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Selenium and Vitamin E Concentrations in a Healthy Donkey Population in Central Italy

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ABSTRACT

Selenium and vitamin E protect the body against oxidative stress. Clinical manifestations of their deficiency in equids include neurologic and muscular symptoms. Despite the importance of donkeys as working and production animals, there is a dearth of scientific data on selenium and vitamin E normal values. Therefore, the aim of this study was to investigate the plasma concentrations of selenium and vitamin E in healthy donkeys belonging to different ages, sexes, and productive phases. Animals were divided into five groups including foals (group A: n = 7, n = 4 males and n = 3 females), weanlings and yearlings (group B: n = 7, n = 2 males and n = 5 females), nonpregnant nonlactating jennies (group C: n = 5), pregnant nonlactating jennies (group D: n = 9), and adult males (group E: n = 9). Plasma samples obtained from each animal were tested for vitamin E and selenium concentration. One-way analysis of variance showed significant differences in selenium concentrations ($P = .001$) between group A and group E. In this study, we found the selenium range for donkeys to be 0.02–0.14 μg/mL, which is lower than the recommended range for horses. The results suggest that donkeys may have a lower selenium requirement than horses. Plasma vitamin E levels were 3.29–12.99 μmol/L, with foals having lower concentrations than adults. Knowing specific reference ranges for vitamin E and selenium in healthy donkeys can help improve our understanding of how to prevent deficiencies that could compromise their overall health and well-being.

1. Introduction

In the last decade, the donkey (*Equus asinus*) population in Italy has increased significantly, reaching close to 53,500 animals [1]. The reason for this significant increase in the population is connected to the rising demand for donkey milk [2] which is suitable for infants who cannot be breast-fed and people suffering from cow's milk protein allergies [3–5]. Donkeys used for dairy production are required to be managed and monitored more intensively than donkeys kept as pets or companion animals [6]. Vitamin and mineral deficiencies may decrease milk production and cause clinical consequences such as compromised health (e.g., equine motor neuron disease, oxidative damage, or decreased immune response) in more extreme deficiencies [7].

Selenium and vitamin E are essential for metabolic pathways and for protection against oxidative stress [8,9]. Selenium is a micromineral or trace mineral that is a functional component of intracellular enzyme glutathione peroxidase. This enzyme combined with selenomethionine acts as a catalyst and forms oxidized glutathione as a free radical. Cytosolic reduction is dependent on the oxidation process which leads to a reduction in free radicals and detoxification of lipoperoxides and hydrogen peroxides [10–12].

Vitamin E, a lipid-soluble vitamin, is considered to be one of the most important antioxidant and free-radical reducers found in cell membranes. It donates reducing equivalents to lipid peroxyl radicals, converting them to less-toxic lipid hydroperoxides, thus protecting unsaturated membrane lipids against oxidative damage [13]. Consequently, vitamin E and selenium work synergistically to reduce oxidative damage [14]. Clinical manifestations of decreased antioxidant activity associated with selenium and vitamin E deficiency in equids include neurological and muscular

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symptoms, in addition to a significant decrease in immune response in horses [8,15–17].

Selenium and vitamin E deficiency has been linked to neuromuscular diseases such as nutritional myodegeneration or commonly referred to as white muscle disease (WMD) and can affect both horse [18] and donkey foals [19]. Despite the importance of donkeys as working and production animals, there is a relative dearth of scientific data on this species compared with other equids such as horses, and therefore, veterinarians encountering donkeys may have difficulties in finding reference intervals for biochemical and hematological blood parameters [6]. Recently, there has been one such study that compared the level of trace minerals in horses and donkeys but not vitamins [20]. The study found a difference in species and gender. Selenium and vitamin E deficiencies may threaten the health status of production donkeys, especially the health of a newborn foal, growing donkey, or lactating jenny. Studies have shown that donkeys compared with horses may react or exhibit different clinical signs of infectious disease [21–26], and considering their physiological features, specific diagnostic [27–31] and therapeutic approaches must be performed in a timely manner when dealing with donkeys because all too often they do not exhibit clinical signs until the disease is quite advanced [32–34].

Considering the lack information related to selenium and vitamin E concentration in donkeys, the aim of this study was to investigate plasma concentrations of selenium and vitamin E in five different classes of healthy donkeys: suckling foals, weanling and yearlings, adult nonpregnant nonlactating jennies, adult pregnant nonlactating jennies, and adult males.

2. Materials and Methods

2.1. Animals

Forty healthy mixed-breed donkeys used for milk production in central Italy were sampled with the owner's consent. Animals were reared in the Lazio region (42°12′N, 12°29′E), an area where the soil is not deficient in bioavailable selenium [35].

All donkeys included in the study were considered to be healthy after a clinical evaluation, and examination was performed by the attending veterinarians. Donkeys were excluded from the study if any significant clinical abnormalities had been detected within the 6 months before sampling the donkey. Animals were divided into five groups according to age, pregnancy, and lactation status. Body condition scores (BCSs) were assigned on a scale of 1–5 [36]. Foals still nursing their dams were assigned to group A (n = 7; n = 4 males and n = 3 females). The foals were aged between 1–4 months (2 ± 0.38 months), and their mean weight was 30–50 Kg. Group B included both weanlings and yearlings (n = 7; n = 2 males and n = 5 females) aged between 6–24 months (14.5 ± 3.4 months), with a mean BCS of 2.7. Group C (n = 5) included nonpregnant nonlactating jennies, aged 4–13 years (8.8 ± 1.5), with a mean BCS of 2.9. Group D (n = 9) included donkeys that were pregnant but nonlactating aged 5–12 years (5.9 ± 0.3), with a mean BCS of 3.0. Group E (n = 9) included all adult males aged 4–14 years (8.3 ± 1.0) with a mean BCS of 3.1.

All groups were housed in covered paddocks with no access to pasture or fresh grass. In group A, the foals were allowed to nurse overnight, and then they were separated for 3 hours (05.00 AM and 2.00 PM) from their dams before milking. All other groups (B–E) were fed forage twice a day (6.00 AM and 6.00 PM) in the amount of 2%–2.5% of body weight on a wet basis. The forage was a grass (meadow)-alfalfa mix and was the sole source of nutrients. The donkeys did not receive any additional concentrates, cereals, or vitamin/mineral supplements.

Sampling was performed in the morning (9–10:00 AM), before feeding, during the month of May 2018. Blood samples were drawn from the jugular vein into 6-mL ethylenediaminetetraacetic acid–containing evacuated blood collection tubes (VACUETTE; Greiner Bio-One GmbH, Kremsmunster, Austria).

All animal treatments, housing, and care described previously were carried out in accordance with the standards recommended by the European Union Directive 2010/63/EU for animal experiments and with the owner's consent.

2.2. Laboratory Analysis

All blood samples were stored in a refrigerated dark box immediately after collection and centrifuged within 30 minutes from collection. For each animal, 1.5-mL plasma aliquot was stored at −80°C until analysis.

2.2.1. Vitamin E Analysis

Vitamin E was assayed using high-performance liquid chromatography (HPLC) (VWR Hitachi Chromaster, Tokyo, Japan). For each sample, 200 μL of plasma were added with 400 μL of precipitating agent and centrifuged at 13000 *g* for 10 minutes before HPLC analysis.

In the analytical session, a standardized calibrator (ClinCal Plasma Calibrator lyophilized for vitamin A and E; RECIPE, Munich, Germany) and a certified control (ClinChek Plasma Control lyophilized for vitamins; RECIPE, Munich, Germany) were used for quantitative determination and to verify the correctness of the calculation, respectively.

2.2.2. Selenium Analysis

Plasma samples were diluted in 1:3 ratio with a solution containing double-distilled water and Triton-X 0.05% (Fluka Chemika; Buchs, Switzerland).

The calibration curve was prepared starting from the selenium standard for AAS Fluka (Sigma-Aldrich, Darmstadt, Germany). Four points of calibration were obtained 0 μg/L, 50 μg/L, 100 μg/L, and 200 μg/L.

The quantification of plasma concentrations of selenium was performed in atomic absorption (Zeeman effect, AA240Z; Agilent Technology, Santa Clara, CA) by using partitioned tubes pyrolytically coated GTA (Agilent Technology, Santa Clara, CA). The sensibility of the analysis was improved by means of selenium ultrAAhc lamp-Se coded (Agilent Technology, Santa Clara, CA). The inert gas required was argon. To reduce or eliminate volatilization or interference in the vapor phase, the palladium was used as modifier (Palladium matrix modifier Fluka; Sigma-Aldrich, Darmstadt, Germany) diluted 1:20 with double-distilled water.

2.3. Statistical Analysis

The Kolmogorov-Smirnov test was applied to assess the distribution of the data. One-way analysis of variance (ANOVA) and Bonferroni's post hoc comparison were performed to find significant differences in selenium and vitamin E concentrations among groups and sex. Ranges were calculated as mean ± 1.96 standard deviation (SD). *P* values < .05 were considered statistically significant. All calculations were performed using the PRISM package (GraphPad Software, San Diego, CA).

3. Results

Mean values and SDs together with minimum and maximum values of selenium and vitamin E concentrations obtained from each group have been reported in Table 1 and Table 2, respectively.

Table 1
Comparing plasma selenium levels in different groups of donkeys indicating a significant difference ($P = .001$) in the youngest group (A) of foals still nursing compared with the adult jacks (E).

Selenium (μg/mL)	Group A[b,a] n = 7	Group B[c] n = 7	Group C[d] n = 5	Group D[e] n = 9	Group E[f] n = 9
Mean	*0.05*	0.08	0.05	0.11	*0.12*
Standard deviation	0.01	0.05	0.00	0.04	0.06
Minimum	0.04	0.03	0.05	0.06	0.04
Maximum	0.06	0.15	0.06	0.20	0.21

[a] Significance ($P = .001$) versus group E.
[b] Suckling foals: 1–4 months of age.
[c] Weanlings and yearlings: 6–24 months of age.
[d] Adult nonlactating jennies: 4–13 years.
[e] Adult pregnant nonlactating jennies: 5–12 years.
[f] Adult jacks: 4–14 years.

The Kolmogorov-Smirnov analysis revealed a normal distribution of the data ($\alpha = 0.05$) observed for both selenium and vitamin E. Significant differences were found in the mean selenium concentrations ($P = .001$) between group A and group E, with group A showing the lowest (0.05 μ/mL) and group E (0.12 μ/mL) showing the highest selenium plasma levels (Table 1). According to the results of the one-way ANOVA, no significant difference was observed in vitamin E plasma concentrations among the studied groups of donkeys (Table 2). No differences were found for selenium or for vitamin E between males and females in group A and B.

4. Discussion and Conclusions

Despite several studies have investigated the effects of animal age and physiological status (growth, pregnancy, lactation) on hematochemical parameters in healthy horses [37–41], to date, few studies dealt with similar information on healthy donkeys [20,42], and no study exists investigating both vitamin E and selenium concentrations in donkeys of different sexes, age, and physiological status. In healthy horses, the adequate reference range for serum vitamin E is considered >4.6 μmol/L, a concentration included between 3.5 and 4.6 is defined marginal, and <3.5 μmol/L is considered as deficient [43]. In our study, an overall mean of 8.13 μmol/L, a mean minimum value of 5.16, and a mean maximum value of 12.40 have been found, and plasma value ranges from 3.29 to 12.99 μmol/L.

Studies on selenium levels in healthy horses, including mares and foals, found the normal serum/plasma selenium reference range to be between 0.10 and 0.26 μg/mL [44–46]. The overall mean selenium concentration was 0.08 μg/mL while the mean minimum and mean maximum values were 0.04 and 0.14 μg/mL, respectively. In our study, the range for selenium was 0.02–0.14 μg/mL suggesting that the selenium concentrations found in donkeys are lower than the range recommended for horses. Such results could suggest a lower selenium requirement for donkeys. A similar trend was observed by Shawaf et al. [20] who found lower selenium concentration in donkeys than in horses. However, in the study by Shawaf et al. [20], the mean selenium values were found to be higher than our results. These differences seen in the two studies may be explained by a dietary difference in the composition that may affect the serum levels of micronutrients. Future studies should consider and evaluate dietary influences by analyzing the diet along with serum or plasma.

Lower plasma α-tocopherol concentrations have been reported in Thoroughbreds than in other horse breeds regardless of gender [47]. In our study, all donkeys were considered to be a mixed breed and a systematic evaluation of breed differences was not applicable. The importance of affordable reference values for donkey foals is essential as deficiencies in selenium and vitamin E have been associated with higher risk for WMD, yellow fat disease, immune dysfunction, and decreased resistance to respiratory infections in equids [17,48–50]. Vitamin E's impact on foal health may depend on several factors including genetic factors, temporal occurrence of deficiency during development, and the duration of deficiency [51]. In foals, weanlings, and yearlings, all seem to have a significantly lower plasma/serum vitamin E concentration than adults [43]. In agreement with a study by Muirhead et al. [8] on Prince Edward Island horses, we found lower selenium concentrations in donkey foals than in adults. A similar trend was also observed by the US Animal Health Laboratory, confirming that neonatal reference values are likely to be considerably lower than those presented for adults [52]. Less than 2% of α-tocopherol is transferred into the dam's milk, and this is likely the cause of lower concentrations of vitamin E found in neonatal foals [53]. As previously suggested for horses, young donkeys might require a different reference range from that of adults [43]. Although no significant difference in vitamin E concentration was observed between groups in our study, foals still had numerically lower vitamin E concentrations than adults.

Low selenium concentrations have been observed in foals aged <30 days showing signs of WMD [9]. A higher incidence of WMD has been seen in foals born to mares who are selenium deficient [8,53]. Higuichi et al. (1989) reported that selenium concentrations tended to be lower in mares having foals affected by WMD [8,54].

Table 2
Comparing mean plasma vitamin E concentration in different groups of donkeys.

Vitamin E (μmol/L)	Group A[a] n = 7	Group B[b] n = 7	Group C[c] n = 5	Group D[d] n = 9	Group E[e] n = 9
Mean	5.92	10.26	7.72	7.81	8.98
Standard deviation	1.24	3.55	2.48	3.00	2.11
Minimum	4.90	5.53	5.53	4.37	5.46
Maximum	8.59	15.47	11.59	15.02	11.33

[a] Suckling foals: 1–4 months of age.
[b] Weanlings and yearlings: 6–24 months of age.
[c] Adult nonlactating jennies: 4–13 years.
[d] Adult pregnant nonlactating jennies: 5–12 years.
[e] Adult jacks: 4–14 years.

Development of WMD is generally seen in nursing foals aged less than one week, and this suggests an association with maternal selenium deficiency [9].

Considering the growing interest and increasing population of dairy donkeys, there is a need for additional information on selenium and vitamin E for both foal and lactating jennies. One study on dairy cows demonstrated that adequate levels of vitamin E have been associated with a lower risk of mastitis [55]. No study, to our knowledge, has investigated the relationship between vitamin E and mastitis in lactating jennies used for dairy production, and such observation merits additional studies to further investigate the implications of selenium and vitamin E on the health of the mammary system and immune response.

Finally, it is unlikely that the collection method could have influenced the concentration of vitamin E because the centrifugation was carried out shortly after the sampling and the samples analyzed immediately after the first thawing, as recommended by other studies [43].

In the present study, we established vitamin E and selenium plasma concentrations in a population of healthy donkeys. The values reported in this study can be considered normal plasma concentrations for healthy donkeys. Future studies should continue to investigate the relationship of vitamin E and selenium with the health of donkeys by focusing on testing different breeds and evaluating the effect of the time of year, season, or stage of lactation on serum or plasma levels [8]. Knowing specific reference ranges for vitamin E and selenium in healthy donkeys at various ages and stages of lactation or pregnancy can help improve our understanding of how to prevent deficiencies that could compromise their overall health and well-being.

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