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Original Research

Effects of Dietary Mineral Intake on Hair and Serum Mineral Contents of Horses

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ABSTRACT

The objective of this study was to determine the effects of dietary mineral intake on serum and hair mineral contents of healthy horses. Twelve registered horses were used in a balanced change over design with three periods (each period, 56 days; the interval between periods, 7 days), four treatments, nine replicates per treatment, and four blocks (two genders and two age groups, <3 and ≥3 years). Two different levels (0% and 2.2% of the diet) of minerals were added to one of two different levels of daily dry matter intakes (50% and 100% of requirements) to make the dietary treatments. Mane hair and serum samples were collected at the end of each period and measured by inductively coupled plasma optical emission spectrometry method. The mineral contents of serum, except copper and strontium, were not affected by dietary treatments ($P > .05$). The effect of dietary treatments on hair calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulfur, selenium, strontium, and zinc concentrations was significant ($P \leq .05$), with higher values for mineral-supplemented diets provided at 100% of their requirements. Except for phosphorus, the effect of age on hair calcium, cobalt, copper, iron, potassium, magnesium, manganese, nickel, lead, sulfur, selenium, strontium, and zinc concentrations and calcium-to-potassium, zinc-to-copper, calcium-to-lead, and sulfur-to-lead ratios were not significant ($P > .05$). For gender, the ratios of calcium to lead, iron to lead, and sulfur to copper in hair were higher in males than females ($P \leq .05$). The hair showed to be a better biological indicator for mineral status in horse than the serum.

1. Introduction

With increasing demand in medical diagnostics and biomonitoring, interest in biological biopsies for toxicology, epidemiology, and assessing individuals' homeostatic state or morbidity is immense [1]. The diets traditionally used for horse nutrition often are imbalanced in mineral, especially regarding macroelement concentrations [2]. Hair analysis has been used to assess the mineral elements status for several decades [3]. The potential use of hair analysis was established in the early 1960s, but the most significant developments have occurred by using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry in the last 20 years [4,5]. Chemical elements accumulate and are eliminated by different routes, including hair [6]. In blood, the minerals undergo homeostatic controls [7]; therefore, the value of routine analyses of whole blood, serum, and urine for bioelements is limited [8], whereas the hair which is built of keratin, a highly resistant protein which prevents loss of internal components of hair and external deposition [7], is a suitable biological candidate for investigating the mineral status in human and animals. On the other hand, hair can be collected easily and requires no special storage considerations such as blood serum [9]. Hair is recommended as a noninvasive biomarker by various institutions—including US Environmental Protection

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Agency (EPA), World Health Organization, and Global Environmental Monitoring Systems of United Nations Environment Programme—to assess the chronic exposure of human to toxic metals [10]. In addition, EPA and the International Atomic Energy Agency accept using hair mineral analysis for measuring the level of toxic and essential metal in human organs [7,11].

There are relationship between essential elements and diseases, metabolic disorders, environmental exposures, and nutritional status [12]. Recent equine studies have demonstrated in contrast to urine and blood analyses, hair analysis can detect and quantify drugs weeks, months, or even years after administration or intake [13]. There are several diseases of horses that may have a direct or indirect causal relationship with diet [14]. Nutritional secondary hyperparathyroidism (NSH) disease tends to occur when horses are fed diets with low in calcium and high in phosphorus. Serum concentrations of both calcium and phosphorus are often normal in diagnostic test for NSH disease [15].

The Ca^{2+} inflow into cells is considered to trigger various diseases, and the hair analysis provides a new diagnosis on cell ion channel gating [16]. The hair mineral analysis provides a new diagnostic tool based on cell ion channels [16]. This would make hair analysis more reliable [17].

Furthermore, it has been established that the level of some elements in biological specimens are related to systemic levels of horses [4]. Exposure of horses to toxic levels of selenium via forage ingestion has been investigated by hair analysis [13,18]. Another potential application of hair analysis is in instances of plant-induced toxicities in horses [18]. Literature has repeatedly shown a relationship between hair mineral analysis and human health [19]. An inverse relationship has repeatedly been shown for calcium, magnesium, iron, zinc, and copper in hair with obesity, diabetes, and the metabolic syndrome, whereas hair sodium has been shown to be increased under these conditions [19].

Miniature horses and ponies have historically been used in equine research studies because of the advantages of their small size [20]. They are easy to handle and less expensive to maintain. Miniature horses are suitable models for traits such as mineral balance and forage utilization [20].

The present study was amid to examine the potential applicability of serum or hair samples as a biological indicator to evaluate the horses responses to (un)sufficient-mineral diets offered daily at 50% and 100% of the requirements recommended by National Research Council [21].

2. Materials and Methods

2.1. Animals and Feeding

Twelve registered Caspian miniature horses with an initial live weight of 163.5 kg were used in this study. Six males (three 1–3 years and three ≥3 years) and six females (three 1–3 years and three ≥3 years) were housed in the premises of the Animal Science Department, Agricultural and Natural Resources Research Center of Guilan, Rasht, Iran. The horses were fed individually from diets formulated on National Research Council [21] recommendation. The experiment was conducted during the months of January–June 2014. After a 60-day adaptation period, the animals were moved to a 3 × 3-m lot and were offered 2% of body weight alfalfa hay daily. During the last 7 days of the adaptation period, the dietary treatments gradually were substituted. During the experiment, horses were housed in covered stalls and were given one half of their daily diet of alfalfa hay and concentrate on 8 AM and 10 AM; and the rest at 4 PM and 6 PM. Housing and management conditions were the same for all animals, and water was available ad libitum. The dietary treatments included were $Diet_{0-100}$, dietary mineral supplementation at 0% and the dry matter intake at 100% of requirement; $Diet_{2.2-100}$, dietary mineral supplementation at 2.2% and the dry matter intake at 100% of requirement; $Diet_{0-50}$, dietary mineral supplementation at 0% and the dry matter intake at 50% of requirement; and $Diet_{2.2-50}$, dietary mineral supplementation at 2.2% and the dry matter intake at 50% of requirement (Tables 1 and 2).

2.2. Hair Mineral Analysis

The method used for hair preparation closely follows that described previously [9,11,13,22,23]. All the chemicals and reagents were of analytical grade from Merck (Darmstadt, Germany). Deionized ultrapure water obtained from a Milli-Q purification device (Millipore Co, Bedford, MA). The standard laboratory ware and glassware were acid washed and rinsed with ultrapure water. Triton X-100 was from Sigma Chemical Co (St-Louis, MO). Multielemental standard solutions were used for ICP-OES, including 10 ppm, 26XSM80B.5L and 10 ppm, 26XSM90C.5L, were obtained from MBH (London, England). The mixed standard solution was obtained by further diluted to desired concentration daily before use [24].

2.3. Serum Mineral Analysis

The serum mineral concentration was measured in the fasting state at the end of each period. A total of 9 mL of the blood samples was collected from the jugular veins in plain vacutainer tubes. After collecting, the blood samples are allowed to clot by leaving them undisturbed at room temperature for at least 1 hour [25]. The serums were removed by centrifuging the clots at 3,000 rpm for 15 minutes. The obtained sera were stored in 1.5-mL microtubes at −80°C until analyzing for mineral concentration [26,27]. Preparation of serum sample for mineral analysis was done by using American Society for Testing and Materials, D4638–03 standard [22].

2.4. Apparatus

In this study, simultaneous ICP-OES, Thermo Scientific iCAP Series 6500, equipped with a charge injection device detector CETAC and Asx-520 Autosampler (England) has been used for determination of elements. Control of the spectrometer is provided by personal computer-based

Table 1
Ingredients composition and chemical analysis of the dietary treatments.

Ingredients and Chemical Analysis	$Diet_{0-100}$[a]	$Diet_{4.86-100}$[a]	$Diet_{0-50}$[a]	$Diet_{4.86-50}$[a]
Ingredients (% of DM basis)				
Alfalfa hay	51.46	49.33	51.46	49.33
Barley grain	46.69	44.75	46.69	44.75
Wheat bran	0.75	—	0.75	—
Dicalcium phosphate	—	1.36	—	1.36
NaCl	—	1.30	—	1.30
Mineral supplement[b]	—	2.20	—	2.20
Vitamin supplement[c]	1.10	1.06	1.10	1.06
Chemical analysis (% of DM basis)				
Dry matter, %	92.79	92.92	92.79	92.92
DE, Mcal/kg	3.054	2.930	3.054	2.930
CP, %	13.041	12.511	13.041	12.511
Lysine, %	0.649	0.622	0.649	0.622
Ca, %	0.895	1.300	0.895	1.300
P, %	0.275	0.522	0.275	0.522
Mg, %	0.251	0.504	0.251	0.504
Na, %	0.062	0.668	0.062	0.668
Cl, %	0.396	1.166	0.396	1.166
K, %	1.643	1.587	1.643	1.587
S, g/kg	234.173	224.525	234.173	224.525
Co, mg/kg	0.239	0.230	0.239	0.230
Cu, mg/kg	13.127	45.525	13.127	45.525
I, mg/kg	0.112	0.678	0.112	0.678
Fe, mg/kg	301.486	289.145	301.486	289.145
Mn, mg/kg	34.578	74.934	34.578	74.934
Zn, mg/kg	28.848	185.792	28.848	185.792
Se, mg/kg	0.387	0.371	0.387	0.371
Vitamin A, IU/kg	2,995.897	2,872.982	2,995.897	2,872.982
Vitamin D, IU/kg	661.017	633.742	661.017	633.742
Vitamin E, IU/kg	99.587	95.492	99.587	95.492
Thiamine, mg/kg	6.780	6.506	6.780	6.506
Riboflavin, mg/kg	3.928	3.769	3.928	3.769

Abbreviations: Ca, calcium; Cl, chlorine; Co, cobalt; CP, crude protein; Cu, copper; DE, digestible energy; DM, dry matter; Fe, iron; I, iodine; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; NaCl, sodium chloride; P, phosphorus; S, sulfur; Se, selenium; Zn, zinc.

[a] Numbers in subscripts for dietary treatments are the supplementation levels of dietary mineral (%) and the level of daily dry matter intakes (% of requirement), respectively. For example, (0–100) indicates the dietary mineral supplementation at 0% and the dry matter intake at 100% of requirements, and so forth.

[b] Mineral supplement composition (DM basis): Ca, 6.5%; Mg, 12%; Na, 4.5%; K, 0.5%; Cu, 1,500 mg/kg; I, 26 mg/kg; Mn, 1,900 mg/kg; and Zn, 7,200 mg/kg.

[c] Vitamin supplement composition (DM basis): vitamin A, 270,000 IU/kg; vitamin D, 60,000 IU/kg; vitamin E, 9,000 IU/kg; thiamin, 600 mg/kg; and riboflavin, 350 mg/kg.

iTEVA software. The wavelengths (nm) used to measure the concentrations of the elements were closely follows that described previously [24].

2.5. Statistical Analysis

This research was conducted using a balanced change over design in three periods (each period extended for 56 days with a 7-day interval between periods), four dietary treatments, nine replicates per treatment, and four blocks (two genders and two age groups, <3 and ≥ 3 years). Two different levels (0% and 2.2% of diet) of minerals were added to one of two different levels of daily dry matter intakes (50% and 100% of requirements) to reached to the dietary treatments [28]. Mane hair and serum samples were obtained at the end of each period. Statistical analyses were conducted using the general linear model procedures of SAS 9.2, version for Windows [29]. The Tukey honestly significant difference test was carried out to compare means. P values lower than .05 were considered as significant.

3. Results

3.1. Hair Mineral Analysis

Mane hair mineral contents (mg/kg, dry weight) are presented in Table 3. Apart from aluminum, nickel, and lead, the mean values of other hair minerals (calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulfur, selenium, strontium, and zinc) and the ratios of calcium to phosphorus, calcium to potassium, zinc to copper, calcium to lead, sulfur to lead, and sulfur to copper were affected by dietary treatments ($P \leq .05$).

Except for phosphorus, the effect of age on hair calcium, cobalt, copper, iron, potassium, magnesium, manganese, nickel, lead, sulfur, selenium, strontium, and zinc concentrations and calcium-to-potassium, zinc-to-copper, calcium-to-lead, sulfur-to-lead ratios were not significant ($P > .05$). For gender, the ratios of calcium to lead, iron to lead, and sulfur to copper in hair were higher in males than females ($P \leq .05$).

Table 2
Daily intakes of nutrients by the Caspian miniature horses from different dietary treatments (DM basis).

Intake (per 100 kg of BW/d)	$Diet_{0-100}$[a]	$Diet_{2.2-100}$[a]	$Diet_{0-50}$[a]	$Diet_{2.2-50}$[a]
CP, g	135.51	135.60	67.75	67.80
DE, Mcal	3.17	3.18	1.59	1.59
Lysine, g	6.74	6.74	3.37	3.37
Ca, g	9.30	14.09	4.65	7.05
P, g	2.85	5.66	1.43	2.83
Mg, g	2.61	5.47	1.30	2.73
Na, g	0.64	7.24	0.32	3.62
Cl, g	4.12	12.63	2.06	6.32
K, g	17.08	17.20	8.54	8.60
S, g	243.33	243.34	121.66	121.67
Co, mg	0.25	0.25	0.12	0.12
Cu, mg	13.64	49.34	6.82	24.67
I, mg	0.12	0.74	0.06	0.37
Fe, mg	313.27	313.38	156.64	156.69
Mn, mg	35.93	81.21	17.96	40.61
Zn, mg	29.98	201.36	14.99	100.68
Se, mg	0.40	0.40	0.20	0.20
Vitamin A, IU	3,113.03	3,113.79	1,556.51	1,556.89
Vitamin D, IU	686.86	686.86	343.43	343.43
Vitamin E, IU	103.48	103.50	51.74	51.75
Thiamine, mg	7.05	7.05	3.52	3.53
Riboflavin, mg	4.08	4.08	2.04	2.04

Abbreviations: BW, body weight; Ca, calcium; Cl, chlorine; Co, cobalt; CP, crude protein; Cu, copper; DE, digestible energy; DM, dry matter; Fe, iron; I, iodine; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; NaCl, sodium chloride; P, phosphorus; S, sulfur; Se, selenium; Zn, zinc.

[a] Numbers in subscripts for dietary treatments are the supplementation levels of dietary mineral (%) and the level of daily dry matter intakes (% of requirement), respectively. For example, (0–100) indicates the dietary mineral supplementation at 0% and the dry matter intake at 100% of requirements, and so forth.

3.2. Serum Mineral Analysis

Apart from copper and strontium, mean values of other minerals in serum were not affected by dietary treatments ($P > .05$). Considering the age and sex effects on serum mineral concentrations, these effects were significant only for copper and phosphorus in the case of age and for copper in the case of sex ($P \leq .05$; Table 4).

4. Discussion

Previous studies showed that the profile of hair mineral imbalance might be useful as a diagnostic tool for the early diagnosis of many diseases [30]. Hair is a metabolically inactive tissue, and its composition reflects levels of trace elements that accumulate in its structure [31] through nutritive blood flow [1].

Because of the potential (sub)clinical implications of knowledge on mineral contents of hair and serum, the present study was aimed to determine the potential applicability of these two biological indicators to determine the mineral status in healthy horses fed diets differing in mineral contents.

Despite the significant effects of dietary treatments on both serum and hair mineral concentrations in this study (Tables 3 and 4), mane hair showed to be a better biological indicator for nutritional imbalances than the serum. Hair composition reflects levels of trace elements that accumulate in the body [31]. Because the minerals undergo

Table 3
The means values of hair mineral analysis in different dietary treatments, age groups, and gender (mg/kg).

Effects of	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	S	Se	Sr	Zn
Dietary treatments[d]																
$Diet_{0-100}$	21.9	1,725^{b}	0.13^{ab}	4.85^{ab}	87.28^{a}	328^{b}	297^{b}	1.2^{bc}	241.7^{b}	0.36	254.7^{bc}	2.296	35,165^{b}	1.084^{b}	5.868^{b}	101.731^{b}
$Diet_{2.2-100}$	16.83	2,405^{a}	0.17^{a}	5.67^{a}	53.83^{b}	508^{a}	420^{a}	4.9^{a}	475.1^{a}	0.56	530.0^{a}	1.854	48,068^{a}	1.674^{a}	7.163^{a}	129.356^{a}
$Diet_{0-50}$	17.44	1,422^{b}	0.08^{c}	4.13^{b}	67.43^{ab}	199^{b}	248^{b}	1.01^{c}	203.3^{b}	0.39	171.8^{c}	2.193	30,729^{b}	0.547^{c}	4.462^{c}	92.740^{b}
$Diet_{2.2-50}$	16.47	1,858^{b}	0.09^{bc}	4.81^{ab}	68.42^{ab}	306^{b}	276^{b}	2.39^{b}	257.4^{b}	0.52	262.0^{b}	2.65	35,709^{b}	0.983^{b}	4.424^{c}	91.720^{b}
SEM	1.83	106.97	0.01	0.2	5.97	32.18	19.00	0.28	27.7	0.073	19.6	0.247	1,391.78	0.079	0.202	4.182
Age groups																
1–3 y	16.520	1,863	0.116	4.86	74.48	333	296	2.42	300.21	0.462	282.8^{a}	2.38	36,453	1.113	5.328	101.92
≥3 y	19.799	1,842	0.118	4.86	64.00	334	324	2.71	288.55	0.453	326.5^{b}	2.11	38,382	1.031	5.631	105.85
SEM	1.298	75.64	0.007	0.14	4.22	22.75	13.44	0.199	19.6	0.05	13.9	0.175	984.14	0.056	0.143	2.957
Gender																
Male	17.889	1,686^{a}	0.109	4.8	65.72	305	301	2.26	283.4	0.453	296.2	2.317	35,275^{a}	0.884^{a}	4.876^{a}	99.05^{a}
Female	18.431	2,019^{b}	0.124	4.92	72.76	362	319	2.88	305.4	0.462	313.2	2.18	39,560^{b}	1.260^{b}	6.082^{b}	108.72^{b}
SEM	1.298	75.64	0.007	0.14	4.22	22.75	13.44	0.199	19.6	0.05	13.87	0.175	984.14	0.056	0.143	2.957

Abbreviations: Al, aluminum; Ca, calcium; Co, cobalt; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; Ni, nickel; P, phosphorus; Pb, lead; S, sulfur; Se, selenium; SEM, standard error of the means; Sr, strontium; Zn, zinc.

[abc] Means within a subcolumn with different superscript letters are significantly different ($P \leq .05$).

[d] Numbers in subscripts for dietary treatments are the supplementation levels of dietary mineral (%) and the level of daily dry matter intakes (% of requirement), respectively. For example, (0–100) indicates the dietary mineral supplementation at 0% and the dry matter intake at 100% of requirements, and so forth.

Table 4
The means values of serum mineral analysis in different dietary treatments, age groups, and gender (mg/kg).

Effects of	Al	Ca	Cu	Fe	K	Mg	Na	P	S	Sr	Zn
Dietary treatments[c]											
$Diet_{0–100}$	2.10	165.80	2.56[a]	12.04	255.08	28.48	4,680	164.41	3,478	1.137[a]	1.473
$Diet_{2.2–100}$	0.48	158.11	2.25[a]	9.23	227.11	29.96	4,425	141.17	3,544	0.994[ab]	1.821
$Diet_{0–50}$	1.05	182.53	1.76[b]	7.25	264.44	34.44	4,941	168.41	3,964	1.221[a]	1.842
$Diet_{2.2–50}$	1.09	160.96	1.70[b]	9.09	224.47	28.1	4,221	149.42	3,355	0.814[b]	2.454
SEM[b]	0.406	9.43	0.189	3.80	13.70	2.16	220.16	11.33	187.7	0.071	0.45
Age groups											
1–3 y	1.55	169.65	2.23[a]	10.79	257.19	29.68	4,584	173.87[a]	3,497	1.093	1.665
≥3 y	0.81	164.04	1.89[b]	8.012	228.36	30.81	4,550	137.83[b]	3,674	0.989	2.129
SEM	0.287	6.67	0.133	2.69	9.69	1.53	155.68	8.01	132.7	0.05	0.32
Gender											
Male	1.168	170.86	1.84[a]	7.53	235.97	31.42	4,710	152.04	3,712	1.022	2.024
Female	1.199	162.83	2.29[b]	11.27	249.58	29.08	4,425	159.66	3,459	1.061	1.771
SEM	0.287	6.67	0.133	2.69	9.69	1.53	155.68	8.01	132.7	0.05	0.32

Abbreviations: Al, aluminum; Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; P, phosphorus; S, sulfur; SEM, standard error of the means; Sr, strontium; Zn, zinc.
[ab]Means within a subcolumn with different superscript letters are significantly different ($P \leq .05$).
[c] Numbers in subscripts for dietary treatments are the supplementation levels of dietary mineral (%) and the level of daily dry matter intakes (% of requirement), respectively. For example, (0–100) indicates the dietary mineral supplementation at 0% and the dry matter intake at 100% of requirements, and so forth.

homeostatic controls in blood [7], the ranges of concentrations are narrow, but in hair, concentrations are broad and reflect long-term metabolic changes.

Hair analysis is beneficial and reflects nutritional imbalances in animals; however, this seems to be stumbling block for some scientists who are not wholly convinced of the validity of hair analysis [32]. The trace element levels in horse tissues are useful for assessment of its nutritional status [4] and environmental exposure to toxic metals [32,33]. The level of trace elements in hair is on detectable level because hair is highly mineralized tissue and can be sampled in relatively high mass [6].

The diagnostic usefulness of hair analysis is confirmed by many authors [1,19,30,34–36], who have proven the correlation between the concentration of basic elements in hair and their concentrations in the body, both in the physiological and pathologic states [37,38]. In addition, hair copper, chromium, iron, and zinc contents provide valuable information for prevention, diagnostics, and treatment of diabetes [30,39] which is usable to equine metabolic syndrome [15]. The studies of other researchers showed that copper in hair correlates significantly with copper in the liver, heart, and kidneys [40]. Similarly, zinc in hair correlates significantly with zinc in bones [40].

Based on the results of the present study, the hair mineral contents of healthy horses fed the balanced dietary treatment ($Diet_{2.2–100}$, Table 3) were in the ranges of Trace Elements Inc reference data [34]. Despite lesser amounts for aluminum, selenium, sodium, potassium, and manganese and greater amounts for lead, calcium, and nickel, the findings of the present study for phosphorus, magnesium, iron, copper, and zinc were similar to the report by Asano et al [23] in Thoroughbred horses.

The results of this study confirmed the suggestion of Nasli et al [41] on scalp hair sample of human as a suitable biological sample for trace element analysis, especially in case of selenium and manganese, because of the high accumulation of these elements in hair.

Our results for gender effect on hair calcium, sulfur, selenium, strontium, and zinc contents are consistent with the results of Asano et al [23] in Thoroughbred horses, with the greater values for male horses. About serum potassium, sulfur, and phosphorus (Table 4) concentrations, the findings of the present study are supported by Kubasova and Kubasov [42] speculation of regulatory mechanisms that keep the horse in a steady state.

In the case of some elements, seasonal changes of diet influence hair mineral contents [7]. The ability of the animal to absorb a mineral may increase during deficiency of it, and it may decrease during its excessive intake. Deficiency or excess of other nutrients may also affect bioavailability of a mineral [43]. In addition, the regulatory mechanisms can keep the plasma level steady, although large amounts of the mineral are absorbed [43].

5. Conclusions

The results of this study have provided baseline data on hair and serum mineral elements that together with information available previously on this topic could be useful for veterinarians and equine nutritionist. Meanwhile, the results of this study clearly answered the main question of the hair being a better biological indicator for mineral status in horses compared with the serum. Finally, future research on the mechanisms and processes involved in mineral accumulation in hair is suggested.

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