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Study on serum macro-mineral concentrations in Kurdish horses and indigenous Mules

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ABSTRACT

Serum biochemical parameters are used for the healthy and diseases status in equines. Interpretation is based on breed of horse, age group and some limits of physiological blood parameters. Determination of serum macro-mineral concentrations and their interrelationships based on spices, gender and age variations in Urmia equines, Iran. Ten ml jugular blood was prepared from 78 Kurdish horses and 22 indigenous mules aged up to 21 years old. Serum calcium, magnesium, phosphorus, sodium, potassium and chloride was evaluated by current laboratory methods. The mineral concentrations between mules and horses statistically were not significant. With the exception of phosphorus and sodium, the mean concentrations in stallions were greater than in mares, but only calcium concentration in mares were lower than in stallions ($P<0.01$). The lowest concentrations for minerals were in equines from 7 to 21years old except for magnesium which was 1-6 years old. The age differences for minerals were not significant except for magnesium which were close to significant difference ($P<0.07$). There were correlations between minerals mainly calcium/magnesium, sodium/chloride, potassium/chloride concentrations. The correlations between calcium/magnesium, sodium /chloride were stronger and higher than others. The concentration of minerals in Urmia equines was normal and not influence by species, gender and age, except for calcium which was low in mares at upper ages and magnesium in lower ages. The correlations among minerals reveal their close cooperation in the physiology of equine body. Therefore, Urmia equines did not show mineral deficiency, but adult mares were probably susceptible to calcium deficiency and magnesium in lower ages.

Keywords: Macro-minerals, Age, Gender, Species, Horse, Mule

INTRODUCTION

Evaluation of mineral concentrations in food [1, 2] and blood [3, 4] are assumed as an easy, safe, proper and cheap method to determine the health of animals [5]. Blood calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl) concentrations in horses were 11.2, 3.1, 1.8 mg/dl, 132, 3 and 98 mmol/l respectively [6]. To investigate equine minerals, understanding the breed, nutrition, age and gender effect on normal physiological condition is necessary [4, 7, and 8]. For example the importance of Ca in metabolic and nutritional disorders of ruminants is well known, but not in equines [9], so this information leads to purification of healthy equines and enhances the capacity and their efficiency in speed, jumping, entertainment and international races [10, 11].

Macro-minerals participate in anatomical, physiological and enzymatic structures. Minerals have vital role in the efficiency of growth, production and reproduction performances [12]. The major duties of Ca are neuromuscular

stimulation and body temperature, Na, k and Cl acid-base balance and tissue fluid, Mg enzymatic and reproduction activities, P energy storage, which means that the health status is directly influenced by the mentioned minerals [6]. Different studies in many aspects of minerals activity and their interrelationships have already been carried out in ruminants though not completely in equines [6].

Variation in macro-mineral concentrations appears during the physiological status following age, nutrition and breed parameters [13] or pathological due to nutritional disorders [7], diarrhea and respiratory deficiency [6], hypomagnesemia [14], Hypophosphatemia [10] and hypocalcemia [15, 16], while variation and diagnosis of the traces is complex and their blood values are considered as the pathological criteria. Thus, the assessment of the blood mineral concentrations in physiological status assumes the diagnostic criteria in horses of the different geographical areas.

The interactions between inter and intra minerals reveal different aspects physiological or pathological status [17]. Direct correlations between minerals is more common than the reverse ones, i.e. between Na/K, Ca/P and Ca/Mg in muscle contraction, bone and teeth composition, Ca/P and Ca/Mg in calved and milking cows [18], and reverse correlation, i.e. Mg/K and [19]. Understanding the relationship among minerals has practical applications such as hypomagnesemia leads to hypocalcemia and hypophosphatemia in equines [10], thus foals are susceptible to rickets and respiratory syndrome [13]. Study on the relationships among minerals in cow is abundant but not in horses and requires further investigation.

The lack of laboratory and clinical features of minerals in Urmia equines has led to possible physiological changes and their relationships which resulted in preparation and regulation of the appropriate food and mineral supplements for health status, enhancing equine population and preventing horses from failing ill. In such criteria the role of species, age and gender may also be addressed [13, 20 and 21]. The aims are to determine the serum mineral concentrations in Urmia equines, the mineral concentrations between horses and mules, mares and stallions at different ages, and finally, the relationships among minerals on the prominent mineral indices important in equines.

MATERIALS AND METHODS

Blood sample and serum extraction

Ten ml blood was collected randomly from jugular vein of 100 horses and mules during the spring and summer 2013 and transferred to the refrigerator at 4°C. Animals were located sporadically or in breeding groups around 45 km from Urmia city. Individual characteristics including identification number, species, gender and ages were registered based on the teeth and inquiry. Horses belonged to Kurd breed and mules were local indigenous. Blood samples were centrifuged for 15 min (3000 rpm), sera were separated into 1.5 ml micro tubes and then placed in freezer (-20 °C).

Overall, there were 100 equines (78 horses and 22 mules), 43 stallions and 57 mares. Age frequency in groups of 1-6, 7-12 and 13-21 years old were 37, 48 and 15, respectively. The number of animals from 1 to 6 were 1, 4, 6, 8, 10 and 8 (37 heads), 7 to 12 were 12, 4, 7, 12, 5 and 8 (48 heads) and 13 to 21 years old were 4, 1, 6, 2, 1 and 1 (15 heads), respectively. Horses and mules were fed forages, dry and wet alfalfa in closed pens or pasture and occasionally consumed barely seed and straw as well. According to the owners claim animals did not receive salt, minerals and electrolyte supplementation. Horses were not pregnant, foaled or in lactation period.

Macro-mineral assessment methods

Serum Ca, Mg, P and Cl were measured by auto analyzer machine (RA-1000, USA) using the appropriate commercial kits (Pars Azmoun, Iran) in mmol/l. Serum Na and K were determined by flame photometer machine using the Na and K standards in mmol/l.

Statistical analysis method

SPSS statistical program was used to determine the mean, standard error and the ranges for macro minerals on the basis of gender and ages in horses and mules. Mean macro minerals were compared by ANOVA and t-test to determine the species, gender and age variations. Correlation tests were run to determine the relationships among minerals.

RESULTS

Table 1 demonstrates the mean, standard error and the range concentrations of macro minerals in equine sera. Serum mineral concentrations in mules was higher than in horses, but statistically not significant, therefore, the

pooled data was used for all equines. Mean serum macro-mineral concentrations (Table 2) in stallions were greater than in mares but statistically Ca concentrations in mares were lower than stallions (P<0.01).

Table 1. Mean±SE concentrations of serum macro minerals` (mmol/l) in horses, mules and overall number of stallions and mares.

Minerals	Horse		Mules		Overall stallions and mares	
	Mean	SE	Mean	SE	Mean	SE
Calcium	2.71	0.01	2.72	0.02	2.71	0.01
Phosphorus	1.79	0.01	1.82	0.02	1.81	0.01
Magnesium	0.73	0.004	0.73	0.003	0.73	0.001
Sodium	137.3	0.43	138.4	0.93	137.5	0.40
Potassium	3.35	0.02	3.35	0.02	3.35	0.02
Chloride	102.7	0.22	103.4	0.26	102.8	0.19

Table 2. Mean±SE and range of serum macro mineral concentrations (mmol/l) in stallions, mares and overall number of stallions and mares

Minerals	Stallions		Mares		Overall stallions and mares	
	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range
Calcium	2.74± 0.013	2.55-2.92	2.68± 0.011	2.48-2.92	2.71± 0.009	2.48-2.92
Phosphorus	1.79± 0.014	1.37-1.92	1.80± 0.009	1.59-1.95	1.80± 0.008	1.37-1.92
Magnesium	0.73± 0.003	0.68-0.77	0.72± 0.003	0.65-0.83	0.73± 0.002	0.65-0.83
Sodium	137.1± 0.56	131.2-144.5	137.9± 0.56	131.0-145.7	137.5± 0.40	131.0-145.7
Potassium	3.36± 0.024	3.15-3.95	3.34± 0.025	3.09-3.95	3.35± 0.017	3.09-3.95
Chloride	102.7± 0.31	99.6-105	102.7± 0.24	99.5-105.8	102.8± 0.19	9.5-105.8

The result of the serum mineral concentrations in 1-6, 7-12, 13-21 years old (Table 3) indicates that the highest and lowest concentrations for minerals were in 1-6 and 13-21 years old except for Na and Cl which in 7-12 years old. The statistical results revealed no age difference among horses and mules except for Mg which were close to significant different (P<0.06).

The correlation results among serum macro-mineral concentrations in horses and mules (Table 4.) showed positive correlation (P<0.05) between Cl/Na (r=0.68) and negative correlations (P<0.05) between P/K (r=0.27) and Cl/K (r=0.32). The correlation coefficient between Ca/Mg and Cl/ Na was stronger and P/K and Cl/K was weaker than others.

Table 3. Mean±SE serum macro mineral concentrations (mmol/l) in different age groups (years)

Minerals/ages	1-6 years[1]	7-12 years[2]	13-21 years[3]	Overall stallions and mares
Calcium	2.70±0.02	2.72± 0.01	2.70± 0.02	2.71± 0.01
Phosphorus	1.79±0.01	1.79± 0.01	1.81± 0.02	1.80± 0.02
Magnesium	0.72±0.003	0.73± 0.004	0.73± 0.007	0.73± 0.002
Sodium	136.9±0.57	137.6± 0.66	138.9± 0.74	137.5± 0.40
Potassium	3.34±0.03	3.36± 0.03	3.34± 0.03	3.35± 0.02
Chloride	102.5±0.33	102.8± 0.29	103.3± 0.24	102.8± 0.19

*[1] *n=37* [2] *n=48* [3] *n=15*

Table 4. Correlations among serum macro mineral concentrations (mmol/l) in horses and mules (n=100)

Minerals	Phosphorus	Magnesium	Sodium	Potassium	Chloride
Calcium	0.05	0.63**	0.63**	0.07	-0.12
Phosphorus		0.18	0.06	-0.27**	0.06
Magnesium			0.16	-0.02	0.03
Sodium				0.02	0.68**
Potassium					-0.32**

*=*P<0.05* ** =*P<0.01*

DISCUSSION

Serum Ca, P concentrations in this study consistent with Radostits *et al* [6] and Miknienė *et al* [4] report and no deficiency was expected in the experimental animals, but Ca was higher and P was lower than that reported by Santos *et al* [22] and Hassan *et al* [23] or Ca was lower and P was higher than Lopez *et al* [21] and Tobey and Anslyn [24] findings. The variations in the mentioned reports are possibly related to the environmental, management and nutritional parameters thus, the local index is crucial in diagnosis of the pathological conditions. The lack of species and age differences in serum Ca was consistent with Samia *et al* [20] findings, while Sevinga *et al* [25] and Ahmed *et al* [16] mentioned low Ca following foals infection and mares retained placenta. In this study serum Ca in mares was lower than stallions the same as Miknienė *et al* findings [4] but placed in normal range that

mares are susceptible to low Ca after foaling and lactation, while not for P. However, the occurrence of physiological hypophosphatemia, hypocalcemia, milk tetany and hyperlipaemia is inevitable following long and severe training, speed, horse riding, transportation [26, 27], pregnancy, parturition and lactation [6, 28]. For this reason scientists recommend the Ca/P ratio in mares 2.5 to 1 and in foal 1.5 to 1, that P is essential in lower ages and Ca is vital in adults [1]. Therefore, according to Grace *et al* [1] findings daily consumption of 9.2 gr Ca and least 3.5 g P in mares and foals respectively, maintains. Serum Ca and P in non pregnant and lactating mares of this study is the result of adequate Ca utilization.

An optimal Mg concentration of equines in this study was consistent with Radostits *et al* [6] which has positive effects on growth and prevents the occurrence of diseases related hypomagnesemia [1]. The species, age and gender results of horses and mules different from Samia *et al* [20] findings that mentioned low Mg in lower ages (foals) or retained placenta in mares [25]. Variations in equine serum Mg in occurrence of disease are more important than P but less than Ca. Serum Mg varies following riding and physical practices [25, 29], during colic, diarrhea and respiratory disorders [13] in adults and suckling foals [1]. Low Mg in diet is associated with hypomagnesemia and hypocalcemia [14]. Since mare's milk is poor in Mg, the continuation of Mg in daily diet is essential and should be 220 mg/day and increases by further physical activities [30].

Serum Na, K and Cl concentrations of the equines were consistent with the Radostits *et al* [6]. Among those minerals hypokalemia is much serious and occurs in equine endotoxemia accompanied by hypocalcemia [10]. The result of age, gender and species variations in serum Na, K and Cl supports Samia *et al* [20] findings, but however, can vary physiologically after riding, severe practices, high speed [23, 29, 31] and pathologically in viral enteritis, colitis, esophageal obstruction and muscular injuries [22]. The correlation between Na and K is negative, whenever serum K decreases, serum Na starts to increase or vice versa the same as seen in urinary tract system, while correlations between Na and Cl were positive and both decline in horses suffering water deficiency and thirst [22], thus access to water and electrolytes is essential before and after practices in horses. Access to food with 1 and 2.1 g/day Na and K, respectively, accompanied by modification of strong breeds and suitable practices improves horses in international competitions [7].

Overall the amounts of minerals in mules were higher than in horses, which means that species did not affect mineral values. This finding was in contrast with Murase *et al* [32] and Sema *et al* [33] results mentioned the breed effect on mineral levels in Arab and Thoroughbred breeds. The age of animals had no influence on macro-mineral levels but as mentioned above Ca in adult ages and magnesium in lower age may be critical for horses.

The main advantage of the study was to determine the relationships among minerals and to identify the premier indices existing in the blood of horses which have not been reported before. The interrelationships among minerals in blood, tissues, bones, muscles, urine and cerebral spinal fluids (CSF) have already been confirmed in ruminants but require further investigation in horses. The correlations between minerals demonstrate an optimal coordination or contrast among minerals. Similar results have been reported by Bigras and Tremblay [18] in cows, but not in horses [34]. Coordination between Ca and P in bone and teeth structure, Na and K in blood osmotic and acid-base balance and coordination of all 4 minerals in muscle contraction could be the result of this study in horses. Among macro-minerals, the role of Ca and K is much more important than Na and P [6] are vital in animal survival.

CONCLUSION

Mineral deficiency is not the case in Urmia horses and mules. Mineral concentrations in horses, mules, mares and stallions in different ages were similar except for Ca in mares, and Mg in foals could be considered the important parameters. The presence of various relationships among minerals will lead to mineral balances in equines dietary program being better prescribed. Further recommendation goes on the evaluation of trace mineral concentrations in which are critical in healthy equines breeding.

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