

## RELATIONSHIP BETWEEN SELENIUM LEVELS in SOIL and MEADOW HAY and GLUTATHIONE PEROXIDASE ACTIVITY in ERYTHROCYTES of OFFSPRINGS of Which DAMS GRAZED on PASTURE

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**Summary:** The relationship between selenium (Se) levels soil and meadow hay and the glutathione peroxidase (GSHPx) activity of erythrocytes in 15±7 day-old lambs in spring season was studied in 12 different districts in region of Kars and its surrounds, in Turkey. The relationship between the levels of Se in soil and meadow hay and activity of GSHPx in erythrocytes was studied in 800 blood samples taken by jugular venapuncture from 15±7 day-old lambs in this geographical area. The blood samples were analysed for the activity of GSHPx and groups named according to adequate GSHPx activity as Group I and inadequate GSHPx activity as Group II. The soil and meadow hay samples were analysed for the concentration of Se. In the three districts where the lambs were found to have adequate GSHPx activity ( $\geq 130$  IU/g Hb) (Group I), the soils contained significantly more Se than in the 3 other districts where the mean levels of GSHPx activity in the lambs were found to be inadequate (between 60 and 130 IU/g Hb) (Group II). The Se levels in meadow hay in the districts in which the GSHPx activity of the lambs was inadequate (Group II) were significantly lower than those of Group I.

These results suggest that soils which have a low clay content and meadow hay which is low in Se yield herbage may predispose grazing sheep to diseases occurred with Se deficiency like nutritional muscular dystrophy.

**Keywords:** Sheep, Se, soil, meadow hay, blood, GSHPx.

### Çayırdaki Otlayan Koyunların Yavrularının Eritrosit Glutasyon Peroksidaz Aktiviteleri ile Çayır Otu ve Toprağın Selenyum Düzeyleri Arasındaki İlişki

**Özet:** Bu çalışmada, Kars ve yöresinin 12 farklı bölgesinde ilkbaharda doğan 15±7 günlük kuzuların eritrosit glutasyon peroksidaz (GSHPx) aktiviteleri ile toprak ve çayır otlarının selenyum (Se) düzeyleri arasındaki ilişki araştırıldı. Bu bölgelerdeki eritrosit GSHPx aktivitesini araştırmak için ilkbahar döneminde doğmuş 15±7 günlük 800 kuzunun v. jugularis'inden vakumlu heparinli tüplerle kan örnekleri alındı. Bu dönemde alınan toprak ve çayır otlarında ise Se analizleri yapıldı. GSHPx aktivitesi yetersiz olan (60-130 IU/g Hb arasında) (Grup II) üç bölgenin toprak Se düzeyi, GSHPx aktivitesi yeterli olan ( $\geq 130$  IU/g Hb) (Grup I) diğer üç bölgenin toprak Se düzeyine göre önemli bir şekilde düşüktü. GSHPx aktivitesi yetersiz (Grup II) olan bölgelerdeki çayır otu Se düzeyleri GSHPx aktivitesi yeterli olan (Grup I) bölgelere göre daha düşüktü.

Bu sonuçlara göre, toprağın kil düzeyinin yetersizliği ve çayır otu ile toprağın Se düzeylerinin düşük olması, bu çayırlarda otlayan koyunların kuzularının beyaz kas hastalığı gibi Se noksanlığı nedeniyle oluşan hastalıklara karşı predispoze hale gelebileceği kanaatini doğurmuştur.

**Anahtar sözcükler:** Koyun, selenyum, toprak, çayır otu, kan, GSHPx aktivitesi.

## INTRODUCTION

The relationship between the intake of plants by animals from soils containing little selenium (Se) and the occurrence of Se deficiency is well known<sup>1,3</sup>. However, the concentration of Se in soils depends on their geological origin of the region<sup>4</sup>. Thus soils derived from recently formed rocks are likely to be deficient in Se, whereas soils formed from sedimentary rocks are rich in Se<sup>5,6</sup>. However, the quantity of Se that plants can absorb from soil is an important factor<sup>7</sup>. Climatic features are important factor for the development of meadow-pasture hay which is the basis of sheep production in the region of Kars.

The determination of blood glutathione peroxidase (GSHPx) activity is a useful method for indirectly

assessing the levels of Se derived by animals from their diet<sup>8</sup>. It is also a good measure of the efficacy of Se supplements, owing to the high correlation observed between the activity of the enzyme and the concentration of Se in blood<sup>9</sup> or in the diet<sup>10-12</sup>.

This paper describes a study of the relationship between Se levels of the soil, meadow hay and the erythrocytes GSHPx activity of the lambs which dams grazing on pastures in 12 different districts in the region of Kars, a major sheep rearing area of Turkey.

## MATERIAL and METHODS

The study was carried out in 12 different districts in the region of Kars, where disorders related to Se

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deficiency, particularly nutritional muscular dystrophy (NMD) in lambs, are very common. The criteria used to select the districts were:

- 1- The size of the flocks (typically between 100 and 1000 head of sheep),
- 2- The soil type (granitic, with a light texture and poor in clay) and
- 3- A previous history of problems associated with Se deficiency.

The diets fed to the herds included cereal grains grown in the same district during the months of low grass production and extensive grazing. The composition of the forage was similar in all the districts.

Blood samples were taken from 15±7 day-old lambs in spring season between 15 and 30 lambs in each district, approximately 5 percent of the animals in the flocks. This work was carried out in lambs as young animals are more liable to suffer from NMD. The samples (5 ml per lamb) were obtained by jugular vena puncture into heparin containing vacutainer tubes. The plasma and erythrocyte samples were stored at -20°C until analysis. At the same time, soil and meadow hay samples were taken from each part or plot of the districts. In order to determine Se levels appropriately collected soil and meadow hay specimens weighing 2 g were fragmented in a teflon bomb as described by Breyer and Gilbert<sup>13</sup>. The amounts of Se were then determined according to the method described by Watkinson<sup>14</sup> and Whetter and Ullrey<sup>15</sup> using a fluorescent spectrophotometer (Perkin Eimer 100). All the chemicals used were of analytical grade and triply distilled water was used throughout. In this work, the recovery rate of Se was found to be 94.5 %. GSHPx activity was expressed in the presence of reduced glutathione (GSH) and cumene hydroperoxide substrates using an end-point direct assay<sup>16</sup> and the activity was expressed as loss of GSH per min. GSHPx activity was expressed in units (one unit is the enzyme quantity that oxidizes 1 µmol GSH per min. in the above system 25 °C).

To study any possible influence of the soil and meadow hay on the availability of Se to the sheep, the six different locations were divided into two groups. Group I (districts in which the lambs had a GSHPx activity of ≥130 IU/g Hb), Group II (districts in which the lambs had marginal or deficient GSHPx values of less than 130 IU/g Hb)<sup>17,18</sup>.

**Statistical Analysis:** The mean values of the Se measured in the soil and meadow hay in the two groups of districts were compared by an unpaired two-tailed Student's t test. The activity of GSHPx in erythrocytes were analyzed and compared using Mann Whitney U test with SPSS software programme (version 9.5). Results were expressed as mean ± standard error (SE). P<0.05 considered to be statistically significant.

## RESULTS

The erythrocytes GSHPx activities of lambs from 12 different districts after 15±7 day of lambing in spring season are shown in Table 1. The erythrocytes GSHPx activity in lambing period in two district had deficient levels, six were marginal and four were adequate levels. In two of the districts the mean activities changed from deficient to marginal, in four of the district the mean activities changed from marginal to adequate.

The mean proportions of Se in the soils and meadow hay of two groups from 12 districts are shown in Tables 2 and 3, respectively. The soils in the districts in which the lambs had adequate GSHPx activity (Group I) contained significantly more clay than the soils from the other group of districts (Table 2). The Se levels in meadow hay in the districts in which the lambs had inadequate GSHPx activity (Group II) were significantly lower than those of Group I (Table 3).

**Table 1.** Mean values (X ± SE) of erythrocytes GSHPx activities (IU/g Hb) of 15±7 day old lambs in the lambing period in selected area.

**Tablo 1.** Belirlenmiş bölgelerde kuzulama döneminde doğan 15±7 günlük kuzuların ortalama (X±SE) eritrosit GSHPx aktiviteleri (IU/g Hb).

Origin of Samples		GSHPx Activity X±SE
Akyaka	1. District <sup>a</sup> (n=67)	69.72±10.9 <sup>M</sup>
	2. District <sup>b</sup> (n=67)	149.92±14.1 <sup>A**</sup>
Merkez	1. District (n=66)	30.11±9.7 <sup>D</sup>
	2. District (n=66)	101.15±14.3 <sup>M**</sup>
Digor	1. District (n=67)	49.53±7.3 <sup>D</sup>
	2. District (n=67)	86.60±13.4 <sup>M*</sup>
Arpaçay	1. District (n=67)	96.14±6.7 <sup>M*</sup>
	2. District (n=67)	180.25±10.2 <sup>A**</sup>
Susuz	1. District (n=66)	74.23±6.3 <sup>M</sup>
	2. District (n=66)	139.95±9.5 <sup>A*</sup>
Selim	1. District (n=67)	99.64±7.5 <sup>M</sup>
	2. District (n=67)	140.30±11.8 <sup>A*</sup>

\*p<0.01, \*\*p<0.001, M: Marginal, A: Adequet, D: Deficient,

a: Districts were distributed according to the low GSHPx activity,

b: Districts were distributed according to the high GSHPx activity.

**Table 2.** Mean  $\pm$  SE Se levels (ppm) in soil from districts where to flocks of lambs had inadequate (<130 IU/g Hb) and adequate activities ( $\geq$  130 IU/ g Hb) of GSHPx.

**Tablo 2.** Yeterli ( $\geq$  130 IU/ g Hb) ve yetersiz (<130 IU/g Hb) eritrosit GSHPx aktivitesine sahip kuzu sürülerinin olduğu bölgelerden alınan toprak örneklerinde Se düzeyleri (ppm).

Origins of Samples	Se Levels of District Where Inadequate GSHPx Activity (n=400) X $\pm$ SE	Se Levels of District Where Adequate GSHPx Activity (n=400) X $\pm$ SE
Akyaka	0.04 $\pm$ 0.002	0.16 $\pm$ 0.03**
Merkez	0.01 $\pm$ 0.003	0.11 $\pm$ 0.04**
Digor	0.08 $\pm$ 0.001	0.13 $\pm$ 0.02*
Arpaçay	0.02 $\pm$ 0.005	0.19 $\pm$ 0.07**
Susuz	0.01 $\pm$ 0.001	0.10 $\pm$ 0.05**
Selim	0.03 $\pm$ 0.002	0.15 $\pm$ 0.02**
Mean Values	<b>0.03</b> $\pm$ 0.002	<b>0.14</b> $\pm$ 0.04

\*p<0.05, \*\*p<0.01

**Table 3.** Mean  $\pm$  SE Se levels (ppm) in meadow hay from districts where the flocks of lambs had inadequate (<130 IU/g Hb) and adequate activities ( $\geq$  130 IU/ g Hb) of GSHPx.

**Tablo 3.** Yeterli ( $\geq$  130 IU/ g Hb) ve yetersiz (<130 IU/g Hb) eritrosit GSHPx aktivitesine sahip kuzu sürülerinin olduğu bölgelerden alınan çayır otu örneklerindeki Se düzeyleri (ppm).

Origins of Samples	Se Levels of District Where Inadequate GSHPx Activity (n=400) X $\pm$ SE	Se Levels of District Where Adequate GSHPx Activity (n=400) X $\pm$ SE
Akyaka	0.08 $\pm$ 0.003	0.39 $\pm$ 0.08***
Merkez	0.07 $\pm$ 0.001	0.35 $\pm$ 0.06**
Digor	0.05 $\pm$ 0.001	0.23 $\pm$ 0.09*
Arpaçay	0.10 $\pm$ 0.008	0.49 $\pm$ 0.08**
Susuz	0.06 $\pm$ 0.004	0.30 $\pm$ 0.03**
Selim	0.07 $\pm$ 0.003	0.45 $\pm$ 0.08***
Mean Values	<b>0.07</b> $\pm$ 0.004	<b>0.35</b> $\pm$ 0.07

\*\*p<0.01, \*\*\*p<0.001

## DISCUSSION

The region of Kars with its mountainous landscape is suitable for sheep rearing, and is one of major sheep breeding area in the north of Turkey. However, in the region, NMD causes heavy economic loss due to the death and weight loss of lambs, particularly when they are first allowed onto the meadow in spring. Likewise, NMD

in farm animals is an important constraint on sheep breeding in many countries of the world<sup>19,20</sup>.

The relationship between blood GSHPx activities and Se content of pasture has been widely studied in sheep and cattle<sup>21</sup>. Reports suggested that assessment of GSHPx activity is an useful means of determining deficient Se levels of flocks. Also it is showed that low Se content of pasture in spring resulting decreased erythrocyte GSHPx activity in lambs<sup>22</sup>. In addition, our unpublished data showed us that GSHPx activity may reflected the Se content of pasture<sup>18</sup>. So, in this research we found significant correlation with Se content of soil and pasture and blood GSHPx activity in lambs of which the dams grazing in these pasture. The Se content of most soils lies between 0.1 and 2 ppm, with only a portion of the Se in soils available to the vegetation they support. The Se concentrations occurring naturally in food and feeds show wide variation, depending on the plant species, and particularly on the Se status of the soils on which they have grown and upon the ability of the plants to uptake the mineral<sup>17,23</sup>. Likewise, McDowell<sup>24</sup> reported that pasture and forage usually contain at least 0.1 ppm Se (dry basis). In our study, the Se levels in soil collected from the disease foci were 0.03 ppm on average and 0.07 ppm (dry weight) in meadow hay, corresponding to the lowest normal levels (Tables 2 and 3, respectively).

Furthermore, it has been reported that Se uptake by plants growing in alkaline soil is higher than that of plants growing in acidic soil; and that Se uptake by plants growing in dry and hot weather conditions is higher than that of those growing in cold or rainy climates<sup>24,25</sup>. In the light of this, and given the fact that the area where this research was undertaken has a long winter season and high rainfall in other seasons, the low levels of Se found in the meadow hay were to be anticipated.

The percentages of Se were significantly different between the districts of groups I and II (Table II). These results are similar to those of Hartley and Grant<sup>8</sup>. However, Counotte and Hartmans<sup>6</sup> who did not find any link between the texture of soils and the mean blood GSHPx activity in lambs may be related to the positive effect of clay on the absorption of selenates and selenites by plants.

These results suggest that soils and meadow hay which have a low clay content, and which are low in Se levels, yield herbage which may predispose grazing sheep to Se deficiency diseases such as NMD.

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