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Evaluation of Various Biochemical Parameters in the Serum and Tissues of Lambs with White Muscle Disease

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ABSTRACT

Background: In this study, the effects of White Muscle Disease (WMD) on certain blood parameters, MDA and GSH levels in liver and thyroid tissues and the thyroid hormones were investigated in sheep.

Methods: Sixteen lambs (8 with WMD, 8 controls) of similar age, 3 to 50 days old, selected from the same region were used in the study. Blood samples were taken from all animals. One ml Vitamin E + Se were injected (I/M) to the lambs with WMD and blood and tissue samples were collected after the treatment as well. The serum Ca, P, T3, T4, albumin, globulin, total protein, glutathione (GSH) values were analyzed by an auto-analyzer. Malondialdehyde (MDA) and GSH amounts of the liver and thyroid tissues were also determined.

Result: The low Ca ion amount in lambs with WMD was found to have increased after injections of vitamin E + Se. P levels followed the same pattern. Total protein, albumin and globulin levels increased after treatment. It was determined that there was a decrease in T3 levels and an increase in T4 levels in patient lamb sera. The levels of GSH in both the liver and thyroid tissues were found to have decreased and statistical significance (at what level) was only found for the liver GSH levels. MDA levels in the hepatic and thyroid tissues were found to have slightly increased in the WMD group. In conclusion, serum differences in Ca, P, albumin, globulin, total protein, T3, T4, GSH, GSH and MDA levels were statistically significant between the groups.

Key words: Blood parameters, Glutathione, Liver, Malondialdehyde, Thyroid, White muscle disease.

INTRODUCTION

White muscle disease (WMD) or muscular dystrophy is an enzootic, nutritional disease caused by selenium and vitamin E deficiency. It has been reported that the disease, which is characterized by hyaline degeneration in skeletal muscle, cardiac muscle and diaphragm, causes great economic losses (Or *et al*. 2003). The disease occurs on a variety of regions with soils like volcanic, deep sedimentary, calcareous and basalitic soils. Soils that produce grazing grasses with low selenium (Se) content are potential causes to predisposition of grazing species to the disease (Hulland, 1985; Mcdowell, 1992; Beytut *et al*. 2002). Selenium and vitamin E deficiencies, or both, can cause growth retardation, decreased wool production, reduced sheep fertility, decreased immune response and white muscle disease. Selenium deficiency is more common in high rainfall areas, while vitamin E deficiency occurs when sheep are fed dry feed for a long time (Kozat *et al*. 2007; Mert *et al*. 2018).

Selenium and vitamin E have important roles in protecting cell membranes against oxidants that cause oxidation of cellular structures in the organism (Van Metre, 2001). The researchers suggested that vitamin E acts as an immune enhancer by increasing disease resistance and also has a positive effect on the feed conversion rate and liver function test of desi laying birds (Abbas *et al*. 2019). Organic selenium supplementation has been reported to reduce the incidence of white muscle disease in lambs born from Awassi sheep (Yavuzer and Bengisu, 2014). Selenium enters the structure of the enzyme glutathione peroxydase (GSH-Px), which is involved in the catabolism of hydrogen peroxide, one of the reactive oxygen species that causes oxidation of unsaturated fatty acids and sulfhydryl groups in cell membranes (Miller *et al*. 1993; Van Metre, 2001; Karapınar and Yılmaz, 2007). In WMD, lipid peroxides and hydrogen peroxide normally occurring in the body cannot be removed from the muscles due to decreased GSH-Px activity, which is caused by deficiencies of Se and antioxidant vitamin E (İmren and Şahal, 1991). As a result, the increased amount of free radicals and hydro- or lipo-peroxides cause

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damage in cells (Değer *et al*. 2008; Pamukçu *et al*. 2001). Osame *et al*. (1990) found a positive correlation between low serum Se and tocopherol levels measured in lambs with white muscle disease and a decrease in GSH-Px enzyme activities. Turgut (1995) reported that the GSH-Px enzyme protects the liver, kidney and pancreas from necrotic degeneration and drew attention to the relationship between the activity of this enzyme and selenium concentration.

The absence of Se and vitamin E causes white muscle disease in lambs. This is in relation to the fact that Ca is required for the muscle contractions. For normal muscle contractions, Ca ions must enter and exit the cell in the required amounts. Excess Ca, however, is toxic to the mitochondria. Peroxidation of the membrane may result from the infiltration of Ca against the sarcoplasm and the formation of mitochondrial lesions in the myocytes. As a result of such a process, cellular energy immediately disappears and myofibrillation degeneration begins, which in turn causes intracellular Ca accumulation. After these events, intracellular enzymes emerge to the extracellular space and blood. The levels of troponin and AST increase. These events are important from clinical and pathological perspectives. As a result, the degenerative cells move on to the necrosis phase which results in a phenomenon called the Zenker's necrosis. If vitamin E levels are low, the process may further be followed by immunodeficiency, which causes secondary infections to occur easily. In such a case, immuno-suppression and bacterial infections can easily be observed as well (Hulland, 1985).

Selenium deficiency can affect thyroid function in sheep, lambs and rats. Selenium deficiency has been reported by some investigators to cause decreased serum T3 concentrations and high serum T4 concentrations (Naziroğlu *et al*. 1998; Ateşşahin *et al*. 2002, Giray *et al*. 2004). Selenium deficiency in chicks decreased circulating T4 and T3 concentrations (Jensen *et al*. 1986).

In this study, the effects of WMD on certain blood parameters and malondialdehyde (MDA) and glutathione (GSH) levels in liver and thyroid tissues and the thyroid hormones were investigated in sheep raised in the Eastern part of Turkey, Van.

MATERIALS AND METHODS

This study was carried out in 2015 and 16 lambs 3 to 50 days old were used as research material. The animals were raised in Özalp, a town of Van, in Se deficit areas. 8 of the lambs were clinically diagnosed with WMD and had all the symptoms typical to it, like the motor disturbances, difficulty in breathing and recurrence during stiffness or movement. The animals with WMD were retained in a separate box. The animals in the control group consisted of 8 lambs, which were also selected from the same area and were in the same age category with the diseased group.

Blood samples were taken from all animals. Vitamin E + Se (Selenium) was injected to lambs with WMD (Yeldif, CEVA-DIF) at the dose of 1 ml / 60 mg of vitamin E, 40 mg of vitamin B1 and 1 mg of Sodium Selenite. Two weeks later, administration of Yeldif was repeated. 7 days after the second injection, blood samples were taken again. The sera obtained were analyzed with an auto-analyzer (Roche Moduler P 800-Yuzuncu Yil University) for the concentrations of Ca, P, T3, T4, albumin, globulin, total proteins and glutathione (Beutler *et al.* 1963; Mert, 1996).

Tissue samples from the liver and thyroid were homogenized and the resulting supernatant was used to determine the amounts of malondialdehyde (MDA) and glutathione. For this purpose, the tissues of the liver and thyroid were homogenized by addition of BHT which were then centrifuged at 10,000 rpm for 15 minutes. The supernatants were analyzed for their GSH content (GSH µmol / g protein) using Ellman's reagent by spectrophotometer (Perkin Elmer) at 412 nm (Fernandez and Videla, 1981). The tissue MDA contents of the liver and thyroid were determined by thiobarbituric acid (TBA) method and measured by spectrophotometry at 532 nm (Xia *et al.* 1994) and the results were presented as nmol / g protein.

Statistical analysis

The Duncan test for multiple comparisons was done and significances were evaluated for all the data obtained from the groups.

RESULTS AND DISCUSSION

Results of all obtained data are shown in Table 1 and Table 2. It was estimated that the sera Ca, T3 ($p<0.01$), P, albumin, globulin, T. protein, T4 and GSH levels ($p<0.05$) were statistically different before and after the treatment (Table 1). While the liver tissue MDA and GSH levels and Thyroid tissue MDA levels before and after treatment were statistically different ($p<0.05$), the difference between thyroid tissue GSH levels before and after treatment was found to be statistically insignificant (Table 2).

In our study, the concentration of Ca ion decreased in WMD lambs, but were increased after the injections of vitamin E + Se. This trend may be due to the intracellular accumulation of Ca and the formation of Zenker necrosis. Similarly, levels of P followed the same pattern, decreasing in lambs with WMD and then reaching normal levels after the treatment.

Conditions that damage tissue integrity such as various infectious diseases, liver disorders, acute inflammatory and proliferative events and trauma can cause changes in plasma protein levels. Changes in blood serum protein fractions of lambs with muscular dystrophy may be due to rapid breakdown of proteins depending on the degree of muscle disorders (Çamaş *et al*. 1976). Or *et al.* (2003), it was found that total protein and albumin levels increased in lambs with WMD. Our study, indicates a positive increase in almost all the parameters after the treatment.

GSH is an important antioxidant compound that protects cells from oxidative damage. The examination of blood GSH-Px activity is an effective marker for blood Se levels.

Table 1: Biochemical analyses of sera of healthy and diseased lambs before and after the therapy.

Parameters	n	Control	Before therapy	After therapy
Ca (mg/dl)	8	9.74±0.89[a]	7.24±0.47[b]	8.02±0.038[c]
P (mg/dl)	8	4.45±0.12[a]	3.50±0.14[b]	4.73±0.11[a]
Albumin (g/dl)	8	1.61±0.09[a]	1.71±0.07[a]	2.30±0.11[b]
Globulin (g/dl)	8	3.32±0.11[a]	3.20±0.17[a]	3.95±0.15[b]
T. Protein (g/dl)	8	5.80±0.21[a]	6.02±0.19[a]	6.91±0.23[b]
T3 (pg/ml)	8	4.82±0.11[a]	2.65±0.06[b]	6.02±0.13[c]
T4 (ng/ml)	8	0.45±0.03[a]	0.60±0.09[b]	0.42±0.04[a]
GSH (mg/dl)	8	0.18±0.18[a]	0.19±0.21[a]	0.23±0.16[b]

a,b,c: The difference between the averages indicated by different letters in the same row are statistically significantly if a and b ($p<0.05$), if and c ($p<0.01$).

Table 2: Biochemical analyses of tissues of healthy and diseased lambs before and after the therapy.

Parameters	n	Control	Before therapy	After therapy
MDA liver (nmol/g protein)	8	0.39±0.10[a]	0.44±0.15[b]	0.37±0.07[a]
GSH liver (µmol/g protein)	8	0.020±0.008[a]	0.018±0.004[a]	0.056±0.005[b]
MDA thyroid (nmol/g protein)	8	0.35±0.06[a]	0.58±0.09[b]	0.38±0.08[a]
GSH thyroid (µmol/g protein)	8	0.42±0.05[a]	0.34±0.04[a]	0.40±0.05[a]

a,b,c: The difference between the averages indicated by different letters in the same row are statistically significantly if a and b ($p<0.05$), if and c ($p<0.01$).

It has been reported that there was a high correlation between serum Se level and blood GSH-Px activity and that GSH-Px in lambs with WMD showed significantly low activity with low serum Se levels (Osame *et al*. 1990). In studies conducted by various researchers, it has been determined that there is a decrease in serum GSH enzyme activity in lambs with white muscle disease (Bildik *et al*. 1996; Or *et al*. 2003). In addition, many studies have shown that glutathione concentrations in the liver and other organs decrease as a result of oxidative stress caused by increased oxygen demand (Broderius *et al*. 1973; Rizzo *et al*. 1994; Nolan *et al*. 1995; Beytut *et al*. 2001; Beytut *et al*. 2002). In our study, tissue levels of GSH in the liver and thyroid were found to have decreased, but statistical significance was only found in the liver GSH content after treatment ($p<0.05$). This decline was due to the reduction of sulfhydryl and peroxyl groups and the increase in oxygen requirements.

MDA levels in hepatic and thyroid tissues were slightly increased in the diseased group, which returned to normal levels after the treatment with vitamin E + Se drugs. A combination of vitamins E + Se prevents the cell membranes from the lipid peroxidation. Increased amount of MDA is the result of insufficient vitamin E and Se in the body. The deficiency in antioxidant compounds causes WMD in sheep. Oxidative stress promotes the increase of MDA in different tissues in the body as well (Değer *et al*. 2008). In our study, MDA levels were also found to have increased in the lambs with WMD, which returned to normal levels after the administration of vitamin E + Se.

In the study investigating the effect of sodium selenite injection on selenium (Se), glutathione peroxidase (GSH-Px), Malondialdehyde (MDA) and cortisol levels; it was determined that the GSH-Px level differed significantly between the groups, sodium selenite injection significantly increased the Se levels in the plasma of Aardi goats compared to the control group (Elsheikh *et al.* 2014).

There is a relationship between Se absorption and the thyroid hormones. Sufficient absorption of alcohol increases the levels of T3 hormone. The development and growth of the body as a whole is also affected by this increase. Deficiency of Se -and/or the WMD itself- causes a decrease in the levels of T3 hormones (Berry *et al.* 1991). In a study investigating the macroscopic and microscopic findings of a case of nutritional cardiomyopathy in a female young camel (*C. dromedarius*), liver congestion and an enlarged thyroid gland were observed. In addition, papillary extensions in epithelium of thyroid follicles due to epithelial hyperplasia were observed (Ozdemir *et al*. 2016).

In the study presented, WMD was found to have caused a decrease in the T3 level ($p<0.01$), but has increased the level of T4 ($p<0.05$) in the diseased lambs. After the vitamin E + Se serum treatment, T4 returned to normal levels. Type 1 deiodinase is a Se-containing enzyme. In the selenium deficiency, the iodinase activity gets inhibited. The circulating T4 levels increase and the concentration of T3 decreases. Serum Se deficiency also may cause iodine depletion (Maia *et al.* 2011).

Conclusion

In conclusion, almost all analyzed biochemical parameters of the serum were found to have changed during WMD. After the administration of vitamin E + Se, however, the altered values returned to their normal states.

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