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ORIGINAL ARTICLE

Quantifying the Impact of Commercial Mineral Supplementation on Equine Serum Concentrations of Iron, Zinc, Copper, and Selenium

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Abstract

Trace minerals are essential for the survival of living organisms. This study aimed to investigate the effects of using mineral supplements on serum concentrations of copper, selenium, iron, and zinc in 24 horses from a club near Tabriz, East Azerbaijan Province. Serum trace element concentrations were measured using photometric methods with a Hitachi 917 biochemical autoanalyzer (for copper, iron, and zinc) and atomic absorption spectroscopy (AAS) with a Varian AA140 atomic absorption spectrometer (for selenium). Ten horses were used as the control group, and 14 horses were used as the treatment group that received supplements. There was no significant difference in serum concentrations of copper, iron, zinc, and selenium between the control and treatment groups ($P > 0.05$). However, a significant correlation was observed between mean serum concentrations of zinc, selenium, and copper before and after supplementation ($P < 0.05$), and there was no significant correlation in mean serum iron concentrations before and after supplementation ($P > 0.05$). The mean serum concentrations of copper, iron, zinc, and selenium in the control and treatment groups were 86.60 ± 3.39 and 83.62 ± 3.09 µg/dL, 171.96 ± 10.47 and 193.19 ± 8.84 µg/dL, 63.71 ± 1.57 and 66.76 ± 1.76 µg/dL, and 106.19 ± 1.29 and 108.81 ± 1.09 µg/L, respectively. The mineral supplements did not significantly affect serum iron concentrations; however, it significantly increased serum copper, zinc, and selenium concentrations.

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Introduction

Trace elements are essential nutrients required in small amounts and play a fundamental role in maintaining the health and well-being of horses. These elements are involved in various physiological processes, including enzymatic activity, immune function, and antioxidant defense (1-3). Deficiency or toxicity of trace elements can lead to a wide range of health problems, such as anemia, muscle weakness, and reproductive disorders (4, 5). The availability of trace minerals in forage is influenced by factors such as plant species, climate, and soil conditions, making supplementation a common practice in equine nutrition (6). However, understanding the balance and interaction between these micronutrients is essential for maintaining optimal horse health and preventing issues related to deficiency or toxicity (7, 8).

Among trace elements, copper, iron, zinc, and selenium are particularly important for equine health. Copper plays a vital role in biological processes as an integral component of enzymes involved in cellular respiration, antioxidant defense, and hematopoiesis (9-11). Iron is essential for oxygen transport in hemoglobin and myoglobin, accounting for approximately 80% of the body's total iron content (12). Zinc acts as a catalyst for over 300 enzymes and serves as a structural component and regulator of gene expression (13). Selenium is incorporated into selenoproteins involved in the body's antioxidant defense system, working synergistically with vitamin E to neutralize harmful free radicals and protect cells from oxidative damage (14, 15).

The results of studies conducted on trace element supplementation in horses have not been conclusive. While some research has reported no significant improvement in growth after supplementation, other studies have observed significant increases in bone mineral deposition in horses fed trace element supplements (16, 17). This contradiction indicates that many questions regarding the role of trace element supplements in horse nutrition remain unanswered. We hypothesized that commercial mineral supplementation would increase serum concentrations of copper, iron, zinc, and selenium in horses. Therefore, the aim of this study was to evaluate the effect of commercial mineral supplements on serum trace element concentrations in horses from a riding club near Tabriz, East Azerbaijan Province, Iran (located at 46°18′00″ E longitude and 38°04′45″ N latitude, at an altitude of 1,365 meters above sea level, with a temperate climate), and to determine whether supplementation can be used as an appropriate strategy to address potential deficiencies of these elements.

Materials and Methods

Animal Selection and Inclusion/Exclusion Criteria

A total of 24 horses were included in this study, comprising 22 males and 2 females. The mean age of the horses was 8.00 ± 3.44 years (range: 3-17 years). The majority of horses were of Arab breed (n=21), with the remaining being Kurd breed (n=3). All horses were clinically healthy and free from internal and external parasitic diseases. All female horses included in the study were confirmed to be non-pregnant. Horses were maintained under uniform management conditions and were used exclusively for horse riding training, ensuring similar activity and physiological states across all subjects. All samples were obtained from a riding club near Tabriz with uniform nutritional and breeding management. Exclusion criteria included relocation of the horse to another stable during the study period and errors in laboratory sample processing.

Feeding and Nutrition

All horses received the same diet throughout the study period, consisting of alfalfa and commercial horse concentrate. Feed was provided uniformly to ensure consistent nutritional intake across both the control and treatment groups.

Study Design

This was a prospective controlled experimental study. In this study, 14 horses received commercial trace element supplements for two months (treatment group), while 10 other horses were selected as the control group and continued their usual diet. Blood samples were taken from all horses on three occasions: before supplementation began, one month after the start of supplementation, and two months after the start of supplementation.

Supplementation

The commercial mineral supplement used in this study contained protein-chelated iron (Fe), cobalt (Co), manganese (Mn), copper (Cu), iodine (I), selenium (Se), zinc (Zn), magnesium (Mg), chromium (Cr), sodium chloride (NaCl), and monocalcium phosphate. The supplement also contained vitamins, flavonoids, probiotics, toxin binders, and herbal extracts. The detailed composition of the supplement is presented in Table 1. Each horse in the treatment group was provided with one 3 kg mineral brick,

administered over the two-month study period. According to the manufacturer's instructions, one mineral brick is sufficient for one month's consumption for each adult horse. To maintain the individual hygiene of the horses, a separate mineral block was used for each horse.

Sample Collection and Analysis

After physical restraint and stabilization of the horses, blood samples were taken from the jugular vein using a 5 mL syringe and a 21-gauge needle. To measure the concentration of trace elements in serum, 6 mL test tubes containing a separator gel (Vacutest Kima, Italy) were used. These tubes accelerate the blood clotting process and separate blood cells from serum by creating a gel barrier. Serum concentrations of Cu, Fe, and Zn were determined by photometric methods using commercial kits (Randox Laboratories Ltd., UK) on a Hitachi 917 biochemical autoanalyzer. To measure the concentration of selenium in horse serum, a VARIAN AA140 atomic absorption spectrometer (Varian, USA) was used with atomic absorption spectroscopy.

Statistical Analysis

Statistical analysis was performed using SPSS software version 26. A repeated measures analysis of variance (ANOVA) was used to evaluate the effects of time (within-subjects factor: before supplementation, one month after, and two months after) and group (between-subjects factor: control and treatment) on serum concentrations of copper, iron, zinc, and selenium. Prior to analysis, the assumptions of the repeated measures ANOVA were tested. Mauchly's test was used to assess sphericity assumption, and when violated, the Greenhouse-Geisser correction was applied. Box's M test was used to evaluate the equality of covariance matrices, and Levene's test was used to assess the homogeneity of error variances. Pairwise comparisons were performed using the Bonferroni adjustment for multiple comparisons. Statistical significance was set at $P < 0.05$, and all results are presented with 95% confidence intervals.

Results

Copper

The analysis results show that there is no significant difference between time × group interaction ($P > 0.05$). In other words, the pattern of changes in copper concentrations over time is similar for both groups (control and treatment). This means that the copper supplement did not have a significant effect on the changes in copper concentrations in the two groups. The mean copper concentration in the three sampling periods in the serum of horses in the control group was 86.60 ± 3.39 µg/dL, and the mean copper concentration in the serum of horses in the treatment group was 83.62 ± 3.09 µg/dL. The results of multivariate tests show that the effect of time on copper concentrations was statistically significant. This means that copper concentrations have changed significantly over time.

Table 1: Composition of the commercial mineral supplement

Component	Minimum Content	Unit
Trace Minerals		
Iron (Fe)	315	mg/kg
Copper (Cu)	90	mg/kg
Zinc (Zn)	360	mg/kg
Selenium (Se)	0.25	mg/kg
Manganese (Mn)	270	mg/kg
Cobalt (Co)	0.9	mg/kg
Chromium (Cr)	0.85	mg/kg
Macro Minerals		
Calcium (Ca)	5300	mg/kg
Phosphorus (P)	4900	mg/kg
Magnesium (Mg)	1125	mg/kg
Sodium chloride (NaCl)	14	%
Vitamins		
Vitamin A	41,250	IU/kg
Vitamin D3	4,125	IU/kg
Vitamin E	825	mg/kg
Vitamin K3	0.36	mg/kg
Vitamin B1	33	mg/kg
Vitamin B6	16.5	mg/kg
Vitamin B12	0.25	mg/kg
Niacin	337.5	mg/kg
Pantothenic Acid	49.5	mg/kg
Folic Acid	54	mg/kg
Biotin	3	mg/kg
Riboflavin	0.033	mg/kg

**The supplement also contains flavonoids, probiotics, toxin binders, and herbal extracts.*

According to the analysis results, with 95% confidence, a significant difference was observed between the data before supplementation and one month after supplementation, and between the data one month after supplementation and two months after supplementation ($P < 0.05$). However, no significant correlation was observed between the data from the first sampling and the third sampling ($P > 0.05$). The mean serum copper concentration

of horses in the control group before supplementation was 83.86 ± 3.82 µg/dL, one month after supplementation was 73.10 ± 6.06 µg/dL, and two months after supplementation was 102.4 ± 83.87 µg/dL. The mean serum copper concentration of horses in the treatment group before supplementation was 89.56 ± 3.49 µg/dL, one month after supplementation was 70.73 ± 5.53 µg/dL, and two months after supplementation was 90.58 ± 4.45 µg/dL (Figure 1).

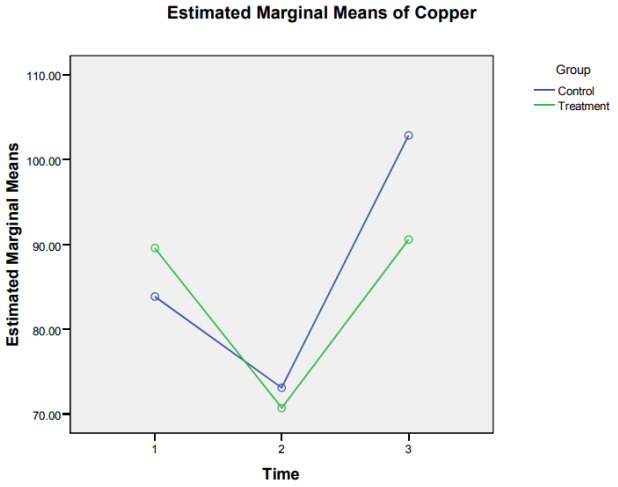

Figure 1. Mean serum copper concentrations (µg/dL) in the control and treatment groups before supplementation, one month after, and two months after supplementation.

Iron

The results show that there is no significant difference between time × group interaction ($P > 0.05$). In other words, the pattern of changes in iron concentrations over time is similar for both groups (control and treatment). This means that the supplement did not have a significant effect on the changes in iron concentrations in the two groups. The mean iron concentration in the three sampling periods in the serum of horses in the control group was 171.96 ± 10.47 µg/dL, and the mean iron concentration in the serum of horses in the treatment group was 193.19 ± 8.84 µg/dL. With 95% confidence, the maximum serum iron concentration in the control group was measured at 193.68 µg/dL and the minimum serum iron concentration was 150.25 µg/dL. With 95% confidence, the maximum serum iron concentration in the treatment group was measured at 211.54 µg/dL and the minimum serum iron concentration was 174.83 µg/dL.

The results of multivariate tests show that the effect of time on iron concentrations was not statistically significant. According to the analysis results, with 95% confidence, no significant correlation was observed between the different time points ($P > 0.05$). The mean serum iron concentration of horses in the control group before supplementation was 150.6 ± 12.25 µg/dL, one month after supplementation was 180.8 ± 12.84 µg/dL, and two months after supplementation was 184.5 ± 18.47 µg/dL. The mean serum iron concentration of horses in the treatment group before supplementation was 183.5 ± 10.35 µg/dL, one month after supplementation was 187.14 ± 10.85 µg/dL, and two months after supplementation was 208.92 ± 15.61 µg/dL (Figure 2).

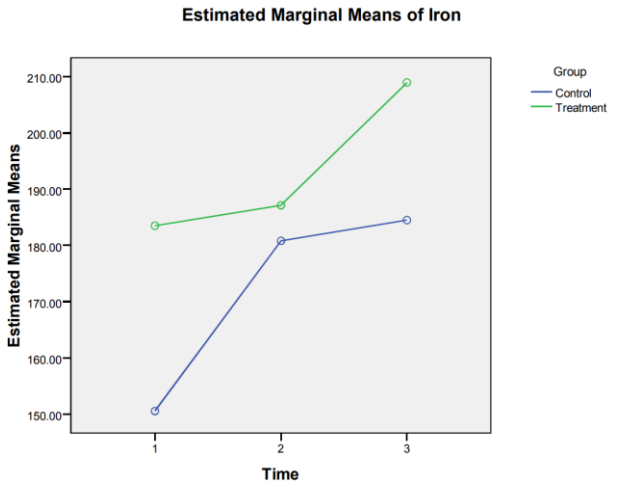

Figure 2. Mean serum iron concentrations (µg/dL) in the control and treatment groups before supplementation, one month after, and two months after supplementation.

Zinc

The results show that there is no significant difference between time × group interaction ($P > 0.05$). In other words, the pattern of changes in zinc concentrations over time is similar for both groups (control and treatment). This means that the supplement did not have a differential effect on the changes in zinc concentrations in the two groups. The mean zinc concentration in the three sampling periods in the serum of horses in the control group was 63.7 ± 1.57 µg/dL, and the mean zinc concentration in the serum of horses in the treatment group was 66.5 ± 1.76 µg/dL. With 95% confidence, the maximum serum zinc concentration in the control group was measured at 67.04 µg/dL and the minimum serum zinc concentration at 60.35 µg/dL. With 95% confidence, the maximum serum zinc concentration in the treatment group was measured at 70.23 µg/dL and the minimum serum zinc concentration at 62.76 µg/dL.

The results of multivariate tests indicate that the effect of time on zinc concentration was statistically significant, and according to the analysis results, a significant correlation was observed between the different time points with 95% confidence ($P < 0.05$). The mean serum zinc concentration of horses in the control group before supplementation was 54.1 ± 2.77 µg/dL , one month after supplementation was

66.2 ± 2.69 µg/dL, and two months after s supplementation was 70.8 ± 3.5 µg/dL. The mean serum zinc concentration of horses in the treatment group before supplementation was 58.5 ± 3.1 µg/dL, one month after supplementation was 70.12 ± 3 µg/dL, and two months after supplementation was 70.87 ± 3.91 µg/dL (Figure 3).

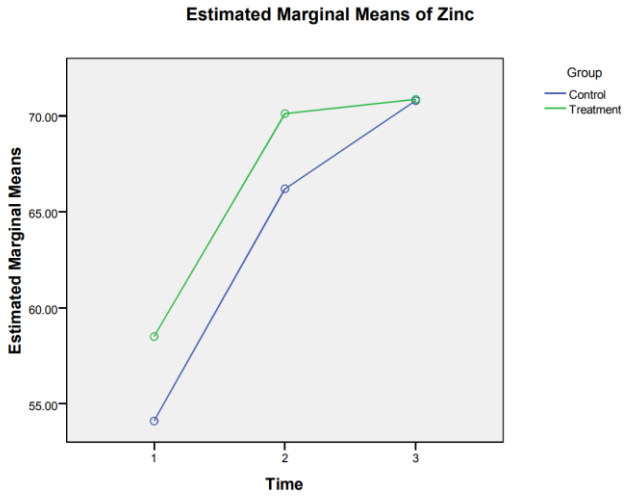

Figure 3. Mean serum zinc concentrations (µg/dL) in the control and treatment groups before supplementation, one month after, and two months after supplementation.

Selenium

The results indicate that there is no significant time × group interaction ($P > 0.05$). In other words, the pattern of changes in selenium concentrations over time is similar for both groups (control and treatment). This means that the supplement did not have a differential effect on the changes in selenium concentrations in the two groups. The mean selenium concentration across the three sampling periods in the serum of the horses in the control group was 106.1 ± 1.29 µg/L, while the mean selenium concentration in the serum of the horses in the treatment group was 108.81 ± 1.09 µg/L. With 95% confidence, the maximum selenium concentration in the serum in the control group was measured at 108.79 µg/L, and the minimum selenium concentration in the serum was measured at 103.41 µg/L. With 95% confidence, the maximum selenium concentration in the serum in the treatment group was measured at 111.08 µg/L, and the minimum selenium concentration in the serum was measured at 106.53 µg/L.

All pairwise comparisons indicate that differences between the mean selenium concentration at different times are statistically significant ($P < 0.05$). The mean selenium concentration in the serum of the horses in the control group before supplementation was 100.74 ± 1.69 µg/L, one month after supplementation was 113.63 ± 2.31 µg/L, and two months after supplementation was 103.94 ± 2.37 µg/L. The mean selenium concentration in the serum of the horses in the treatment group before supplementation was 100.73 ± 1.42µg/L, one month after supplementation it was 115.19 ± 1.95 µg/L, and two months after supplementation was 110.5 ± 2 µg/L (Figure 4).

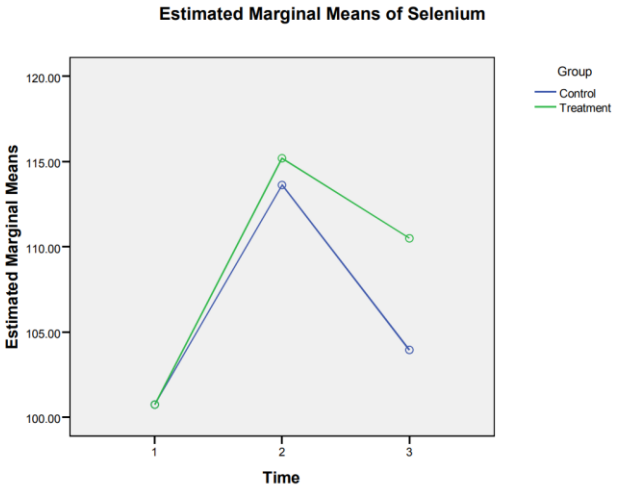

Figure 4. Mean serum selenium concentrations (µg/L) in the control and treatment groups before supplementation, one month after, and two months after supplementation.

Discussion

Copper is an essential micronutrient for horses that plays a significant role in bone growth and antioxidant processes. Copper deficiency has been directly associated with skeletal disorders in horses, highlighting the importance of cooper supplementation (23). Copper, along with selenium and zinc, is necessary for the function of antioxidant enzymes in horses. While recent studies have emphasized the importance of micronutrients, including copper, in supporting skeletal health in foals, the specific copper requirements for different horse populations remain to be defined. Consequently, further research is necessary to optimize the use of copper supplements in horse diets. Research by Pearce et al. (1998) on the use of copper supplements in horses has shown varying effects on blood and tissue copper concentrations. While copper supplementation in horses has been shown to increase hair copper concentrations, particularly when using mineral forms such as copper sulfate, its impact on plasma copper concentration and ceruloplasmin activity is inconsistent (24). The study by Gordon et al. (2013), which investigated the effects of various feed additives, including chelated minerals, on copper metabolism in horses found no significant differences in copper digestibility or serum copper concentration between the treatment and control groups (25). Research by Pearce et al. (1998) has demonstrated that copper supplementation in pregnant mares can increase liver copper concentration in foals;

however, it has little to no effect on plasma copper concentrations or ceruloplasmin activity in either the mare or the foal. Similar findings have been observed in foals fed copper supplements, where liver copper concentrations increased but had no impact on plasma copper or ceruloplasmin activity (24). Contrary to findings in horses, a study conducted by Saxena et al. (2010) on crossbred calves showed a significant increase in serum copper concentrations following the use of copper supplements (26). The results obtained from the current study did not show a significant relationship between serum copper concentrations in the control and treatment groups; however, a significant correlation was observed between serum copper concentrations before and after supplementation. Nevertheless, based on the results obtained in the present study, the supplement did not have a significant effect on serum copper concentrations in the treatment group compared to the control group, although the mean serum copper concentration in horses at the end of this study showed an increase.

Iron is an essential micronutrient in equine physiology and plays a vital role in oxygen transport and DNA synthesis (27). While iron deficiency is uncommon in adult horses, excessive supplementation despite its ineffectiveness in improving athletic performance is common (27). Serum ferritin serves as a reliable indicator of body iron stores and reflects iron concentrations in the liver and spleen (28). Exercise requirements significantly impact iron metabolism in horses and affect iron, ferritin, and transferrin concentrations (29). Research on iron supplementation in horses has yielded varying results regarding its impact on serum iron concentrations. While some studies, such as that by Harvey et al. (1987), reported changes in serum ferritin concentrations in foals following iron supplementation (30), other studies, such as that by Cooter and Mowbray (1978), failed to demonstrate a significant impact on serum iron, total iron-binding capacity, or hemoglobin concentrations in adult horses (31). These findings emphasize the importance of precise iron management in equine nutrition and the need for thorough evaluation before the use of supplements. One possible reason for not observing a significant difference in serum iron concentrations between the experimental groups in the present study may be attributed to the limited assessments focused solely on serum iron concentrations.

Zinc is an essential micronutrient with a significant impact on various physiological functions in horses. It acts as a valuable serum marker for inflammatory status, which is observed to decrease in serum zinc concentrations under disease conditions (32). The use of zinc supplements in the diet has shown immune-modulating effects in horses, with different zinc compounds eliciting varying responses. Zinc chloride hydroxide has been shown to increase glutathione concentration and decrease white blood cell counts, while zinc methionine enhances the proliferative activity of peripheral blood mononuclear cells (33). The bioavailability of zinc from various compounds is a vital factor, as supplementation can lead to increased zinc concentrations in the hooves (34). According to the study by Omidi et al. (2018), serum zinc concentrations in healthy Thoroughbred horses typically range from 41 to 79 µg/dL, with fluctuations observed post-exercise and in horses with specific illnesses. While plasma zinc concentrations are relatively stable across age, sex, and horse type, animals with metabolic disorders often exhibit higher zinc concentrations compared to control groups (33, 36). According to the study by Murase et al. (2013), dietary zinc supplementation has been shown to increase zinc content in mane hair without significantly affecting plasma zinc concentrations (32). Consistent with the findings of the present study, no significant correlation was observed between serum zinc concentrations in the control and treatment groups and the intake of supplements containing this element; however, a significant correlation was noted between serum zinc concentrations before and after supplementation.

Research by Montgomery et al. (2012) has shown that selenium supplementation, particularly with organic forms, can effectively increase plasma selenium and red blood cell concentrations in horses (35). In the research by Omidi et al. (2018), horses exhibiting crib-biting behavior had lower serum selenium concentrations compared to control groups, indicating a potential relationship between selenium deficiency and this behavioral disorder (36). According to the study by Wyganowska et al. (2017), selenium supplementation in horses effectively increased blood selenium concentrations (37). According to the study by Richardson et al. (2006), both organic and inorganic sources of selenium can increase serum selenium concentrations; however, organic forms may be more effective in the short term (38). In the study by Shellow et al. (1985), dietary selenium supplementation led to a linear increase in whole blood and serum selenium concentrations, reaching its maximum after 5 to 6 weeks (39). However, the relationship between selenium supplementation and glutathione peroxidase activity is less clear, with some studies finding no significant effect on plasma glutathione peroxidase activity. According to the study by Maylin et al. (1980), oral selenium supplementation at 1 mg/day can increase blood selenium concentrations above those associated with muscle degeneration in horses and foals (40). Another study by Gordon et al. (2013) showed that horses receiving

chelated mineral and organic selenium had lower whole blood selenium concentrations compared to the control group (25). The results of the present study indicated that although selenium supplementation did not have a significant effect on the difference between the control and treatment groups, a significant increase in the serum selenium concentrations was observed after supplementation compared to baseline.

In a study conducted by Ghorbani et al. (2015) on 12 Caspian horses, the effect of mineral supplementation on the concentrations of trace elements in serum and mane hair was evaluated using inductively coupled plasma atomic emission spectrometry. The results indicated that while mineral supplementation had a significant effect on the concentration of some elements in hair, no significant changes were observed in the concentration of most elements in serum, with only the changes in copper and strontium concentrations being significant due to the mineral supplement. The effect of mineral supplementation on the concentrations of calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulfur, selenium, strontium, and zinc in hair was found to be significant (41). These findings suggest that hair is more sensitive to changes in trace element compared to serum. A study conducted by Neustädter et al. (2018) on three Thoroughbred horses examined the effect of a mineral supplement with reduced trace element content on serum concentrations of copper, zinc, and selenium. The results showed that reducing the trace element content in the diet did not have a significant effect on the serum concentrations of these elements. These findings indicate that under the conditions of this study, the reduction of trace element content in the diet of Thoroughbred horses did not negatively affect the balance of trace elements in the body (42).

In the present study, no significant correlation was observed between serum of copper, iron, zinc, and selenium concentrations in the treatment and control groups of horses. However, the mean concentrations of zinc, selenium, and copper in the serum after supplementation were significantly higher than those before supplementation. Although the sample size examined was small, based on the obtained results, the mineral supplements did not have a significant impact on increasing serum iron concentrations; however, they played a role in increasing the concentrations of the trace elements copper, zinc, and selenium in the serum. Additionally, examining the interactions between elements in the mineral supplement on each other's absorption, including the effect of zinc on copper absorption or the effect of molybdenum on reducing copper absorption, may provide new insights into this topic.

Limitations and Recommendations for Future Studies

This study has some limitations that should be considered when interpreting the results. Due to resource constraints, horses of varying ages (3-17 years) were included, and the sample included both sexes (22 males and 2 females) and two breeds (Arab and Kurd). Future studies would benefit from using a more homogeneous sample with standardized age, sex, and breed to minimize potential confounding variables. Additionally, this study measured only serum concentrations of trace elements. Since the liver is the primary storage organ for copper and serum concentrations may not fully reflect total body status, future studies should consider measuring liver copper concentrations and ceruloplasmin activity. Similarly, to obtain a more comprehensive assessment of iron status in horses, future studies should evaluate additional parameters such as total iron binding capacity (TIBC), transferrin saturation, and ferritin levels alongside serum iron measurements. It should also be noted that baseline serum iron concentrations were higher in the treatment group (183.50 ± 31.79 µg/dL) than in the control group (150.60 ± 47.01 µg/dL) before supplementation began. This difference in baseline values may have influenced the interpretation of iron supplementation effects and should be considered when evaluating the results.

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Authors' Contributions

Fereydon Rezazadeh: Conceptualization, Investigation, **Amirreza Kenaroudi:** Farm study, **Mohmmad Toloui:** Investigation, **Majid Ebrahimi Hamed:** Supporting material for study.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

All excremental procedures involving in this study were conducted in accordance with the ethical standards and guidelines for the care and use of laboratory animals

established by the Ethical Committee of the University of Tabriz, Iran.

Conflict of Interest

The authors declared no potential conflicts of interest.

Consent for Publication

Not applicable.

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