Glutathione peroxidase responses in mature horses following the withdrawal of an organic selenium supplement

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ABSTRACT: The study comprised 25 mature horses and incorporated five dietary treatments; a negative control (C: 0.085 ppm of Se in the diet), 3 levels of organic Se supplementation (S2, S3 and S4; 0.2, 0.3 and 0.4 ppm of Se on DM respectively) and one positive control supplemented with Na selenite (N3: 0.3 ppm of Se on DM). Diets were offered for 112 days (supplementary period) after which horses were blood sampled to determine GSH-Px activity. Sampling was repeated at 28 day intervals for the following 112 days (post-supplementary period). Increased GSH-Px activity was observed with ascending concentrations of dietary Se following the supplementary period. GSH-Px activities are still notably higher at the end of the post-supplementary period in S2, S3, S4 and N3 when compared to C, although rates of decline were greatest in those treatments with the highest levels of selenium supplementation. Furthermore, GSH-Px activity was better maintained in those animals that had received organic Se following the withdrawal of Se supplements. Therefore, from a nutritional viewpoint, organic forms of Se are superior to selenite as GSH-Px activity is maintained during periods of Se inadequacy.

Key words: Organic selenium, Selenomethionine, Glutathione peroxidase, Horse.

INTRODUCTION – It is generally accepted that in biological systems Se participates in various physiological functions as an integral part of a range selenoproteins. The mammalian Se-containing selenoproteins are divided into three groups (Behne and Kyriakopoulos, 2001); proteins with non-specific incorporation of Se; specific Se-binding proteins; specific proteins containing Se in the form of selenocysteine (SeCys) which includes the Glutathione Peroxidases (GSH-Px). When selenomethionine (SeMet) is available in the diet, non specific incorporation of SeMet in place of methionine into general proteins is a way of preserving Se for future use in the body (Surai, 2006). Selenium in form of SeMet becomes available for selenium protein synthesis when those selenium containing proteins are catabolised in proteasomes and the SeMet converted to H2Se (Schrauzer, 2003). The aim of this study was to evaluate the GSH-Px responses in horses following the withdrawal of either organic or inorganic Se supplement.

MATERIAL AND METHODS – The study comprised 25 mature horses (mean age 13.6 ± 4.79 years) sourced from the same herd. The study period lasted 224 days during which time all horses were offered the same feeds and diet (on average 6 kg/head per day of grass hay and 3 kg/head per day of concentrate). The study was of a randomised complete block design and comprised two distinct and consecutive experimental periods: a 112 day continuous supplementary period and 112 day post supplementary period. During the supplementary period all animals received the same basal diet that differed only in either Se source (organic vs inorganic) or concentration. Organic Se was obtained from Saccharomyces cerevisiae CNCM I-3060 containing 63% of SeMet and 34-36% of low molecular weight seleno-components (Sel-Plex®); as inorganic selenium Na selenite was used. Treatments comprised a negative control (C: background Se only), 3 different levels of Sel-Plex® supplementation (S2, S3 and S4; 0.77, 1.62 and 2.47 mg/head/day of Se respectively, to achieve final dietary Se concentrations of 0.2, 0.3 and 0.4 mg/kg DM respectively); one positive control supplemented with Na selenite (N3: supplementation with 1.62 mg Se/head/day to achieve a final dietary Se concentration of 0.3 mg/kg DM). The Se was offered daily supplied to each...
horse using specific premix containing Ca carbonate for each treatment. Animals were blocked by live weight and then randomly allocated to one of the five dietary treatments. Treatments were also balanced to account for gender, age and activity differences. At the end of the 112 day supplementary period horses were blood sampled (T0) using Li-heparin treated tubes (Monovet tube Se-free). Samples stored on ice whilst awaiting assessment of haemoglobin status and GSH-Px activity using the Ransel kit (Randox, UK). The sampling procedure was repeated at 28 day intervals throughout the post-supplementary period. A monthly pooled sample of the diets and their compositional ingredients were analyzed to determine Se content (ICP-MS) and to calculate metabolisable energy content (ME). The quantity of feed offered to each individual horse was daily recorded; nutrient intakes, obtained from these data, were monthly calculated. Results obtained during the post-supplementary period were analyzed using the Mixed Models procedure of SAS. The statistical model included treatment and time. Statistical tests were undertaken for main effects and first order interactions. The GSH-Px values following Se withdrawal of each horse were used to calculate a 2nd degree regression (time as independent variable). A first derivative was calculated at each time using the coefficients estimated with the regression for each horse and subsequently analyzed using the Mixed Models procedure. The model included treatment and time and GSH-Px values at T0 were used as a covariate term.

RESULTS AND CONCLUSIONS – The mean background Se content of the diet was 0.085 mg/kg DM, whereas the Se content during the same period for treatments S2, S3, S4 and N3 were 0.181, 0.290, 0.395 and 0.288 mg/kg DM respectively. The mean crude protein content of the diets was 121.6 ± 12.40 g/kg DM. The mean calculated ME content of the diets was 2.16 Mcal/kg DM with values ranging between 2.12 and 2.20 Mcal/kg DM. At the completion of the period of Se supplementation (T0) values of GSH-Px were significantly higher in all those treatments that had been supplemented with Se when compared to the unsupplemented control (Table 1). Furthermore, GSH-Px activity was significantly greater at T0 in treatments S3 and S4 when compared to S2. Despite there being no significant difference in GSH-Px activity at T0 between organic and inorganic Se sources it should be noted that GSH-Px activity was numerically higher in those animals receiving an organic Se source when compared to an inorganic supply (S3 vs N3).

Following the withdrawal of Se supplementation GSH-Px activity decreased in all treatments. The slow and prolonged reduction in GSH-Px activity following the withdrawal of supplementary Se is related to the rate of turnover of red blood cells. The lifespan of equine erythrocytes is 140-150 days. Furthermore, GSH-Px activities are still notably higher at T112 in those animals that had received Se supplementation when compared to the negative control at the same time point, although rates of decline were greatest in those treatments with the highest levels of selenium supplementation, possibly reflecting the rate of erythrocytes substitution.

Table 1. Activity of GSH-Px (U/g of Hb) following the withdrawal of Se supplementation (T0) in horses that had previously received diets containing either no additional Se (C), or 0.77, 1.62 and 2.47 mg/d of organic Se (S2, S3 and S4, respectively) or 1.62 mg/d of inorganic Se (N3).

| Time | C          | S2        | S3        | S4        | N3        | SED (a) |
|------|------------|-----------|-----------|-----------|-----------|---------|
| T0   | 170.2\textsuperscript{aA} | 243.3\textsuperscript{bAB} | 306.6\textsuperscript{cB} | 300.4\textsuperscript{cB} | 283.0\textsuperscript{bcB} | |
| T28  | 155.0\textsuperscript{aA} | 253.9\textsuperscript{bB} | 304.0\textsuperscript{bB} | 320.7\textsuperscript{cB} | 297.9\textsuperscript{bB} | |
| T56  | 141.9\textsuperscript{aA} | 247.3\textsuperscript{bB} | 294.9\textsuperscript{bB} | 294.4\textsuperscript{cB} | 276.1\textsuperscript{bcB} | 21.5727 |
| T84  | 140.1\textsuperscript{aA} | 235.0\textsuperscript{bB} | 293.5\textsuperscript{cB} | 264.4\textsuperscript{bB} | 255.8\textsuperscript{bcB} | |
| T112 | 159.3\textsuperscript{aA} | 221.7\textsuperscript{bAB} | **255.1\textsuperscript{bB}** | **236.0\textsuperscript{bAB}** | **212.3\textsuperscript{bAB}** | |
| SED (b) | | | | | | 15.3979 |

\textsuperscript{**}: P<0.01; \textsuperscript{***}: P<0.001 at the left of the mean indicate the differences within column (T0 vs T112).

Means on the same row without right common superscripts differ significantly (\textsuperscript{a,b}: P<0.05; \textsuperscript{A,B}: P<0.001).

SED (a): standard error of difference between two treatment means of 5 horses at a given time.

SED (b): standard error of difference between two time means of 5 horses for the same treatment.

Glutathione peroxidase activity in blood following the withdrawal of selenium supplementation was better preserved when organic Se was used when compared to a comparable level of inorganic Se; at T112 GSH-Px activities were greater (P=0.08) in those animals supplemented with organic Se (S3) when compared to inorganic Se (N3).
with values 255.1 and 212.3 U/g Hb respectively. Table 2 shows the rates of decline in GSH-Px activity following withdrawal of selenium supplementation. These results, covariate adjusted using GSH-Px values observed at T0, show an increase in the rate of decline of GSH-Px activity at sequential 28 day intervals following the withdrawal of Se in those treatments supplemented with Se (Table 2). The rate of decline in GSH-Px activity was not affected significantly by the levels of Se supplementation although S4 shows numerically higher values than S2 (P=0.12 at T112) and S3 (P=0.16 at T112). In addition, the sustained GSH-Px activity following the withdrawal of selenium supplements seen in animals that had received an organic selenium supplement was confirmed; at T112 the reduction rate in GSH-Px activity was -0.86 U/g Hb day⁻¹ in S3 compared to -1.65 U/g Hb day⁻¹ in N3 (P=0.06). Furthermore, rate of declination was also greater in N3 at T112 when compared to S2 (P<0.05), although there were no appreciable differences between N3 and S4. The slower rate of decline observed in organic Se supplementation could suggest a higher incorporation of Se, as SeMet, in various proteins in the body in order to preserve Se for future use (Surai, 2006). In humans the average whole body half-life of SeMet is higher (252 d) than selenite (102 d), indicating a re-utilization of SeMet in the body (Patterson et al., 1989).

Table 2. Rate of decline in GSH-Px activity (U/g Hb per day) following the withdrawal of Se supplementation (T0) in horses that had previously received diets containing either no additional Se (C), or 0.77, 1.62 and 2.47 mg/d of organic Se (S2, S3 and S4, respectively) or 1.62 mg/d of inorganic Se (N3).

| Time | C     | S2    | S3    | S4    | N3    | SED (a) |
|------|-------|-------|-------|-------|-------|---------|
| T0   | -1.18a| 0.26b | 0.38b | 0.47b | 0.56b |         |
| T28  | 0.79  | 0.01  | 0.07  | -0.01 | 0.01  |         |
| T56  | -0.40 | -0.26 | -0.24 | -0.50 | -0.54 | 0.4538  |
| T84  | -0.01b| -0.51a| -0.55a| -0.98a| -1.09a|         |
| T112 | -0.38a| -0.77a| -0.85a| -1.46a| -1.65a|         |
| SED (b) | 0.42 |       |       |       |       |         |

Means on the same row without right common superscript differ significantly (a,b: P<0.05; A,B: P<0.001).<br>SED (a): standard error of difference between two treatment means of 5 horses at a given time.<br>SED (b): standard error of difference between two time means of 5 horses for the same treatment.

In conclusion the supplementation with organic vs inorganic Se did not affect ultimate GSH-Px activities achieved at the end of the supplementation period. However, organic Se supplementation would appear to preserve GSH-Px activity following withdrawal of Se supplementation. Therefore, from a nutritional viewpoint, organic forms of Se supplements are superior to selenite in maintaining GSH-Px activity during periods of Se inadequacy.

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