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Time-dependent effect of selenium supplementation on the relationship between selenium concentrations in whole blood and plasma of sheep

J.B.J. van Ryssen[a,*], R.J. Coertze[a], M.F. Smith[b]

[a] Department of Animal & Wildlife Sciences, University of Pretoria, Pretoria 0002, South Africa
[b] Stats4science, P.O. Box 50505, Moreleta Village 0097, Pretoria, South Africa

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ABSTRACT

The ratio between selenium (Se) concentrations in pairs of plasma and whole blood of sheep after commencement of Se supplementation was calculated from data obtained from 10 independent investigations. The diets in the studies consisted of a variety of ingredients, from the individual feeding of feedlot diets to grazing trials on Se-deficient pastures. Means from 51 treatments, derived from 179 collections at various stages after commencement of Se supplementation, were used to calculate the ratios. In one study it was found that plasma Se concentration reached steady-state within 4 days of commencement, while Se in whole blood reached steady-state only at the collection 60 days after supplementation commenced. Using the 179 pairs, the ratio of whole blood and plasma stabilized at about 50 days after commencement when inorganic Se was supplemented, and at about 60–70 days when the Se was in the organic form. The ratios stabilized when they were between 2:1 and 3:1, and remained practically constant from 50 days post onset of supplementation, when plasma Se constituted 0.445 of whole blood Se concentration for the inorganic Se source and 0.410 when the Se source was organic. It was concluded that after commencement of Se intake, plasma Se concentration remains relatively constant from within 4 days of onset, while whole blood Se concentration apparently reaches steady-state only after approximately 50 days. Before 50 days, whole blood Se seems not to be reliable to predict the Se status of an animal. In a situation where Se intake is stable and Se requirements do not fluctuate, a bloodplasma ratio of between 2:1 and 3:1 would be a guideline to indicate that whole blood Se and plasma Se concentrations could both be used to assess the Se status of sheep.

1. Introduction

The concentration of selenium (Se) in whole blood, plasma/serum and the liver of animals, as well as glutathione peroxidase (GSH-Px) activity in the erythrocytes, is widely used by diagnostic laboratories to predict the Se status of animals (Gerloff, 1992). Such values have been published in tables and guidelines, using criteria such as deficient, marginal, adequate, high and toxic ranges (Puls, 1994; Kincaid, 1999, 2008; Underwood and Suttle, 1999). Such a classification implies that there should be a relationship between the criteria of Se status. For example, deficiency can be expected within specific ranges of concentrations of Se in serum/plasma, whole blood and the liver.

One of the most convenient animal tissues to sample for diagnostic purposes is blood on which Se concentrations can be measured in plasma/serum and in whole blood.

* Corresponding author. Tel.: +27 12 4206017; fax: +27 12 4203290.
E-mail address: jvryssen@up.ac.za (J.B.J. van Ryssen).

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Glutathione peroxidase activity in haemoglobin or erythrocytes can be measured, but the repeatability of results between laboratories is low (Gerloff, 1992). Therefore, this assay is used less frequently.

In the early 1990s controversy was rife in the scientific literature between laboratories as to whether plasma/serum Se or whole blood Se was the preferred fluid to use in assessing the Se status of animals (Maas et al., 1992, 1993; Thompson and Ellison, 1993). For instance, Maas et al. (1993) pointed out some limitations in using serum Se concentrations, viz. the unknown effect of haemolysis and the greater variability in serum Se values in relation to blood Se values, especially at low concentrations. This issue was not really resolved and laboratories world-wide still use whole blood Se, or plasma/serum Se, or both for diagnostic purposes (Waldner et al., 1998).

It is well recognised that the information provided by whole blood Se status represents a different time frame in the nutritional history of the animal from plasma/serum Se concentrations (Waldner et al., 1998). Selenium concentration responds more slowly to changes in Se intake in whole blood than in plasma/serum, because Se is incorporated in erythrocytes at the time of erythropoiesis (Nicholson et al., 1991), and changes very little over the lifespan of the cell. A complete response in whole blood to Se supplementation would require a timespan equal to the lifespan of the erythrocyte, which in cattle can range from 135 to162 days and in sheep from 131 to 157 days (Kaneko, 1980), while Wright (1965) recorded an average of 157 days in sheep. On the other hand, Se concentrations in plasma/serum responded more quickly to changes in Se intake. This demonstrates why plasma concentrations would reflect more accurately the current level of Se intake of an animal and whole blood Se over the long term (Gerloff, 1992; Maas et al., 1993; Thompson and Ellison, 1993; Whelan et al., 1994; Hall, 2006; Kincaid, 2008).

What is unclear from these arguments is exactly what is meant by short-term and long-term Se status. We were afforded the opportunity to use data collected from 10 experiments conducted under the supervision of the main author over approximately 12 years in which the Se concentrations were measured in both plasma and whole blood for the duration of the study. This enabled us to compare the relationship between whole Se and plasma Se and to observe how time after commencement of Se supplementation affected this relationship.

2. Material and methods

2.1. Source of experimental data

In a study by Cronjé (2004, unpublished results) blood and plasma samples were collected on day zero (pre-supplementation) and then on days 1, 2, 4, 8, 16, 30, 60 and 90 after commencement of supplementation. The results of the two treatments ($n = 11$/treatment) in which inorganic Se was supplemented are presented in Fig. 1. At the onset of the study, the Se concentration in whole blood was 56 ng/g, suggesting a marginal deficiency (Puls, 1994), and the supplemented treatments consisted of high doses of Se, calculated to be 2.5 mg Se/kg feed and 4 mg Se/kg feed, from day 1 onwards.

In a further investigation the association between the Se concentrations in whole blood and plasma were calculated from data obtained from 10 independent trials (Table 1) in which the Se concentration in both whole blood and plasma were determined. The ratios between whole blood Se and plasma Se concentrations were calculated for all data at all the stages after commencement of Se inclusion in the diets. The total number of pairs was 179 (Table 2), subdivided into source of selenium and number of days after commencement of Se supplementation. When the original studies were conducted, ethical approval was obtained at the institutions where the studies were conducted.

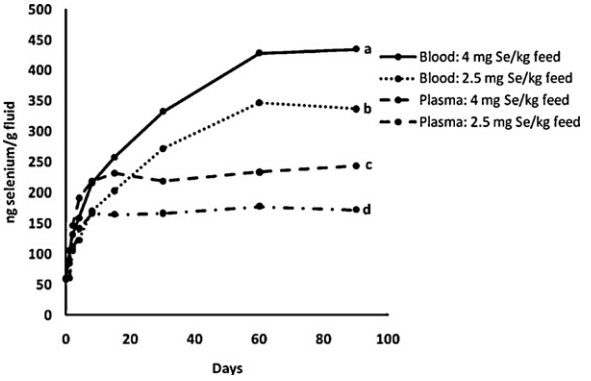

Fig. 1. Changes in selenium (Se) concentrations in plasma and whole blood after commencement of selenium supplementation. Means with different letters (a,b,c,d) differ at $P < 0.0001$. From day 2 onwards differences in plasma Se concentrations between dietary levels of Se were significant at $P < 0.001$. From day 8 onwards differences in whole blood Se concentrations between dietary levels of Se were significant at $P < 0.001$.

In all the studies, Merino type sheep were used, ranging in age from 5 months to *ca.* 18 months. Dietary levels ranged from 0.07 mg Se/kg to 6.4 mg Se/kg DM (Table 1).

In seven of the trials, the sheep were fed individually in pens, and in three trials weaned lambs were kept on pastures. The duration of the trials ranged from 44 to 150 days (Table 1). The individually fed animals received constant levels of dietary Se per treatment for the duration of the study, though the diets between trials differed substantially in ingredient composition (Table 1). It was not possible to establish the dietary Se level in the grazing trials because some treatments entailed Se fertilization of the pasture and parenteral supplementation of Se, though the blood Se:plasma Se ratios were used in the calculations.

Pre-experimental treatments varied. In some cases animals were depleted, others started with low selenium reserves; and in some studies an adaptation period was used. Measurements on whole blood and plasma collected at day zero therefore represented a pre-experimental feeding regimen, and were not included in the calculations.

2.2. Sources of selenium

In most of the experimental treatments, inorganic Se, as sodium selenite ($NaSe_2O_3$), was used as the supplement (Table 2). However, the raw diet ingredients did contain Se, presumably in an organic form. This natural Se would have contributed something to the results. In some treatments, organic sources of selenium were supplemented, viz. a commercially available selenoyeast or natural organic Se in Se fertilized pasture and in the raw feed ingredients (Table 2). Since the organic sources differed, factors such as ruminal degradation of the organic Se might have differed between sources.

2.3. Sample preparation and analytical procedures

In all trials, lithium heparin was used as the anti-coagulant, though whole blood Se concentration was not corrected for packed cell volume (PCV). Visible haemolysed plasma samples were rejected. In the chemical analysis, blood and plasma samples were weighed and expressed on a weight basis because this was found to be more repeatable than measuring blood and plasma volume. In the first number of experiments the Koh and Benson (1983) fluorometric technique was used for the Se assay, and this was followed by the continuous hydride generation atomic absorption method, read at an absorbency of 196 nm and lamp energy of 16 mA. To verify the accuracy of the Se assay, internal laboratory bovine livers, calibrated against a bovine liver sample (no. 1577b) from the National