STUDIES ON SERUM $\alpha$-TOCOPHEROL, SELENIUM LEVELS AND CATALASE ACTIVITIES IN LAMBS WITH WHITE MUSCLE DISEASE

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Abstract

This study was conducted to evaluate the effects of combined deficiencies of Se and vitamin E on catalase activity in order to investigate the possible interactions between Se, $\alpha$-tocopherol, and catalase in lambs with white muscle disease (WMD). Ten healthy and fifteen WMD affected lambs of the Akkaraman breed were used. Serum $\alpha$-tocopherol and Se levels, and catalase activity were analysed. The catalase activity as well as $\alpha$-tocopherol and Se levels in lambs with WMD before the treatment (day 0), were found to be lower than those of the control group (P<0.001). Therefore, the catalase activity should be taken into consideration in lambs with WMD.

Key words: lamb, white muscle disease, catalase, $\alpha$-tocopherol.

White muscle disease (WMD) or nutritional muscular dystrophy (NMD) in lambs is an enzootic and nutritional disease, which results from selenium and vitamin E deficiencies. This disease has been reported in many countries of the world, as well as from the Turkish province of Anatolia, especially its central, eastern, and south-eastern parts (1, 24, 25).

Vitamin E is essential for such body functions as growth, reproduction, prevention of various diseases, and protection of the integrity of tissues. The metabolic function of selenium is closely linked to vitamin E. Both vitamin E and selenium act to protect biological membranes from oxidative degeneration. The most serious effect of selenium and vitamin E deficiency is tissue degeneration (18).

Selenium is also an integral component of the glutathione peroxidase (GSH-Px) in the organism (22). GSH-Px neutralises the effects of hydrogen peroxide and lipid hydroperoxide, which cause cell protein destruction and necrosis (3). Selenium and vitamin E are somewhat related, because vitamin E acts to protect cells from the harmful effects of oxidising agents. Vitamin E also acts as an antioxidant (13, 25). Vitamin E and the selenium-containing GSH-Px are an integral part of the antioxidant system present in all cells (18).

In WMD, lipid peroxidation and hydrogen peroxide occurring normally in the organism were not withdrawn from muscles due to a decreased GSH-Px activity, caused by selenium and antioxidant vitamin E deficiencies (14). Elevated free radicals and hydro- or lipoperoxides cause damage in cells (14, 21). Selenoenzyme GSH-Px, simultaneously with superoxide dismutase and catalase, remove elevated free radicals and hydro- or lipoperoxides from cells (21). Antioxidant defences are composed by non-enzymatic hydro and liposoluble compounds like vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiois, and enzymes, such as superoxide dismutase (SOD), catalase (CAT), and GSH-Px (8, 26). The enzymatic and non-enzymatic systems operate together to counteract the action of prooxidants in muscle tissues (8). GSH-Px and CAT are the unique enzymes scavenging hydroperoxides and therefore acting in combination with SOD (15).

A large number of studies have been carried out concerning creatin kinase, aspartat aminotransferase, alkaline phosphatase, lactate dehydrogenase (13, 23), and GSH-Px (24) activities, and vitamin E level (16) in animals with WMD. However, there is not any comprehensive work performed that relates to other antioxidant enzymes in animals with WMD, such as SOD and CAT. In this study, it was therefore decided to
determine the selenium and vitamin E levels, and CAT activities in WMD affected lambs. This is the first study determining CAT activity in lambs with WMD.

**Material and Methods**

The study was carried on 25 Akkaraman lambs. Out of these, 15 lambs, on the basis of clinical and laboratory examinations, were diagnosed as WMD affected, and the remaining 10 were healthy animals, originating from the Ozalp District of Van Province, Turkey. All the lambs were under control for 30 d during the study under the same feeding conditions.

Blood samples were collected from the jugular vein into serum test tubes for biochemical analysis prior to the treatment (at 0 d) and on day 30 after the treatment. The blood samples were centrifuged at 1700 x g for 15 min to obtain sera. The lambs with WMD were treated with Yeldif (injectable solution, Dif ®), a preparation containing 20 mg of sodium selenite, 1200 mg of vitamin E, and 800 mg of vitamin B1, with the dosage at 1 ml per lamb. Yeldif was administered twice, at two-week intervals.

CAT (EC 1.11.1.6) activity was determined according to Goth method (10). Serum Se content was measured by fluorophotometric method (17). Serum α-tocopherol level was determined by high performance liquid chromatography method (19).

The results were analysed by using a one way ANOVA and a Dunnet multiple comparisons test was used to compare control group with the other lambs. P<0.05 was considered significant. The data was expressed as means ± SD (standard deviation).

**Results**

In clinically examined WMD lambs, there were observed generalised weakness, stiffness, deterioration of skeleton muscle, loss of weight, and prostration (only in 4 lambs). The biochemical findings obtained before and after the study are summarised in Table 1.

Serum CAT activity and levels of α-tocopherol and selenium in lambs with WMD before treatment (day 0) were found to be significantly lower than those of control group (P<0.001), whereas after treatment (d 30) these differences were not found to be significant (P>0.05).

**Discussion**

White muscle disease occurs in the areas with soils as different as deep volcanic sedimentary, calcareous, and basaltic. The soils that produce pasture grasses and legumes low in selenium content are obviously capable of predisposing susceptible grazing species to the disease (13). Selenium deficiency in the feed of farm animals also leads to disorders in the antioxidant metabolism (4). As it had been reported that the region where this study was carried out has deep volcanic sedimentary, calcareous, and basaltic soils (11). It is difficult to reach a clear diagnosis for WMD on the basis of the clinical symptoms alone (4), because WMD may be confused with several other diseases, such as enzootic ataxia, cerebro-cortical necrosis, polyarthritis, listeriosis, Borna disease, pneumonia, and mineral element balance disorders (23). An indispensable and rapid aid to diagnose WMD is to determine in animals specific enzymes (23, 24) or plasma/serum selenium and vitamin E levels (23).

Selenium is undoubtedly an essential trace element for nutrition (7) and its deficiency manifests itself as a dysfunction of various organs and tissues in many species (5). Several researchers reported that selenium and vitamin E levels in lambs with WMD were significantly lower than in healthy lambs (1, 2, 6, 16). In another study, selenium levels in humans with skeletal muscle disorders were also significantly lower than in healthy ones (5). In this study, statistical analysis revealed that selenium values in lambs with WMD were significantly lower than those of the control group. These results are comparable with findings of other researchers (1, 2, 5, 6, 16).

Several investigators (1, 2, 6, 20) reported that vitamin E and selenium deficiency play the most important role in the occurring of white muscle disease. Vitamin E (α-tocopherol) is a major membrane-bound antioxidant (7). In another study, it was reported that vitamin E levels were significantly lower in lambs with WMD compared to healthy lambs (16).

**Table 1**

Mean selenium and α-tocopherol levels and catalase activity in lambs with WMD and in control group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Before treatment (0 d)</th>
<th>After treatment (30 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (kUI⁻¹)</td>
<td>14.16 ± 0.99*</td>
<td>9.29 ± 0.91</td>
<td>13.60 ± 1.23</td>
</tr>
<tr>
<td>α-tocopherol (µg/mL)</td>
<td>0.311 ± 0.08*</td>
<td>0.109 ± 0.02</td>
<td>0.292 ± 0.03</td>
</tr>
<tr>
<td>Se (ng/mL)</td>
<td>59.99 ± 11.55*</td>
<td>26.26 ± 9.55</td>
<td>58.23 ± 7.51</td>
</tr>
</tbody>
</table>

* P<0.001; ±SD
In this study however, while the mean serum α-tocopherol level in lambs with WMD was found to be 0.109±0.02 µg/mL on day 0 and 0.292±0.03 µg/mL on day 30 after the treatment, those of the control group were 0.311±0.08 µg/mL. When the serum α-tocopherol levels of lambs with WMD were compared with those of the control group (day 0) and after the treatment (day 30), it was found to be significantly lower than α-tocopherol level of the controls. However, no significant difference was found on day 30 after treatment. This is similar to the findings of Keleş et al. (16).

Vitamin E and Se, through the action of the selenoprotein glutathione and glutathione peroxidase, have important antioxidant functions within cells (6). Glutathione peroxidase, depending on selenium level in the organism, plays an important role in the reduction and detoxication of lipid peroxides. As for vitamin E, it prevents hydrogen peroxide occurring from lipid peroxidation (14, 21).

Aerobic organisms also synthesise numerous antioxidant enzymes and other proteins in an attempt to minimise oxidative damage (7, 9). Both glutathione peroxidase and catalases detoxify hydrogen peroxide by reducing it to water and oxygen (7). Hydrogen peroxide (H₂O₂) levels in muscle cells appear to be controlled principally by the CAT. The CAT is considered to be the principle safeguard against H₂O₂. Since peroxidases in general, are limited to the utilisation of specific hydrogen donors with only slight activity, glutathione peroxidase is found at a very low concentration in muscle tissue (12).

As it is known, the most important metabolic role of selenium in mammalian species is its function in the active site of the selenoenzyme glutathione peroxidase. This enzyme, together with SOD and CAT, protects cells against damage caused by free radicals and hydro-or lipoperoxides (21). In this study, serum CAT activity of the lambs with WMD was slightly lower (9.29±0.91 kUI⁻¹) than the serum CAT activity of control group (14.16±0.99 kUI⁻¹). However, on the 30th day of the therapy (13.60±1.23 kUI⁻¹), the difference was not statistically significant. It seems that the reason for the reduction of CAT activity is caused by its usage in the remove of increased hydrogen peroxides and lipid peroxides from the skeletal muscles.

In conclusion, the CAT activity, together with serum Se and vitamin E levels, could be useful criterion for the diagnosis and prognosis of WMD in sheep.

References
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