Investigations have been conducted on Slavonia during a three year period on six locations, two per each year during June (in the first year = Vinkovci and Beli Manastir; in the second year = Valpovo and Djakovo; in the third year Slatina and Donji Miholjac). Six samples from the soil, pasture and sheep wool, as well as 15 sheep blood samples were taken from each locality. There was a deficit of selenium content in the soil on four locations in the second and third year (0.18; 0.14; 0.10 and 0.07 mg/kg) and an adequate content on locations Vinkovci and Beli Manastir in the first year (0.98 and 0.71 mg/kg). The locality Valpovo was characterized by the highest selenium concentration in pasture, blood and wool of sheep, as well as by the lowest blood enzymes activities (ALT, AST, CK, LDH) when compared to other locations. Selenium concentration in pasture was under the critical range on all locations (from 0.006 to 0.03 mg/kg DM), except the locality Valpovo in the second year (0.05 mg/kg DM). Serum selenium concentrations (from 0.035 to 0.082 mg/L) on all locations were below the adequate range for adult sheep and differed among locations. Activities of enzymes in the blood of sheep (AST: 3.59, CK: 4.93 and LDH: 6.87 µkat/L) showed severe selenium deficiency. Selenium concentrations in the wool were under the critical level on all locations (from <0.0002 to 0.06 mg/kg). The results regarding selenium in wool showed that, apart from blood selenium and activities of plasma enzymes (AST, CK, LDH), wool should also be taken in account as a selenium status indicator.

Key words: blood, enzyme blood activity, selenium, sheep, soil, pasture, wool
Scotland, Australia) contain 0.1 to 0.6 mg/kg selenium in the soil (Gupta and Gupta, 2000). Forages in many areas of the world do not provide adequate dietary selenium for livestock, whereas in other areas selenium concentrations in some grasses are high and can result in animal toxicity (Herdt et al., 2000). Panonian region is an area with selenium deficit in both soil and plants (Krajinović, 1984; Čuvardić et al., 1997; Jovanović et al., 1998; Pešut et al., 2004; Antunović et al., 2005). Selenium deficiency occurs in large areas of many countries and is largely restricted to grazing ruminants (including sheep) that have little or no access to concentrated feeds. Diseases associated with selenium deficiency have been widely documented and include white muscle disease, poor reproduction, reduced daily gain and immune function etc (Surai, 2006). Koening et al. (1997) demonstrated that selenium absorption and retention were distinctly greater in sheep fed diets rich in concentrates than in sheep fed diets with high forage content. Selenium content of soils, forages, and animal tissues can be used as diagnostic tools for determining the selenium status in ruminants (Puls, 1994). Although the selenium status in different regions of the world has been determined (McDowell, 1997), in Croatia there is little information on this element (Gavrilović, 1982; Antunović et al., 2005). The two basic methods for the assessment of selenium status in organism are the determination of selenium concentration in the blood (plasma or serum) and measurement of glutathione peroxidase activity in whole blood (Pavlata et al., 2002). The aim of the investigation was to determine the selenium content in soil, pasture, wool, blood plasma enzymes activity, as well as which is the best selenium status indicator for sheep.

MATERIALS AND METHODS

Locations, animals and diets

Investigations have been conducted on Slavonia and Barania areas (Croatia) in a three year period in the summer season. During three years 6 locations were analyzed, two per each year (in the first year: Vinkovci and Beli Manastir; in the second year: Valpovo and Djakovo; in the third year: Slatina and Donji Miholjac) showed on Figure 1. Sheep farm herds of more than 100 heads were held free on pasture (summer) and in stables (winter). The sheep were on average 3 years old in the first phase of pregnancy, crossbreed Zackel x Merinolandschaft. Mean
body weight of sheep was 58.5 kg. During summer the sheep grazed pastures (*Lolium perenne, Lolium italicum, Phleum phleoides, Trifolium repens and Dactylis glomerata*) and had meadow hay at offer. During the winter season the ewes were kept in stable boxes and fed a grain mixture (60% of oat, 30% of maize and 10% of soybean grits) 300 g daily and meadow hay (*ad libitum*). The sheep were reared under semi-intensive feeding systems and primarily fed on fodders produced on those sites.

**Sampling and analyses**

Six samples from pasture soil, pasture and wool including 15 sheep blood samples were taken from each locality. The samples were taken at the same time during each year (in the first week in June). Soil samples from each locality were taken by an auger at 30 cm depth (reaching the plant root system). Each sample of soil and pasture was composed of 10 sub-samples covering area of approximately 1ha pasture. Representative samples of soil and pasture which had been taken at the selected locations were dried and grounded. Soil pH in 1 M KCl was from 4.2 to 5.6. Dry matter content was determined at 105 °C. Wool samples were taken after wool shearing with an electric shear and were kept in sealed plastic bags (approximately 10 g of wool per animal). Each wool sample was composed of 6 sub-samples. Dirt and grease were removed by thoroughly washing the wool in hexane and ethanol.

Samples of soil, pasture and wool underwent microwave assisted acid digestion using concentrated nitric, perchloric and chloric acids (high pure) according to the method for Microwave Sample Preparation-MARS 5 in order to complete sample decomposition. Determination of selenium content was done by Continuous Hydride Generation Atomic Absorption method on Perkin-Elmer 2380 MHS-10.

Blood was collected from the jugular vein (10 mL) into sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium). After that, the serum was separated by centrifugation (10 min) at 3000 revolutions/min and placed into the auto analyser Olympus AU 640. Serum enzyme activities (ALT – alanine aminotransferase, AST – aspartate aminotransferase, CK – creatine kinase and LDH – lactate dehydrogenase) were measured.

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture, Forestry and Water Management.

**Statistical processing**

The values obtained from the studied indicators were processed by using the general linear model STATISTICA (StatSoft Inc., 7.1) procedure. The differences in investigation years between locations were statistically tested by Duncan's post hoc test.
RESULTS AND DISCUSSION

While analyzing the obtained values it was noticed that selenium shortage was found on four locations (Table 1) in Slavonia, respectively on Valpovo, Djakovo, Slatina and Donji Miholjac locations in the second and third year. All other concentrations (locations Vinkovci and Beli Manastir during the first year) exceeded the value of 0.5 mg/kg dry matter. The highest selenium concentration in pasture, blood and wool was determined in the locality Valpovo when compared with other locations (Table 1 and 2). In our country, preliminary studies of soil and animal feed samples (Gavrilović, 1982; Antunović et al., 2005) indicated the presence of low selenium levels in the area of Slavonia. Soil selenium content is highly variable. Soils containing less than 0.5 μg/g of total selenium are considered as deficient in this element (Mayland et al., 1989). Selenium partitioning is affected by soil pH and redox potential, content of sesquioxides, clay, organic matter and microbiological activity (Jayaweera and Biggar, 1996).

Table 1. Selenium content in soil and pasture on investigation locations during a three year period

<table>
<thead>
<tr>
<th>Year</th>
<th>Locality</th>
<th>Kind of measure</th>
<th>Soil (mg/kg DM)</th>
<th>Pasture (mg/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± s</td>
<td>Mean ± s</td>
</tr>
<tr>
<td>1st</td>
<td>Vinkovci</td>
<td></td>
<td>0.98 ± 0.340</td>
<td>0.020 ± 0.010</td>
</tr>
<tr>
<td></td>
<td>Beli Manastir</td>
<td></td>
<td>0.71 ± 0.110</td>
<td>0.030 ± 0.010</td>
</tr>
<tr>
<td>2nd</td>
<td>Valpovo</td>
<td></td>
<td>0.18 ± 0.090</td>
<td>0.050 ± 0.020a</td>
</tr>
<tr>
<td></td>
<td>Djakovo</td>
<td></td>
<td>0.14 ± 0.010</td>
<td>0.030 ± 0.001b</td>
</tr>
<tr>
<td>3rd</td>
<td>Slatina</td>
<td></td>
<td>0.10 ± 0.030</td>
<td>0.006 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>D. Miholjac</td>
<td></td>
<td>0.07 ± 0.001</td>
<td>0.007 ± 0.004</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.363</td>
<td>0.024</td>
</tr>
<tr>
<td>Critical level</td>
<td></td>
<td></td>
<td>0.500*</td>
<td>0.050**</td>
</tr>
</tbody>
</table>

a, b – means with different superscript letters differ significantly (p<0.05); s – standard deviation; DM – dry matter; * according to Mayland et al. (1989); ** according to Whelan et al. (1994a)

The selenium cycle in the food chain of land animals and humans starts from soils and includes herbage and animal sources ultimately dependent on its assimilation from the soil (Surai, 2006). Indeed, soils are the major source of selenium for pastures and therefore for animals eating those pastures. In areas where selenium responsive diseases in livestock occur, the pasture and forage concentrations are generally below 0.05 mg/kg dry matter (Whelan et al., 1994b). Dietary selenium requirements of sheep are generally quoted to be between 0.1 and 0.3 mg/g dry matter (Puls, 1994). However, under grazing conditions no
response to selenium supplementation of sheep is expected at dietary concentrations of >0.03 mg/kg dry matter selenium (Grace and Clark, 1991).

Table 2. Selenium content in blood and wool of sheep on investigation locations during a three year period

<table>
<thead>
<tr>
<th>Year</th>
<th>Locality</th>
<th>Kind of measure</th>
<th>Blood (mg/L)</th>
<th>Wool (mg/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± s</td>
<td>Mean ± s</td>
</tr>
<tr>
<td>1st</td>
<td>Vinkovci</td>
<td></td>
<td>0.042 ± 0.010</td>
<td>0.006 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>Beli Manastir</td>
<td></td>
<td>0.036 ± 0.010</td>
<td>Nd</td>
</tr>
<tr>
<td>2nd</td>
<td>Valpovo</td>
<td></td>
<td>0.082 ± 0.030A</td>
<td>0.060 ± 0.0060A</td>
</tr>
<tr>
<td></td>
<td>Djakovo</td>
<td></td>
<td>0.071 ± 0.020B</td>
<td>0.008 ± 0.0005B</td>
</tr>
<tr>
<td>3rd</td>
<td>Slatina</td>
<td></td>
<td>0.035 ± 0.020</td>
<td>0.003 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td>D. Miholjac</td>
<td></td>
<td>0.037 ± 0.007</td>
<td>0.004 ± 0.0010</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>0.057</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td>Level</td>
<td></td>
<td>0.12-0.15*</td>
<td>0.027**</td>
</tr>
</tbody>
</table>

A, B – means with different superscript letters differ significantly (p<0.01); s – standard deviation; DM – dry matter; Nd – not detectable <0.0002; * adequate levels-according to Aitken (2001); **critical level – according to Grace and Clark (1991)

With regard to selenium concentrations in pasture taken from the investigated locations it was noticed that the critical range of 0.05 mg/kg dry matter was exceeded by only one locality (Valpovo) in the second year. All other investigations indicated selenium deficiency in pasture (Table 1). Lower selenium content in the pasture is probably the result of a higher acidity of the pasture soils. In the case of acidic soils or poor soil aeration, selenium can form insoluble complexes with iron hydroxide and thus becomes poorly available. Only 47% of labelled selenium was transferred from the soil (pH 6) to ryegrass leaves. Increased pH to 7 results in increased selenium assimilation to 70% (Haygarth et al., 1995), because at higher soil pH, selenate is the dominant selenium from which is weakly bound to soils and is more available to plants (McBride, 1994). When compared with the locality Djakovo a significantly higher (p<0.05) in pasture and a higher (p<0.01) selenium concentration in blood and wool were determined on the Valpovo locality in the second year (Table 1 and 2). Concentration of selenium in pasture was from 0.029 to 0.073 mg/kg dry matter (Van Ryssen et al., 1999). Panousis et al. (2007) recorded 0.07 µg/g dry matter selenium in pasture, and White and Rewell (2007) from 0.025 to 0.254 mgSe/kg DM.

In our investigation serum selenium concentrations (from 0.035 to 0.082 mg/L) were below the adequate range (0.12-0.15 mg/L) for adult sheep (Aitken, 2001) and reference range of 120-500 ng/mL (Menzies et al., 2004) and differed among locations (Table 2). As similar trend for selenium content in
pasture and blood of cows was determined by Muratovic et al. (2007). Serum is most sensitive to dietary selenium and it is the preferred measure of selenium status in sheep (Whelan et al., 1994b). Bickhardt et al. (1999) regarded in blood plasma of sheep selenium values <0.08 mg/L as deficiency. Ramis et al. (2001) in Western Pomerania region of Poland determined even lower selenium concentrations in sheep blood (<10 µg/L). Similar results were found by Pilarczyk et al. (2007). Selenium-deficient cattle herds were detected in almost all regions of the Czech Republic (Pavlata et al., 2002). Blood concentrations of selenium were significantly higher in sheep reared under intensive feeding systems than those on the semi-intensive one (Panousis et al., 2007).

Results of wool selenium concentration (Table 2) showed a deficit of this element in the fifth investigated location. Satisfactory selenium concentration in wool samples on the Valpovo locality can be explained by an adequate selenium concentration in pasture (Table 1). Ramirez-Perez et al. (2000), in Columbia, found in the wool of sheep 0.205 mg/kg selenium. Dietary selenium level affected wool selenium content (Davis et al., 2008). Cristaldi et al. (2005) reported increased selenium in the hair of feedlot steers as dietary selenium was increased. Gabbedy (1971) recognized sheep to be the most sensitive index of selenium deficiency in Western Australia. Adequate selenium concentrations in the wool were from 0.027 to 0.57 mg/kg (Grace and Clark, 1991). To deficiency of selenium in the feed, animals respond by mobilization of tissue reserves. Wool accumulates selenium and other elements. It has been suggested that wool reflects the trace element status of the sheep at the time of wool follicle development (Lee and Grace, 1988). Indeed, hair selenium reflected long-term selenium status (Thomas, 2004).

Table 3. Enzymes activity in blood of sheep on investigation locations during a three year period

<table>
<thead>
<tr>
<th>Year</th>
<th>Locality</th>
<th>ALT Mean ± s</th>
<th>AST Mean ± s</th>
<th>CK Mean ± s</th>
<th>LDH Mean ± s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Vinkovci</td>
<td>0.47 ± 0.10</td>
<td>3.76 ± 0.54</td>
<td>4.96 ± 0.58</td>
<td>8.45 ± 1.45</td>
</tr>
<tr>
<td></td>
<td>Beli Manastir</td>
<td>0.55 ± 0.09</td>
<td>4.05 ± 0.64</td>
<td>5.65 ± 0.64</td>
<td>7.38 ± 1.47</td>
</tr>
<tr>
<td>2nd</td>
<td>Valpovo</td>
<td>0.48 ± 0.10</td>
<td>2.94±0.44</td>
<td>3.90±0.38</td>
<td>4.90±1.35</td>
</tr>
<tr>
<td></td>
<td>Djakovo</td>
<td>0.40 ± 0.06</td>
<td>3.49±0.49</td>
<td>4.52±0.37</td>
<td>6.43±1.63</td>
</tr>
<tr>
<td>3rd</td>
<td>Slatina</td>
<td>0.51 ± 0.08</td>
<td>3.68 ± 0.52</td>
<td>5.31 ± 0.56</td>
<td>7.14 ± 1.45</td>
</tr>
<tr>
<td></td>
<td>D. Miholjac</td>
<td>0.43 ± 0.10</td>
<td>3.62 ± 0.61</td>
<td>5.27 ± 0.54</td>
<td>7.02 ± 1.50</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.47</td>
<td>3.59</td>
<td>4.93</td>
<td>6.87</td>
</tr>
<tr>
<td>Level*</td>
<td></td>
<td>0.50</td>
<td>1.00-4.67</td>
<td>1.17-5.00</td>
<td>3.97-7.33</td>
</tr>
</tbody>
</table>

A, B – means with different superscript letters differ significantly (p<0.01); a, b – means with different superscript letters differ significantly (p<0.05); s – standard deviation; *according to Kaneko et al. (1997)
We determined the increased activity of ALT, AST, CK and LDH in the blood of selenium deficient sheep (Table 3). The lowest activity of blood enzymes during investigation was determined on the Valpovo locality in the second year. Hence, this locality was determined by a significantly lower activity of CK, AST and LDH when compared to Djakovo locality. A similar trend, as in our investigation, was observed by Sobiech and Kuleta (2002) and Faixova et al. (2007) in blood serum of selenium deficient lambs. Bickhardt et al. (1999) determined that the activity of blood enzymes (aspartate aminotransferase and glutamate dehydrogenase) in the blood of sheep a better indicator of selenium deficiency than the activity of creatine kinase.

In conclusion, apart from blood selenium deficiency and activities of enzymes (AST, CK and LDH) in blood, in terms of the obtained results, wool selenium concentrations (from 0.0002 to 0.06 mg/kg) can be taken as a selenium supply indicator. Based on the achieved results, we concluded that Slavonia is an area with selenium deficiency both in soil (0.363 mg/kg) and pasture (0.024 mg/kg) and therefore Se should be regularly added into sheep rations.

ACKNOWLEDGEMENTS:
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KONCENTRACIJA SELENA U ZEMLJIŠTU, BILJKAMA ZA ISPAŠU, KRVI I VUNI OVACA

ANTUNOVIĆ Z, STEINER ZD, VEGARA M, ŠPERANDA MARCELA, STEINER ZV* i NOVOSELEC J

SADRŽAJ

U ovom radu su prikazani rezultati istraživanja sprovedenih tokom juna u Slavoniji, u tri uzastopne godine. U prvoj godini, ispitivanja su izvedena u Vinkovcima i Belom Manastiru, u drugoj u Valpovu i Đakovu i u trećoj u Slatini i Donećkom Miholjcu. Sa svakog lokaliteta je bilo uzeto po šest uzoraka zemljišta, biljaka za ispašu i vune i 15 uzoraka krvi ovaca. U drugoj i trećoj godini je dokazan deficit selena u zemljištu na četiri lokacije (0,18, 0,14, 0,10 i 0,07 mg/kg) i zadovoljavajući nivo samo u Vinkovcima i Belom Manastiru prve godine (0,98 i 0,71 mg/kg). Lokalitet Valpovo se karakterisao najvećim sadržajem selena u biljkama za ispašu, vuni i krvi ovaca kao i najmanjom aktivnošću enzima ALT, AST, CK, LDH u poređenju sa drugim lokalitetima. Koncentracija selena u biljkama za ispašu je bila ispod kritičnog nivoa na svim lokalitetima (0,006 do 0,03 mg/kg SM) osim u Valpovu (0,05 mg/kg SM). Koncentracija selena u krvi je bila ispod donjih granica za odrasle ovce i razlikovala se između pojedinih lokaliteta. Aktivnost enzima u krvi ovaca (AST: 3,59, CK: 4,93 i LDH: 6,87 µkat/L) je ukazivala na veliki deficit selena. Koncentracija selena u vuni je bila ispod kritičnog nivoa na svim lokalitetima (od 0,0002 do 0,06 mg/kg). Naši rezultati ukazuju da se, osim određivanja koncentracije selena u krvi i aktivnosti enzima AST, CK, LDH, njegov sadržaj u vuni može uzeti kao relevantan parametar statusa ovog mikroelementa.