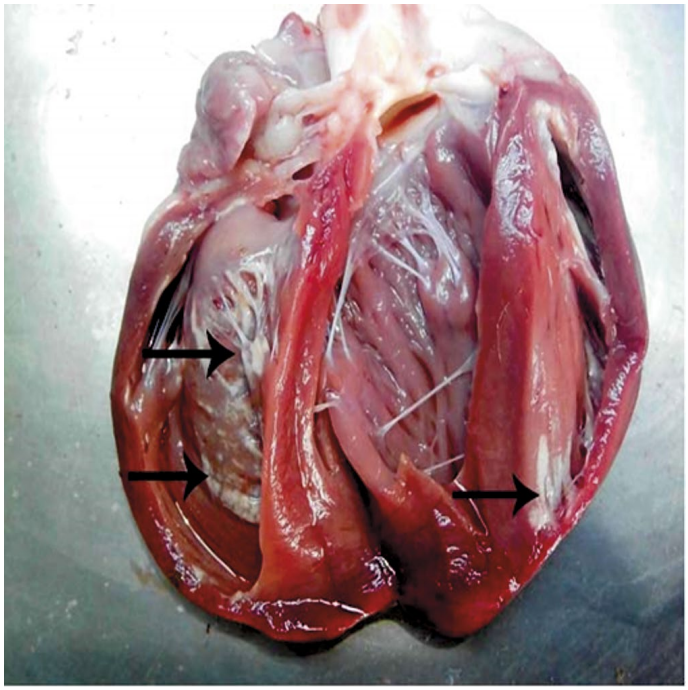

Fig. 1. Zenker's necrosis in heart muscle (black arrow)

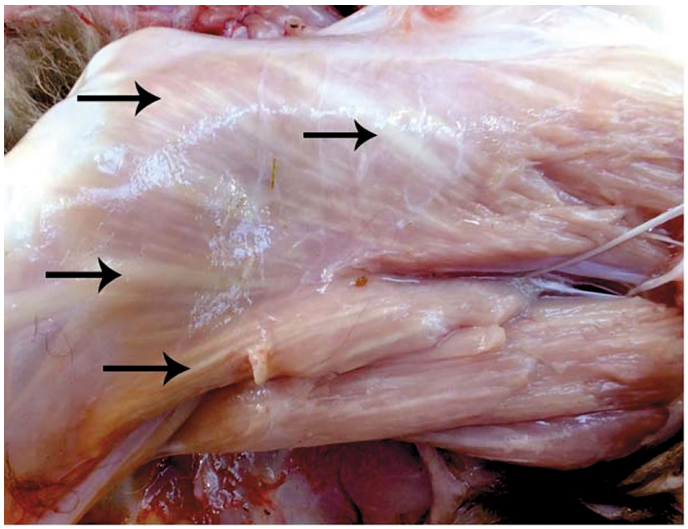

Fig. 2. Zenker's necrosis in gluteal muscle (black arrow)

Fig. 3. Gluteal muscles (section side) Zenker's necrosis in lambs with WMD

In the pathological examination 30 lambs were used. Macroscopically Zenker's necrosis was observed in the heart muscle of 17 lambs, in gluteal and chest muscle of 6 lambs and in the heart and gluteal muscle of 7 lambs (Fig. 1-3). Histopathological examinations of both groups have also been done. In the WMD the heart and gluteal muscles showed similar appearances, such as swollen muscles, homogeneous pink and pycnotic nuclei, hyperemic and hemorrhagic vessels were observed. These histopathological findings were similar to previous studies. However, in this study, densities of densely calcined and mononuclear cells were found to be higher than those of the control (Fig. 4, 5).

The status of Vitamin D is better determined by serum 25 (OH)D as opposed to 1.25 (OH)2D. Its long circulating half life (~3 weeks versus ~8 hours), the concentration of 25(OH)D is 1000 times higher in circulation compared to 1.25 (OH)2D (ng/mL vs pg/mL) and the production of 1.25 (OH)2D is mainly under the influence of PTH which tightly regulates calcium levels. Thus, levels of 1.25 (OH)2D could be elevated in individuals with severe vitamin D deficiency in order to maintain normal serum calcium levels. As a mediator of cardiovascular disease, it is believed that 25 (OH)D is thought to be the best biomarker describing vitamin D status, although this has not been proven (7). Vitamin D3 levels decreased in diseased lambs but no statistical significance was found ($p \geq 0.05$).

Treatment of the cardiac form of white muscle disease is usually ineffective. The muscular form of the disease can be treated with additional selenium and/or vitamin E Aksakal et al. (1) tried to understand the effects of vitamin A, vitamin E, and Se in the ethology of WMD on sheep and feed them a with different diet supplemented with vitamin A + E + Se. They concluded that vitamin A, like vitamin E, helps maintain normal blood parameters in physiological levels and can be also used prophylactically. Vitamin E alone has been reported to be more effective than selenium alone in the prevention of WMD (17). A ewe can receive vitamin E prior to lambing. A therapeutic dose of two to four weeks before lambing works well. Although there were

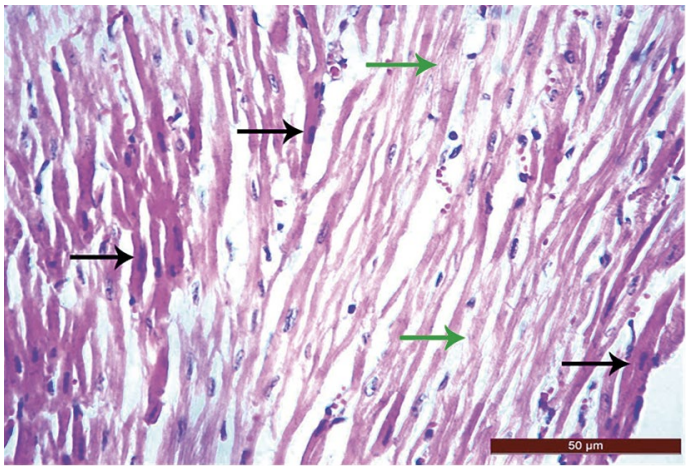

Fig. 4. Hyaline degenerations (green arrow) and Zenker's necrosis (black arrow) at heart muscle. H&E Bar: 50 µm

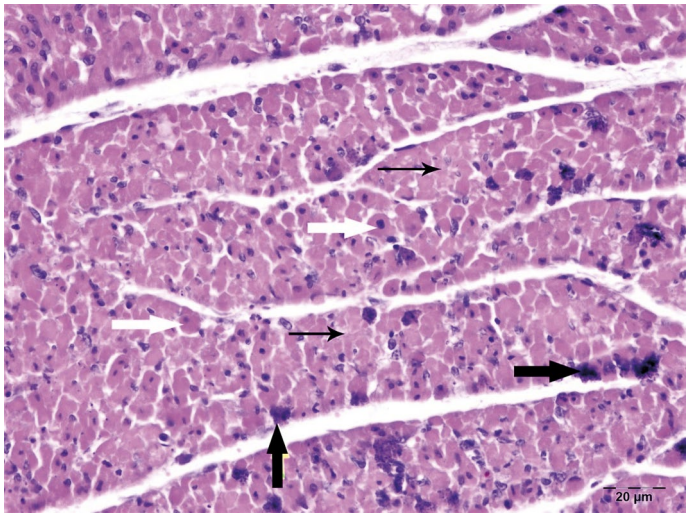

Fig. 5. Hyaline degenerations (thin arrows), Zenker's necrosis (write arrows) and dystrophic calcification (Thick black arrows) at heart muscle. H&E Bar: 20 µm

some lambs with lesions in their research, vitamin E maintained the plasma enzyme activities at low levels in the sheep examined. In contrast, selenium alone did not maintain the activities of plasma enzymes at low levels. Of the tissues examined, the highest activity of glutathione peroxidase was found in the heart. Vitamin E had no influence on blood selenium levels or on the tissue activity of glutathione peroxidase.

Vitamin D is an important pro-hormone for the optimal absorption of intestinal calcium for the mineralization of bones. Since the vitamin D receptor is present in several tissues, there has been interest in evaluating other potential functions of vitamin D, particularly in cardiovascular diseases. Vitamin D deficiency is associated with an increased risk of cardiovascular disease, including hypertension, heart failure and ischemic heart disease. Ca is also an important parameter for WMD pathogenesis. Excess amounts of Ca will cause an excessive contraction of the myofibrils, then a degeneration will form in muscles. Vitamin D deficiency will promote these degenerative processes (7).

In conclusion, all vitamins analyzed in serum were low in lambs with WMD. Affected sheep will improve if they are transferred to a pasture containing green feed. Vitamin E in green feed usually corrects the deficiency in a week. White muscle disease can be successfully treated by administering selenium/vitamin E injections. To prevent significant economic losses of farmers, our results support the importance of a balanced diet and vitamins in livestock.

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