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Assessment of selenium status in alpaca

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ABSTRACT

The aim of this study was to determine the relationship between selenium (Se) concentration (which is used as direct method for diagnostics of selenium status) and activity of glutathione peroxidase (indirect method) in whole blood of alpacas and thus compare the suitability of use of these two diagnostic methods. A further objective was to determine Se status in relation to different age groups of alpacas and different Se supplementation. A total of 257 alpacas (196 adults and 61 crias) from 18 farms were included in this study. The farms differed in size and the use of Se supplement. The age of animals ranged from 4 days up to 16 years old. Blood samples were collected for measurement of whole blood Se concentration and glutathione peroxidase (GSH-Px) activity. The activity of GSH-Px is given in μkat/l of whole blood and μkat/g of hemoglobin. The relation between concentration of Se and GSH-Px activity and the effect of sex, age and feeding pattern on these parameters were statistically evaluated. Mean (±SD) Se concentration was 84.1 ± 35.3 μg/l and ranged from 27.9 μg/l to 225.6 μg/l for all age groups. We found significant difference ($P < 0.001$) between the group of crias (<1 year of age) and the group of adults: mean 67.8 ± 25.1 μg/l vs. 89.1 ± 36.5 μg/l, respectively. The mean activity of GSH-Px (210.2 ± 162.9 μkat/l or 1.71 ± 1.31 μkat/g of hemoglobin) ranged from 22.0 μkat/l to 840.2 μkat/l in the whole blood or from 0.20 μkat/g to 6.53 μkat/g of hemoglobin, respectively. There was no significant influence of age on GSH-Px activity. Evaluation of Se status by dividing the alpaca farms according to different Se supplementation pattern showed significantly higher mean blood Se concentration and GSH-Px activity on farms where Se supplement is fed regularly (Se 101.8 ± 35.1 μg/l, GSH-Px 284.7 ± 181.9 μkat/l) in comparison with farms not using Se supplement (Se 50.4 ± 17.8 μg/l, GSH-Px 114.7 ± 101.7 μkat/l) or feeding Se supplement only occasionally (Se 79.6 ± 28.1 μg/l, GSH-Px 170.3 ± 120.5 μkat/l). We found significant but not very close correlation between the two parameters ($r = 0.35$; $P < 0.01$) for Se concentration vs. GSH-Px activity. The results of this study indicate that the practical use of indirect method of Se status determination in alpaca is limited. Therefore we recommend measurement of both the Se concentration and the activity of GSH-Px in order to characterize the Se status exactly. Furthermore the data from this study indicate that age-specific reference ranges are appropriate for blood Se concentration in alpacas.

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1. Introduction

Selenium (Se) is one of the essential nutritional elements protecting the organism from oxidative damage. Since 1973 when Se was identified as a functional component in the cellular antioxidant, glutathione peroxidase (GSH-Px), a number of other biological functions of Se were described. Apart from GSH-Px, several selenoproteins have been identified, the most important including iodothyronine deiodinase, selenoproteins P, W, R, T, N, thioredoxine reductase and others (Papp et al., 2007).

Selenium deficiency and therefore suboptimal levels of GSH-Px activity causes a range of clinical and subclinical problems. The best known clinical form of Se deficiency in farm animals is nutritional muscular dystrophy, described in ruminants, horses as well as in camels (El-Khouly et al., 2001; Faye and Seboussi, 2009; Ludvikova et al., 2005a; Pavlata et al., 2001). Other Se responsive conditions reported include: illthrift in lambs and calves, lowered fertility and embryonic death in sheep, retained placenta, metritis and cystic ovaries in cows, and decreased immunological function (Gyang et al., 1984; Levander, 1986; Andrieu, 2008). Llamas and alpacas develop signs and syndromes similar to those of cattle and sheep. Heat stress seems to increase Se requirements causing the animals on marginal Se intake to be more prone to hyperthermia (Fowler, 2010).

There are regions of Se deficiency in the Czech Republic. Both ruminants (Pavlata et al., 2002) and humans (Kvicala et al., 1995) in the Czech Republic were found to be deficient in Se. The Se status determination by direct measurement of Se concentration and the clinical signs of its deficiency have not yet been described in alpaca neither in the Czech Republic nor – to authors' knowledge – anywhere in Europe.

The samples used for measurement of Se status in the field are those that can be conveniently obtained from the animal. Whole blood and serum or milk concentration of Se, and whole blood GSH-Px activity, are commonly used in the diagnostics of Se deficiency (Pavlata et al., 2000, 2012; Suttle, 2004). Since GSH-Px becomes a component of erythrocytes already in the stage of erythropoiesis, its activity in whole blood indicates long-term Se status, while blood plasma Se concentration rather reflects the actual status (Gerloff, 1992). When an animal's diet fails to meet its needs for selenium, four overlapping phases of deprivation can often be distinguished: (a) depletion, when stores in the tissues or body fluids are diminishing; (b) deficiency, when selenium levels normally kept within close limits in transport pools fall; (c) dysfunction, when selenium-dependent functions in the tissues or body fluids become rate-limited and (d) disorder, when livestock appear abnormal or perform poorly. The transition between phases is often gradual and consequently, disorders develop gradually, with only the most vulnerable individuals being visibly affected. Assessment of Se deficit is difficult and practitioners should judge from responses to Se treatment whether a given biochemical standard is helpful (Suttle, 2004).

Many authors confirmed a positive correlation between GSH-Px activity and Se concentration in whole blood, for example, this has been demonstrated by Pavlata et al. (2000) in cattle, Ludvikova et al. (2005b) in horses, Misurova et al. (2009) in goats, and Pavlata et al. (2012) in sheep in the Czech Republic. There are studies from UK, Switzerland and Italy measuring GSH-Px activity of alpacas as indirect determination of Se status due to fact that this assay is relatively easier and cheaper comparing with assay for total blood Se with which it correlates (Foster et al., 2009; Hengrave Burri et al., 2005; Morgante et al., 2001). There are studies on dromedary camel supporting the theory of positive correlation (Abdel Rahim, 2005; Faye and Seboussi, 2009; Seboussi et al., 2008), however similar studies for South American camelids are scarce (Dart et al., 1996; Hill et al., 1992). The studies done on llamas and alpacas are using only small number of animals and moreover no study describing the correlation between blood Se concentration to GSH-Px activity was done – to our knowledge – on South American camelids in Europe.

Furthermore we took into consideration works on camelids indicating that age specific reference ranges are necessary for blood Se concentration (Herdt, 1995; Van Saun, 2008) and that blood Se concentration of llamas <1 year old is significantly lower than values in llamas >1year old (Smith et al., 1998).

The objective of our work is therefore to compare the direct and indirect method for diagnostics of Se deficiency in blood of alpacas to find out the best method for practical use. Further objective was to determine blood Se concentration and GSH-Px activity in relation to different sex and age groups of alpacas (animals <1 year of age and animals >1 year of age). To determine the effect of various feeding practices on Se status and GSH-Px activity this study used wide range of alpaca farms with different use of Se supplementation.

2. Materials and methods

2.1. Animals/farm

A total of 257 alpacas from 16 farms in the Czech Republic and 2 farms in Germany (the main suppliers of alpacas to Czech Republic) were included in this study. Mean (±SD) age of animals was 3.8 ± 3.2 years and ranged from cria 4 days old up to female 16 years old. Farms varied from small (4 alpacas) to larger size (>60 alpacas) to reflect a range in size and feeding patterns used in typical alpaca operations. The most common alpaca farm in the Czech Republic consists of one or two breeding males and three to six females. Farms having more than twenty animals are exception. To obtain larger number of animals kept under similar condition, 2 German farms were included in our study.

The farms differed in Se intake (see Table 1) to obtain the widest possible range of blood Se concentrations and the GSH-Px activities. As it was neither possible to analyze Se in the hay and pasture forage on each farm nor to determinate the amount of mineral supplement being consumed by individual alpacas, we only divided, for purposes of clarity, the farms into three groups by the use of mineral supplement containing Se. Group A was fed regularly (apart from pasture and hay) the supplement made for llamas/alpacas, which contains 2 mg Se per kg of dry matter. The recommended daily intake is 450 g of supplement per 50 kg of body weight, therefore we calculated for average adult alpaca the intake >1 mg of se daily. Group B received the same supplement but in smaller amount and not regularly. We estimated from the described practice that animals were receiving about 40% of recommended daily dose. Animals from group C received only pasture/hay and occasionally small amount of grain for energy but no trace mineral supplementation.

In preparation for sample collection we requested the owners to fill up the form asking about farm details (number of animals, feeding pattern, the age, sex, medical history and origin of individual animals). All animals