Published December 4, 2014

Metabolic and hematological profiles in mature horses supplemented with different selenium sources and doses[1,2]

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ABSTRACT: This study was conducted to determine the effects of dietary Se source and dose on metabolic and hematological profiles, and their relationships with oxidative status in horses. Twenty-five mature horses were blocked by BW and randomly allocated to 1 of 5 dietary treatments: negative control (CTRL: 0.085 mg of Se/kg of DM), 3 different dietary concentrations of organic Se provided by Se yeast (SY02, SY03, and SY04 containing 0.2, 0.3, and 0.4 mg of total Se/kg of DM, respectively), and 1 positive control provided by sodium selenite (SS03 containing 0.3 mg of total Se/kg of DM). Horses were fed the same basal diet (6 kg of grass hay and 3 kg of concentrate per horse daily) and received their respective treatments for a continuous period of 112 d. Jugular venous blood samples were collected before the morning feed on d 0, 28, 56, 84, and 112. Whole blood was analyzed for hematological profile, and plasma was analyzed for metabolites of energy, protein, and mineral metabolism; enzymatic activities and metabolites related to liver and muscle damage; and markers of inflammatory and oxidative status. Plasma metabolites related to energy, protein, and mineral metabolism, acute phase proteins, and enzyme activities related to hepatocellular, hepatobiliary, and muscle damage were not affected by Se source or dose. There were no differences among treatments in either reactive oxygen metabolites or thiol group concentrations in plasma. However, a linear decrease ($P < 0.01$) in plasma total antioxidants was observed with increasing Se yeast supplementation. Furthermore, total antioxidant concentrations were less in SY03 than SS03 horses ($P < 0.05$), and were less in SY03 and SY04 than CTRL horses ($P < 0.05$). These results could be interpreted as an improvement in the preventive antioxidant systems of horses fed Se yeast. Total white blood cell count was not affected by treatment. There was a tendency for horses receiving greater concentrations of Se yeast to have greater lymphocyte counts ($P = 0.09$), with greater lymphocyte counts in blood of SY03 vs. SS03 horses ($P < 0.05$). Despite the lack of effect of Se source and dose on markers of inflammatory and liver status, the hematological profile seems to indicate an immunomodulatory action, as shown by mild changes in the white blood cell populations in response to Se yeast inclusion.

Key words: hematological profile, horse, oxidative status, selenium yeast, sodium selenite

J. Anim. Sci. 2010. 88:650–659
doi:10.2527/jas.2009-1855

INTRODUCTION

Selenium plays immunomodulatory and anti-inflammatory roles in the animal. A major immunostimulatory effect of Se is Se-induced, upregulated expression of the α and β subunits of the IL-2 receptor, which are expressed especially in T and B lymphocytes, increasing the ability of these cells to respond to IL-2 (McKenzie et al., 2002). The anti-inflammatory effects of Se rely on its ability to influence the redox state of the cell and the ability of Se compounds to remove reactive oxygen species (McKenzie et al., 2002).

Selenium supplements can be in 2 forms: inorganic such as Na selenite (Na_2SeO_3) or organic such as selenomethionine (**SeMet**), which is the predominant form of Se within seleno yeasts (**Se yeast**). Selenomethionine, unlike selenite, is actively transported by methionine transporter mechanisms across intestinal membranes during absorption and can be nonspecifically incorpo-

[1]This research was funded by European Union Regulatory Affairs Department, Alltech France.
[2]The authors thank G. Brigati (Veterinary Office Cristella, Cremona, Italy) for veterinary assistance, and G. M. D'Aragona and E. D'Aragona (Agricultural Company Croara Vecchia, Piacenza, Italy) for their contribution to this research.
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Received January 31, 2009.
Accepted October 21, 2009.

rated into body protein (Schrauzer, 2003). Irrespective of source, Se must undergo a metabolic transformation before its assimilation into selenocysteine and subsequent incorporation into selenoproteins (Weiss, 2003).

Results of studies on the effects of Se supplementation on hematological variables are few and often contradictory. In lambs, total erythrocyte count and osmotic resistance of red blood cells (**RBC**) were greater in animals supplemented with Se yeast, whereas white blood cell (**WBC**) counts were less than those fed the basal diet (Faixová et al., 2007). Similarly, Horton et al. (1978) reported decreased leukocyte counts in the blood of Se-supplemented ewes and lambs. Conversely, lactating dairy cows, offered a diet containing different concentrations of Se as Seyeast or as Na_2SeO_3, showed no treatment effects regardless of the Se source or dose (Juniper et al., 2006).

The aim of this study was to evaluate the effect of the graded addition of Se yeast to the diet of mature horses and comparable doses of organic and inorganic Se supplements on hematological profile, enzyme activities, and plasma oxidative status, together with markers of inflammatory status.

MATERIALS AND METHODS

The research protocol and the animal care were in accordance with the European Community Council Directive guidelines for animals used for experimental and other scientific purposes (European Community, 1986).

Animals and Husbandry

Horses used in this study were raised in a private herd located in Piacenza province (Italy). A total of 25 mature Italian Saddle horses were selected on the basis of good health, age (mean age 13.6 ± 4.8 yr), and activity (slightly exercised). All horses were housed in a single barn with each horse occupying a single box stall. Description of housing and husbandry was reported previously (Calamari et al., 2009). All horses were individually fed and received the same basal diet. Feeding management and diet composition were also reported previously (Calamari et al., 2009). Representative samples of hay and concentrate feed were taken weekly during the study and pooled on a monthly basis for the analyses. Each horse was individually fed, and refusals were recorded daily.

Experimental Design

The study was conducted as a randomized complete block design composed of a continuous period of 112 d (from November 30, 2005, to March 23, 2006), during which all horses received the same basal diet that differed in only Se source (Se yeast vs. Na_2SeO_3) or dose of yeast [Sel-Plex, Se yeast (*Saccharomyces cerevisiae* CNCM I-3060) containing 63% SeMet (Alltech, Nicholasville, KY)]. Before beginning of the study, all horses received the same basal diet without additional Se for 2 mo. Horses were then blocked by BW and randomly allocated to 1 of 5 dietary treatments with 5 horses per treatment: negative control (**CTRL**; background Se only), 3 different concentrations of Se yeast supplementation [**SY02**, **SY03**, and **SY04**; 0.77, 1.62, and 2.47 mg of Se/(animal·d), respectively, to achieve 0.2, 0.3, and 0.4 mg of total Se/kg DM], and 1 positive control supplemented with Na_2SeO_3 [**SS03**; 1.62 mg of Se/(animal·d) to achieve 0.3 mg of total Se/kg of DM; Retorte GmbH, Röthenbach a.d., Pegnitz, Germany]. The mean age and the mean BW of horses of each treatment were reported previously (Calamari et al., 2009). All Se supplements were offered daily to each individual horse by top-dressing the concentrate fed each morning using 6 $g \cdot d^{-1}$ of Ca carbonate as a carrier.

Measurements and Sampling

Blood samples were obtained before the morning feed (0800 h) at d 0 ($\mathbf{T_0}$), 28 ($\mathbf{T_{28}}$), 56 ($\mathbf{T_{56}}$), 84 ($\mathbf{T_{84}}$), and 112 ($\mathbf{T_{112}}$). At each sample point, 3 blood samples were collected by venipuncture from a jugular vein: two 7.5-mL Li-heparin-treated tubes (Monovet tube Se-free, containing 15 IU of Li-heparin/mL, Sarstedt, Princeton, NJ) and one 3-mL K_3EDTA-treated tube (Venoject, containing 2.1 g of K_3EDTA/mL, Terumo, Leuven, Belgium). Blood samples were immediately placed into an ice-bath where they were stored until they were processed within approximately 30 min after collection.

Laboratory Analyses

Selenium Content in Feeds and Whole Blood. Selenium content of the specific premixes and composite feedstuffs was determined after wet mineralization (acid hydrolysis) according to Laurent (1997) using inductively coupled plasma mass spectrometry (Elan 6100, Perkin Elmer, Norwood, MA). The first Li-heparin-treated tube was used to measure total Se and glutathione peroxidase (**GPX-1**) activity as described by Calamari et al. (2009).

Plasma Metabolic Profile. The second Li-heparin-treated tube was centrifuged (3,500 × *g* for 15 min at 10°C), and plasma was separated and immediately stored at –20°C until analysis. Blood metabolites were analyzed at 37°C by an automated clinical analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA). Analysis for glucose, urea, calcium, inorganic phosphorus, magnesium, total protein, albumin, total bilirubin, and creatinine were conducted using commercial kits (Instrumentation Laboratory) and zinc (Wako Chemicals GmbH, Neuss, Germany). Enzymatic analyses for total cholesterol and triglycerides were conducted using commercial kits (Instrumentation Laboratory). A potentiometric system with specific electrodes was used to determine Na, K, and Cl. Plasma thiol groups (**SHp**) were analyzed using commercial kit (Diacron

International, Grosseto, Italy) as described by Bernabucci et al. (2005). Total plasma antioxidants (**TA**) were analyzed using a commercial kit (Oxy-Adsorbent test, Diacron International, Grosseto, Italy), according to the methods of Trotti et al. (2001). The Oxy-Adsorbent test assesses the antioxidant power of the plasma barrier by measuring the ability of the barrier to oppose the massive oxidant action of hypochlorous acid (**HClO**). Results are expressed as micromoles of HClO per milliliter remaining after the reaction. Total plasma reactive oxygen metabolites (**ROM**) were measured using a commercial kit (d-ROMs test, Diacron International) as described by Bernabucci et al. (2005). Results are expressed as milligrams of hydrogen peroxide per 100 mL of plasma. This reagent kit measures not only ROM existing in the matrix, but also the species developing during the Fenton reaction (Oriani et al., 2001). Kinetic analysis was adopted to determine activity of glutamate dehydrogenase (**GDH**, EC 1.4.1.2) using a commercial kit (Randox Laboratories, Antrim, UK), and alkaline phosphatase (EC 3.1.3.1), aspartate aminotransferase (**AST**, EC 2.6.1.1), γ-glutamyltransferase (**GGT**, EC 2.3.2.2), L-lactate dehydrogenase (**LDH**, EC 1.1.1.27), alanine aminotransferase (**ALT**, EC 2.6.1.2), and creatine kinase (**CK**, EC 2.7.3.2) using commercial kits (Instrumentation Laboratory). Ceruloplasmin and haptoglobin were determined with reagents prepared according to the method reported by Bertoni et al. (1998). Total Se in plasma was determined according to the method reported by Calamari et al. (2009).

Hematological Profile. The K_3EDTA-treated tube of whole blood was used to measure hematological profile using a Cell-Dyn 3700 hematology analyzer (Abbott Diagnostici, Roma, Italy). Measurements were total RBC number (millions/μL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (fL), mean corpuscular hemoglobin (**MCH**; pg), MCH concentration (g/dL), width of RBC volume distribution (**RDW**; %), total WBC number (thousands/μL), neutrophils (thousands/μL and % of WBC), lymphocytes (thousands/μL and % of WBC), monocytes (thousands/μL and % of WBC), eosinophils (thousands/μL and % of WBC), basophils (thousands/μL and % of WBC), total platelet number (thousands/μL), and mean platelet volume (fL).

Statistical Analysis

Results were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) according to Littell et al. (1998). Data were tested for normal distribution using the Shapiro-Wilk test. Variables not normally distributed were subjected to log-transformation to obtain a normal distribution of the values before statistical analysis.

Treatment, time, and treatment × time interaction were included in the statistical model. The random variable was horse within treatment. Pretreatment period data ($\mathbf{T_0}$) were used as a covariate. Each variable analyzed was subjected to 3 covariance structures: autoregressive of first order, compound symmetry, and spatial power. Using the Akaike information criterion and Schwarz Bayesian criterion, the spatial power was the covariance structure that best fitted the model. The dose response to concentration of Se yeast in the diet was considered for treatments CTRL, SY02, SY03, and SY04. If a F-test or linear dose effect was $P < 0.15$, all possible pairwise comparisons of treatment means were made using PDIFF option of SAS and also noted in tables when $P < 0.05$. Within a table, sources of variation that had no effect ($P > 0.15$) were noted as not significant; otherwise, the actual P-value was reported. Simple Pearson correlations, across and within treatment, were calculated among the variables for the entire period using CORR procedure of SAS and reported when $P < 0.01$.

Table 1. Values of Se concentration of the diet (mean ± SD of monthly measurements) in each treatment during the experiment

Treatment[1]	Se additive	Total, mg/kg of DM
Negative control[1]	None	0.085 ± 0.052
Organic Se (SY02)[2]	Se yeast[3]	0.182 ± 0.053
Organic Se (SY03)[2]	Se yeast[3]	0.290 ± 0.053
Organic Se (SY04)[2]	Se yeast[3]	0.395 ± 0.053
Positive control (SS03)[2]	Na selenite	0.288 ± 0.053

[1]The estimated value before the start of the study was 0.10 mg of Se/kg of DM.
[2]The intended dietary Se contents were 0.20, 0.30, 0.40, and 0.30 mg of total Se/kg of DM for SY02, SY03, SY04, and SS03, respectively.
[3]Sel-Plex Se yeast (Alltech, Nicholasville, KY).

RESULTS

The concentration of total Se in the negative control diet was 0.085 mg of Se/kg of DM, which was similar to estimated value before the beginning of the study (Table 1). Total dietary Se concentrations of Se-supplemented treatments were also similar to values estimated before the beginning of the study. Chemical and nutritive characteristics of the diet used in this study were reported previously by Calamari et al. (2009). The results on Se status (total blood Se, total plasma Se, and GPX-1) obtained in this study have been already reported (Calamari et al., 2009).

Plasma Metabolites

Plasma metabolites related to energy and protein metabolism (Table 2) and mineral metabolism (Table 3) were not affected by Se source or dose. Furthermore, no differences among treatments were observed in clinical health status. There was a trend for an overall treatment effect on Na ($P = 0.086$) and also a treatment × time interaction for Cl ($P = 0.03$), but there seemed to be no clear effect of the treatment.

Table 2. Effect of Se sources and doses on plasma metabolites related to energy and protein metabolism for each experimental group of mature horses[1]

	Dietary treatment[2]					
Item	CTRL	SY02	SY03	SY04	SS03	SED
Glucose, mmol/L	5.64	5.66	5.71	5.50	5.62	0.15
Total cholesterol, mmol/L	2.76	2.67	2.74	2.69	2.73	0.10
Triglycerides, mmol/L	0.349	0.388	0.396	0.359	0.353	0.036
Urea, mmol/L	5.97	5.90	5.93	6.02	5.91	0.29
Creatinine, μmol/L	133.3	133.3	129.9	132.6	132.3	3.5
Total protein, g/L	67.2	65.2	66.8	64.9	63.9	1.7
Albumin, g/L	37.90	37.17	38.09	36.95	36.76	0.56

[1]No effect of treatments was observed. CTRL is control diet; SY02, SY03, and SY04 are Se yeast diets (Sel-Plex Se yeast; Alltech, Nicholasville, KY); and SS03 is Na selenite diet. Least squares means, n = 5 horses.

[2]Diet provided a total of 0.085 mg of Se/kg of DM by natural feedstuffs without Se supplementation. Se yeast provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM for SY02, SY03, and SY04, respectively. Na selenite (SS03) provided 0.29 mg of total Se/kg of DM.

Acute Phase Proteins and Enzyme Activities

Inflammatory status appeared not affected by Se source and dose as indicated by ceruloplasmin and haptoglobin plasma concentrations (Table 4). In addition, there appeared to be no effect of time or a treatment × time interaction with respect to inflammatory status variables. Plasma CK activity was not affected by treatment or time, and there was no treatment × time interaction, indicating no effect of Se source or dose on muscle metabolism (Table 5). This was further confirmed by the activities of AST and LDH, which were also not affected by treatment and time, with no treatment × time interaction. Plasma ALT, AST, and GDH activities were unaffected by treatment and time, and there was no treatment × time interaction, indicating no notable effect of Se source or Se dose in a time-dependent manner on hepatocellular damage. Plasma alkaline phosphatase activity did not differ among treatments. Plasma GGT tended to have a greater value in CTRL horses than those receiving treatment SS03 ($P = 0.058$). Despite these differences, total bilirubin concentrations were not affected by Se source or dose.

Oxidative Status of Plasma

There were no differences in oxidative status-related markers, ROM, or SHp concentration, among experimental groups (Table 4). However, SY03 and SY04 horses had less plasma TA than horses from treatment CTRL ($P < 0.05$), and less plasma TA ($P < 0.05$) were observed in those animals receiving the Se yeast supplement when compared with a comparable dose of selenite (SY03 vs. SS03). Plasma TA decreased linearly as Se yeast supplementation increased. A correlation was observed between plasma ROM and ceruloplasmin ($r = 0.86$; $P < 0.01$) and between plasma ROM concentration and ALT activity ($r = 0.41$; $P < 0.01$).

Table 3. Effect of Se sources and doses on plasma concentration of the monitored minerals for each experimental group of mature horses[1]

	Dietary treatment[2]						*P*-value		
Item	CTRL	SY02	SY03	SY04	SS03	SED	Tr[3]	Tr × T[4]	Linear dose effect[5]
Ca, mmol/L	3.25	3.20	3.25	3.14	3.25	0.05	NS	NS	NS
P, mmol/L	1.14	1.09	1.16	1.11	1.08	0.07	NS	NS	NS
Mg, mmol/L	0.93	0.93	0.90	0.90	0.94	0.02	NS	NS	NS
Na, mmol/L	140.0[a]	141.0[ab]	141.1[ab]	141.6[b]	142.0[b]	0.6	0.086	NS	NS
K, mmol/L	3.75	3.54	3.72	3.72	3.71	0.17	NS	NS	NS
Cl, mmol/L	105.5	104.9	105.3	105.8	105.5	0.5	NS	0.003	NS
Zn, μmol/L	10.87	10.53	10.52	9.88	10.44	0.55	NS	NS	NS

[a,b]Within a row, means without common superscripts differ ($P < 0.05$).

[1]CTRL is control diet; SY02, SY03, and SY04 are Se yeast diets (Sel-Plex Se yeast; Alltech, Nicholasville, KY); and SS03 is Na selenite diet. Least squares means, n = 5 horses. NS = not significant ($P > 0.15$).

[2]Diet provided a total of 0.085 mg of Se/kg of DM by natural feedstuffs without Se supplementation. Se yeast provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM for SY02, SY03, and SY04, respectively. Na selenite (SS03) provided 0.29 mg of total Se/kg of DM.

[3]Tr: treatment effect.

[4]Tr × T: treatment × time interaction.

[5]Linear dose effect including CTRL, SY02, SY03, and SY04 treatments.

Table 4. Effect of Se sources and doses on positive acute phase proteins and oxidative status markers in plasma of mature horses[1]

	Dietary treatment[2]						*P*-value		
Item	CTRL	SY02	SY03	SY04	SS03	SED	Tr[3]	Tr × T[4]	Linear dose effect[5]
Ceruloplasmin, μmol/L	3.50	3.28	3.42	3.35	3.24	0.25	NS	NS	NS
Haptoglobin, g/L	0.55	0.58	0.58	0.55	0.66	0.06	NS	NS	NS
Globulin, g/L	29.51	28.18	28.74	28.06	26.86	1.35	NS	NS	NS
Thiol groups, μmol/L	286.9	277.2	291.2	269.6	287.0	14.8	NS	NS	NS
ROM, mg of H_2O_2/dL	13.61	13.54	13.56	13.22	13.02	1.20	NS	NS	NS
Total antioxidant, μmol of HClO[6]/mL	246.8[b]	234.5[ab]	220.9[a]	218.1[a]	244.1[b]	11.4	0.029	NS	0.009

[a,b]Within a row, means without common superscripts differ ($P < 0.05$).

[1]CTRL is control diet; SY02, SY03, and SY04 are Se yeast diets (Sel-Plex Se yeast; Alltech, Nicholasville, KY); and SS03 is Na selenite diet. Least squares means, n = 5 horses. NS: not significant ($P > 0.15$). ROM: reactive oxygen metabolites.

[2]Diet provided a total of 0.085 mg of Se/kg of DM by natural feedstuffs without Se supplementation. Se yeast provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM for SY02, SY03, and SY04, respectively. Na selenite (SS03) provided 0.29 mg of total Se/kg of DM.

[3]Tr: treatment effect.

[4]Tr × T: treatment × time interaction.

[5]Linear dose effect including CTRL, SY02, SY03, and SY04 treatments.

[6]HClO = hypochlorous acid.

Hematological Responses

Total WBC number was not affected by treatment (Table 6). The number of lymphocytes increased slightly (linear, $P = 0.095$) as Se yeast supplementation increased. Greater values of lymphocytes were observed in SY03 and SY04 when compared with SS03 horses ($P < 0.05$), whereas eosinophil values were greater in SY02 and SY03 than SS03 horses ($P < 0.05$). Leukocyte population when expressed as a percentage of WBC reflected the differences in absolute values. Differences in the other hematological measurements, even those with statistically significant differences (MCH and RDW) did not seem to follow a treatment-related trend.

Variations in lymphocyte number and neutrophil:lymphocyte ratio throughout the experiment are shown in Figure 1. The most noticeable difference in lymphocyte counts with respect to time was observed at d 84, when the $\log_{10}$ of the absolute value was less in SS03 than SY02, SY03, or SY04 ($P < 0.01$; Figure 1a). The main difference for neutrophil:lymphocyte ratio with respect to time was observed at d 56, and the $\log_{10}$ of the ratio was less in SY04 than in SY02 or SS03 ($P < 0.01$; Figure 1b).

DISCUSSION

Selenoproteins are involved in a wide range of physiological functions (e.g., thyroid activity, muscular metabolism, antioxidant activity, immune response, and erythrocyte stability), mainly by their role as part of enzymes (Surai, 2006). In the present research, the pos-

Table 5. Effect of Se sources and doses on enzymes activity and total bilirubin in plasma of mature horses[1]

	Dietary treatment[2]						*P*-value		
Item	CTRL	SY02	SY03	SY04	SS03	SED	Tr[3]	Tr × T[4]	Linear dose effect[5]
CK, U/L	172.6	154.3	148.9	180.6	152.5	20.0	NS	NS	NS
AST, U/L	289.5	301.3	293.5	299.6	277.1	16.0	NS	NS	NS
LDH, U/L	611.0	597.3	536.3	579.4	562.2	47.8	NS	NS	NS
ALT, U/L	6.61	7.79	6.32	7.04	6.33	0.61	NS	NS	NS
GDH, U/L	1.82	2.24	0.63	1.25	0.58	0.85	NS	NS	NS
AP, U/L	81.89	77.89	88.50	76.00	72.39	6.91	NS	NS	NS
GGT, U/L	22.59[b]	19.80[ab]	22.46[b]	20.59[ab]	19.05[a]	1.32	0.058	NS	NS
Total bilirubin, μmol/L	32.05	28.27	30.86	29.05	31.37	3.03	NS	NS	NS

[a,b]Within a row, means without common superscripts differ ($P < 0.05$).

[1]CTRL is control diet; SY02, SY03, and SY04 are Se yeast diets (Sel-Plex Se yeast; Alltech, Nicholasville, KY); and SS03 is Na selenite diet. Least squares means, n = 5 horses. NS: not significant ($P > 0.15$). CK: creatine kinase activity; AST: aspartate aminotransferase activity; LDH: L-lactate dehydrogenase activity; ALT: alanine aminotransferase activity; GDH: glutamate dehydrogenase activity; AP: alkaline phosphatase activity; GGT: γ-glutamyltransferase activity; U: unit of activity.

[2]Diet provided a total of 0.085 mg of Se/kg of DM by natural feedstuffs without Se supplementation. Se yeast provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM for SY02, SY03, and SY04, respectively. Na selenite (SS03) provided 0.29 mg of total Se/kg of DM.

[3]Tr: treatment effect.

[4]Tr × T: treatment × time interaction.

[5]Linear dose effect including CTRL, SY02, SY03, and SY04 treatments.

Table 6. Effect of Se sources and doses on hematological profile in mature horses[1]

Item	Dietary treatment[2]						*P*-value		
	CTRL	SY02	SY03	SY04	SS03	SED	Tr[3]	Tr × T[4]	Linear dose effect[5]
White blood cell (WBC) count, $\log_{10}$ (thousands/mL)	3.74	3.77	3.76	3.73	3.74	0.02	NS	NS	NS
Neutrophils	3.53	3.54	3.53	3.48	3.54	0.03	NS	NS	NS
Lymphocytes (LYM)	3.27^{ab}	3.28^{ab}	3.30^{b}	3.31^{b}	3.23^{a}	0.03	0.114	NS	0.095
Monocytes	2.15	2.11	2.11	2.08	2.09	0.11	NS	NS	NS
Eosinophils	1.73^{ab}	1.83^{b}	1.92^{b}	1.78^{ab}	1.64^{a}	0.09	0.059	NS	NS
Basophils	1.23	1.15	1.05	1.06	1.22	0.16	NS	NS	NS
Neutrophil:LYM ratio, $\log_{10}$ [NEU (thousands/mL):LYM (thousands/mL)]	0.26^{b}	0.26^{ab}	0.23^{ab}	0.17^{a}	0.31^{b}	0.05	0.075	NS	NS
WBC count differential, %									
Neutrophils	61.44^{b}	60.59^{ab}	59.30^{ab}	56.06^{a}	64.03^{b}	2.40	0.056	NS	NS
LYM	34.12^{a}	34.80^{ab}	35.61^{ab}	38.39^{b}	31.97^{a}	2.00	0.067	NS	NS
Monocytes	3.63	3.06	2.75	3.33	3.18	0.64	NS	NS	NS
Eosinophils	1.04	1.24	1.72	1.30	0.93	0.34	NS	NS	NS
Basophils	0.35	0.35	0.28	0.31	0.32	0.09	NS	NS	NS
Red blood cell (RBC) count, millions/μL	6.96	7.19	7.42	7.35	7.04	0.23	NS	NS	NS
Hemoglobin, g/100 mL	12.98	13.05	13.24	13.43	12.96	0.37	NS	NS	NS
Hematocrit, %	33.92	34.34	35.37	35.19	34.12	1.13	NS	NS	NS
Mean corpuscular volume, fL	48.39	48.12	48.18	48.10	47.90	0.35	NS	NS	NS
Mean corpuscular hemoglobin, pg	18.67^{c}	18.16^{ab}	17.87^{a}	18.26^{b}	18.45^{bc}	0.16	0.002	0.087	NS
Mean corpuscular hemoglobin concentration, g/100 mL	38.10	38.11	37.81	38.08	37.82	0.33	NS	NS	NS
RBC distribution width, %	23.20^{a}	24.02^{b}	23.13^{a}	23.47^{ab}	22.94^{a}	0.37	0.081	NS	NS
Platelets, thousands/μL	97.24	95.38	90.62	94.67	93.57	5.33	NS	NS	NS

$^{a–c}$Within a row, means without common superscripts differ ($P < 0.05$).

[1]CTRL is control diet; SY02, SY03, and SY04 are Se yeast diets (Sel-Plex Se yeast; Alltech, Nicholasville, KY); and SS03 is Na selenite diet. Least squares means, n = 5 horses. NS: not significant ($P > 0.15$). NEU: neutrophils; LYM: lymphocytes.

[2]Diet provided a total of 0.085 mg of Se/kg of DM by natural feedstuffs without Se supplementation. Se yeast provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM for SY02, SY03, and SY04, respectively. Na selenite (SS03) provided 0.29 mg of total Se/kg of DM.

[3]Tr: treatment effect.

[4]Tr × T: treatment × time interaction.

[5]Linear dose effect including CTRL, SY02, SY03, and SY04 treatments.

sible cascade of consequences, which can arise from differences in Se availability in mature horses, were addressed by studying hematological and metabolic profile and oxidative stress markers.

To our knowledge, only a few papers on the metabolic profiles of mature horses maintained on mild physical activity (not for racing) are available. However, a comparison of our results with those with comparable horses used in those studies indicates a good nutritional status of the animals in our experiment. Particularly, plasma glucose values agree with those reported by Calamari et al. (1990) and by Stull and Rodiek (1988) with different diets. Plasma creatinine values were not affected by Se supply and were in the reference range reported by Amory et al. (2005). Inflammatory status was not affected by source or dose of Se supplement, as indicated by the lack of any difference in plasma ceruloplasmin and haptoglobin concentrations. Values of haptoglobin were less than those reported by Hanzawa et al. (2002) in Thoroughbred horses, whereas those of ceruloplasmin agree with those reported by Calamari et al. (1990) in Italian trotter horses.

Enzyme activities that reflect cellular damage, because these enzymes are released into the circulatory fluid when cell membrane integrity is damaged, were not affected by the treatment in this study. The activity of AST was not affected by treatment and was well within published normal ranges (Falaschini et al., 2005; Harvey et al., 2005; Pieralisi and Comazzi, 2006). Plasma CK activity was not affected by treatment; however, the effect on this enzymatic activity is generally demonstrable only in some physiological phases and when Se is considerably deficient in the diet (Arthur, 1988). Plasma ALT activity was not affected by treatment, but a comparison of absolute values with reference values is difficult because recently published reference values for ALT in horses are for trained trotters (Valle et al., 2006) rather than older saddle horses used in this study. Consequently, ALT enzyme activities seem to be less than published values. In addition, LDH activity was not affected by dose or source of Se used in this study, even though the absence of any reference value makes it impossible to make any comparison with other horses.

Among the other enzyme activities, only GGT activity was affected by treatment, this is different to the observations made by Juniper et al. (2006) and Faixová et al. (2007) who did not report any differences on this plasma enzymatic activity when using Se supplements. However, total bilirubin concentration was not affected

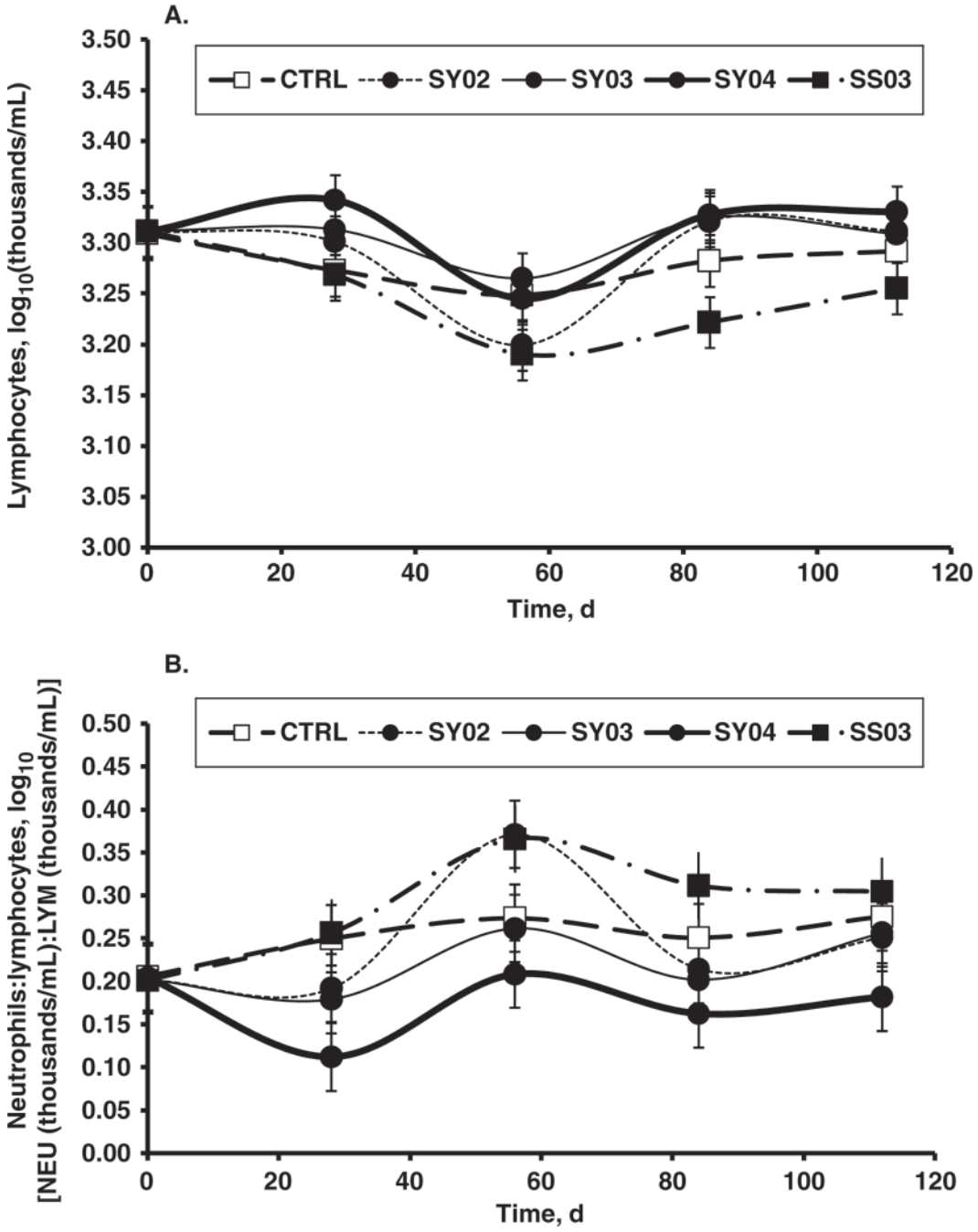

Figure 1. Blood lymphocyte count [A, $\log_{10}$(thousands/mL)] and neutrophil:lymphocyte ratio [B, $\log_{10}$(neutrophils, thousands/mL:lymphocytes, thousands/mL)] during the supplementation period in horses that received diets containing no additional Se (CTRL: □ and dashed line), Se yeast (Sel-Plex Se yeast; Alltech, Nicholasville, KY) that provided 0.18, 0.29, and 0.39 mg of total Se/kg of DM (SY02: ● and thin dotted line; SY03: ● and thin continuous line; and SY04: ● and bold continuous line, respectively), or Na selenite that provided 0.29 mg of total Se/kg of DM (SS03: ■ and dashed-dotted line). Values are least squares means, and vertical bars represent SEM; n = 5 horses. Lymphocyte counts at d 84: SS03 vs. SY02, SY03, or SY04, $P < 0.01$; neutrophil:lymphocyte ratio at d 56, SY04 vs. SY02 or SS03, $P < 0.01$.

by treatment, indicating no evidence of hepatobiliary injury (Boone et al., 2005). Furthermore, the GDH activities were not affected by treatment, time, or treatment × time interaction, indicating no effect of the supplementation treatment on hepatocellular damage (Boone et al., 2005).

The absence of treatment effects on enzyme activities related to muscle cellular damage could be explained by the experimental conditions of the current study. Horses were only slightly exercised, which did not result in any increases in markers of muscle cellular metabolism, thus avoiding any changes in these enzyme activities that are related to cell membrane integrity (Peter, 1988; Kozat, 2007). The biochemical role of Se in the protection of cell membranes and organelles from oxidative damage is well documented (Surai, 2006). It is this function that helps maintain muscle and vascular integrity. In the current study, the Se values of CTRL horses throughout the study and those on Se treatment at T_0 were indicative of marginal Se status. Treatment

means for total plasma Se in horses at T_0 (86.0 ng/g; Calamari et al., 2009) were below the reference range of 130 to 160 ng/mL for adult horses reported by Stowe and Herdt (1992), but above concentrations considered as deficient (8 to 53 ng/mL) for horses (Puls, 1994). The values of total Se in whole blood observed at T_0 were similar to the minimum of the reference range of 182 to 240 ng/mL reported for adult horses by Stowe (1998). As reported previously (Calamari et al., 2009), at the end of the supplementation period (T_{112}), the total Se content of whole blood was 178.0 ± 16.1, 346.2 ± 15.7, 448.5 ± 15.8, 488.9 ± 16.1, and 381.5 ± 15.9 ng/g for CTRL, SY02, SY03, SY04, and SS03, respectively. A linear dose effect and source effect were observed with greater Se contents in those horses supplemented with Se yeast (SY03) at T_{84} and T_{112} when compared with those receiving a comparable dose of selenite (SS03).

In the equine species, an exercise-induced imbalance in favor of oxidants has been described in several experiments (Mills et al., 1997; Deaton et al., 2002; Kirschvink et al., 2002), as well as in field investigations (Hargreaves et al., 2002; Marlin et al., 2002). Exercise-induced oxidative stress is believed to contribute to accelerated muscle fatigue and muscle fiber damage, leading to exercise intolerance and poor performance in different animal species (Sen and Packer, 2000), and Se nutrition is of interest due to its effects on exercise tolerance (Avellini et al., 1999). de Moffarts et al. (2005) reported that, in intensely trained horses and horses that have raced the day before blood collection, the long-term oral supplementation with antioxidants could partially counterbalance the oxidative stress induced by exercise by improving the hydrophilic, lipophilic, and enzymatic antioxidant blood capacity. The activity levels of the horses involved in this study were very slight (slightly exercised); thus, the experimental conditions in the current study were not so stressful to induce possible benefits against oxidative damages.

No differences among experimental groups in ROM or SHp levels were observed, although the results seems to be inconsistent with the reduced neutrophil concentration in SY04 horses, which had a less TA concentration than CTRL and SS03 horses. The correlations between plasma ROM and ceruloplasmin concentrations and between plasma ROM concentration and ALT activity seem to indicate how plasma ROM are related, within the context of this study, to liver activity. Local inflammatory response can be excluded by the absence of any relationships with blood neutrophil concentration.

Questions arise on the response timing of antioxidant concentrations to increasing pro-oxidative activity and the meaning of TA concentrations in plasma because very few data are available on the kinetics of TA. Generally, cytokines increase oxidant production and antioxidant defenses, thus minimizing damage to the host (Grimble, 2001). Therefore, our observations seem to indicate that SY04 horses had a better inflammatory status that had less impact on these cytokine activities.

Total plasma antioxidants decreased in a linear fashion to the graded addition of Se yeast to the diet. Furthermore, decreased TA values in Se yeast (SY03) vs. selenite (SS03) were observed in this study. Because measuring different antioxidant molecules separately is not practical, the evaluation of antioxidant status based on total antioxidative capacity of plasma samples is widely used as an indicator of oxidative stress (Boldižárová et al., 2005).

The interpretation of plasma TA variations in this experiment is difficult, also considering the increased variability observed by Balogh et al. (2001) when antioxidant capacity was assessed using different methods. Boldižárová et al. (2005) hypothesized that a long-term feeding of Se yeast could result in nonspecific incorporation of SeMet into general body proteins, which can be continuously released into the free AA pool and subsequently splitting into larger amounts of selenide (Suzuki and Ogra, 2002). The continuously formed selenide of SeMet origin is constantly bound to albumin, which was reflected in decreased plasma TA in lambs (Boldižárová et al., 2005). However, we are not able to confirm this hypothesis from our results.

Castillo et al. (2006) suggested that the decrease in serum TA is not necessarily an undesirable condition when the production of reactive species (which would be reflected in malonaldehyde values; not analyzed in the current study) decreases. The reduction of plasma TA in horses fed Se yeast in the current study could be interpreted as an improvement in their preventive antioxidant systems in terms of the free radical formation and chain-breaking antioxidants. The dose effect of Se on GPX-1 observed in Se yeast treatments (Calamari et al., 2009) indicates that the preventive system of free radical formation was improved with increased Se supply. Nevertheless, GPX-1 activity did not differ between SY03 and SS03 (Calamari et al., 2009). Furthermore, the absence of differences in ROM values among treatments seems to indicate a minor effect of preventing free radical formation on the reduction of plasma TA. Because Se (glutathione and thioredoxin systems) is also involved in the line of defense consisting of chain-breaking antioxidants (Surai, 2006), it could be hypothesized that the reduction of plasma TA could be a consequence of an improvement of the chain-breaking antioxidants not detected with the analysis of TA in plasma (i.e., intracellular systems as phospholipid hydroperoxide GPX or GPX-4 and thioredoxin system). Further studies are needed to confirm this hypothesis.

Poor antioxidant defense is associated with enhanced inflammation and overproduction of PGE_2, resulting in suppression of lymphocyte activity and proliferation (Grimble, 2001). The greatest lymphocyte count and least neutrophil:lymphocyte ratio in SY04 horses of this study seem to indicate that an improved antioxidant defense was active. It is postulated that dietary Se status, which in turn determines tissue Se concentration, plays an important role in the regulation of arachidonate metabolism, affecting the 5-lipoxygenase pathway.

This may be one of the biochemical mechanisms underlying the inhibition of lymphocyte proliferation and the decrease in resistance to infectious diseases observed in Se-deficient animals (Cao et al., 1992).

The overall hematological results indicated that RBC, hemoglobin, and hematocrit were within the lower limits of published normal ranges, and mean corpuscular volume, MCH, and MCH concentration were within the upper limit of published normal ranges, whereas RDW was always greater than the published upper limit (Gavazza et al., 2002). The least value of MCH in SY03 treatment seems attributable to the elevated RBC count in that group, but is not followed by a proportional increase in hemoglobin. However, from a biological point of view, this result does not represent a signal for problem related to erythropoiesis. Leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil values were within the lower limit of normal published ranges, whereas platelet counts were below the lower limit of normal published ranges (Gavazza et al., 2002), even though it should be noted that very little hematological data are available in aged Saddle horses.

In the current study, WBC number was not affected by treatment, which is consistent with Juniper et al. (2006) on lactating dairy cows. In our study, a trend for an effect of treatment on lymphocytes and neutrophils population was observed. This was particularly evident in the comparison between SY03 and SS03 throughout the experimental period and could agree with a possible inhibitory effect of Na_2SeO_3 on lymphocytes (Spyrou et al., 1996). The mild effect on the population of lymphocytes and neutrophils affected the neutrophil:lymphocyte ratio.

Considering the shared housing and feeding of animals, increased numbers of eosinophils in SY02 and SY03 horses compared with those of SS03 horses is not attributable to the different responses against parasites. Conversely, eosinophil count variation is often related to different sensitivity against chemical substances, which can evoke allergic reactions, leading to histamine production (Poli and Bonizzi, 2000). However, the lack of difference between SY04 horses and the other Se-yeast supplemented horses indicated no effect of the supplementation on allergic reaction.

In conclusion, the results of this study indicated that source and concentration of dietary selenium did not affect inflammatory status and liver status in terms of the hepatocellular and hepatobiliary status. Enzyme activities that reflect cellular damage were not affected by the treatment used in this study. The linear decrease in plasma total antioxidants with increasing Se yeast supplementation and the reduced TA values in horses fed Se yeast could be interpreted as an improvement in their preventive antioxidant systems in terms of the prevention system of free radical formation and chain-breaking antioxidants. Hematological profiles were generally within normal published ranges. Despite the lack of an effect of Se source and dose on markers of inflammatory and liver status, the mild effect on the population of lymphocytes and neutrophils seems to indicate an immunomodulatory action, as indicated by changes in the WBC populations in response to Se yeast inclusion.

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