Hoof Quality: Correlation Between Calcium, Phosphorus, Copper & Zinc Levels in the Hoof Shavings and Blood Levels of Arabian Horses in Saudi Arabia

Mohammad Racin Abdin-Bey

Department of clinical studies,
College of Veterinary Medicine & Animal Resources
King Faisal University, Al-Hasa, Saudi Arabia

Abstract

Samples of hoof horn and blood were taken from 27 horses.

The concentrations of calcium, phosphorus, copper and zinc in the plasma and hoof keratin were determined by Atomic Absorption Spectrophotometer (AAS).

The mean (±SD) concentrations of the samples were determined and then the mineral concentration levels of the hoof wall were plotted against the plasma levels.

There was no significant relation found for calcium and phosphorus levels between plasma and hoof. But there was a significant linear relation established between the concentration of copper in plasma and hoof horn. Thus blood levels of copper may be sufficient to determine whether the hoof horn contains an adequate level of this necessary trace element or not.

On the contrary, zinc levels were recorded within narrow boundaries in the blood, while in the hoof a wider array of results were found. Considering the homoeostasis of zinc levels in the blood this study suggests the superiority of hoof samples over blood samples as an indication for sufficient supply of horses with zinc.

Key words: *Arabian horses, hoof, hoof composition, surgery, trace minerals, hoof-shavings*

Introduction

The hoof is one of the most 'burdened' body-parts of the horse. On one hand its outer part is largely responsible for protecting the hoof from external influence such as abrasive, noxious substances or infectious agents. On the other hand, hoof-horn must be flexible enough to withstand thousands of pounds of impact and to absorb and divert the shock sustained on them. Therefore the hoof-horn should be rigid as well as elastic. Several factors could influence the physical and chemical properties of the hoof, decreasing its hardness, toughness and viscoelasticity. The structure of the keratin forming

part of the hoof could be changed due to hereditary factors (Jossek, 1991), race and environment (Baggott, 1982) or nutrition (Vermunt, 1990).

The wall and the sole of the hoof are composed of keratinous hoof material that is produced continuously; its epidermal structure consists of microscopic tubes formed by the germinative layer of the epidermis above the corium papillae. It is made up of amino acids, such as cysteine and methionine (Grosenbaugh and Hood 1992), minerals including calcium, phosphorus, magnesium and the trace elements zinc, copper, selenium (Demertzis, 1978, Davis and Mertz, 1987; Kempson, 1991) and vitamins including biotin, folic acid, Vitamin A and E (Comben *et al.,* 1984).

Since the hoof horn is a dynamic tissue, only provision of adequate and good quality food, containing the correct supplement, results in continuous growth of high quality hoof-horn.

Calcium, copper, zinc and magnesium are important in relation to claw affections (Johnson and Schugel, 1994). According to Mülling et al. (1999), minerals are essential for activation of enzymes that are prerequisite for physiological keratinization and cornification of the claws. Thus deficiencies in one or more elements could induce structural alterations in the hoof build-up, a reduction in the horn cell quality that serves as a major parameter of hoof health.

Calcium plays an important role in the regulation of cellular differentiation and desquamation of epidermal keratinocytes and influences the hardness of keratins by virtue of its presence and the crystal structure of its complexes (Vicanova *et al.,* 1998). It is an essential element acting as an enzyme cofactor or activator during the process of keratinization. Copper is important in the incorporation of disulphide bonds into the protein molecules which form a major part of the keratin mix. Such bonds determine the physical properties of keratin (Davis and Mertz, 1987). Zinc is known to be of importance in keratin synthesis and hoof horn formation and it plays an important role in making the hoof more resistant to stress (Banting, 1978).

Blood mineral concentration is an important indicator of the nutritional status of an animal and changes in its concentration may affect the hoof mineral content (Moor *et al*., 1975; Smart *et al*., 1992)

Arabian horses are famous for their excellent hooves. However information about the correlation between its plasma mineral and hoof minerals is lacking.

This study aimed to find a correlation between blood plasma levels of calcium, phosphorus, zinc and copper and their occurrence within the hoof-horn of Arabian horses.

Material and Methods

Twenty-three (23) Arabian and four (4) Anglo-Arab horses from different locations in the eastern province of Saudi Arabia were used in this study. By gender there were 18 mares and 9 stallions and their age ranged between 2 and 26 years. All horses were in apparent health and had no clinical signs of any diseases.

After a thorough cleaning of the hoof with water it was left to dry and the samples were then collected. The hoof horn material was collected from all four hooves of each animal and from all parts of the hoof wall using a special hoof rasp.

The hoof horn samples were dried at a constant temperature of 115^{o} C for 24 hours, and the dried samples were ashed in a Muffle Furnace at 550^{o} C for another 18 hours, the ash content expressed as a proportion of dry matter.

Two grams of dried horn samples were then prepared by wet ashing in 15 ml of a 20% nitric acid and filled up with deionized water to complete 100 ml.

Calcium, phosphorous, zinc and copper were determined using Atomic Absorption Spectrophotometer, Perkin-Elmer, model 3280, USA.

Blood samples (10 ml) were collected in lithium heparin tubes from all horses by venepuncture; plasma was subsequently separated for the study. These samples were later analyzed for calcium, phosphorous, zinc and copper.

Additional samples (5 ml) collected in lithium heparin tubes were used for hematology. These samples were analyzed for RBC, WBC, Hb and PVC and the results were within the normal range.

Results and Discussion

The values of the median and mean±SD of calcium, phosphorous, copper and zinc found in hoof-shavings and plasma are given in Table (1). Since median and average values are in close proximity to each other, a normal distribution of values can be assumed. The interquartile range, compiled of the middle 50% of our findings, shows no extreme outlyers that could undermine the validity of the statistical result.

Table (1)
Median and Mean concentrations for calcium, phosphorus, copper and zinc found in hoof-shavings and in blood plasma respectively

	Median	Mean ± SD	Range	Interquartile Range
Hoof-shavings				
Ca (mg/dl)	1,8	2,47 ± 1,70	0,3 - 6,8	1,1 - 3,7
P(µg/dl)	253,4	278,24 ± 150,37	80 - 676,7	177,8 - 341,5
Cu(µg/dl)	23	24,77 ± 11,42	15 -70	20 - 24
Zn(µg/dl)	150	157,48 ± 36,49	108 - 258	127 - 180
Blood plasma				
Ca(mg/dl)	11,75	11,44 ± 2,06	6,31 - 13,96	10,96-12,69
P(mg/dl)	1,922	2,23 ± 0,84	0,57 - 4,02	1,56-2,64
Cu(µg/dl)	90	99,26 ± 26,01	75 - 200	85-105
Zn(µg/dl)	140	139,1± 6,05	130 - 150	135-145

While the hoof-shavings values display a wider range among the samples, the ranges of mineral in the plasma are narrower (Tab 1a).

Table (1a)
SD in percent of the mean

	Ca	P	Co	Zn
Hoof-shavings	68	54	46	23
Plasma	18	37	26	4.

The blood plasma levels are within well established data ranges for calcium and phosphorus (Ca: 8,01-12,12 mg/dl -corresponding to 2-3,5 mmol/l- and P: 1,5-4,0 mg/dl) and within ranges established by other previous studies for copper and zinc.

In comparison to findings in the previous study by Abdin-Bey (2001), where only young horses (up to 2 years) were considered, the mean calcium level was higher by nearly 10 percent and the zinc plasma level was closer to the lower end of the previously established range (Tab.2).

Table (2)
Blood Plasma. ***Findings and reference parameters***

	Present Study	**Abdin-Bey 2001**	**Stubley *et.al.* 1983 ***	**Knickel *et.al.* 1995**
Ca (mg/dl)	11,44	10,47	-	8-12
P (mg/dl)	2,23	-	-	1,5-4,0
Cu (µg/dl)	99,26	50-118	79 / 101	
Zn (µg/dl)	139,1	110-310	111 / 170	

*Group 1/2

Regarding the concentration found in the hoof-shavings (Tab.3), only the copper contents correspond to previous findings by Weiser *et al.*(1965) and Sasimowski *et al*. (1987). Phosphorus was slightly lower and zinc considerably lower than their findings.

On the other side, calcium levels were well above the reference parameter.

Table (3)
Hoof-shavings. ***Findings and comparison***

	Present Study	**Weiser 1965 ***	**Sasimowski 1987**
Ca (µg/g DM)	1230 =2,47(mg/dl)	294/359	60-470
P (µg/g DM)	139 =278,24(µg/dl)	154/209	250-430
Cu (µg/g DM)	12 =24,77(µg/dl)	12/12	3-11
Zn (µg/g DM)	79 =157,48(µg/dl)	139/146	115-177

* Different values from newly grown hoof horn and old hoof horn; DM=Dry Matter

The possible explanation of differences in the level of these trace elements and minerals could be attributed to environmental, nutritional or breeds differences which may suggest that the hooves of Arabian horses is of a higher quality than other breeds, however further studies are needed to verify.

Relating the mineral contents of the hoof-shavings to the plasma concentrations is the aim of this study, achieved by plotting hoof-shavings findings against blood plasma.

There was significant relationship of copper level in the blood and hoof-shaving but the significant linear relationship between these levels was not observed in case of calcium, zinc and phosphorus (Fig.1-4).

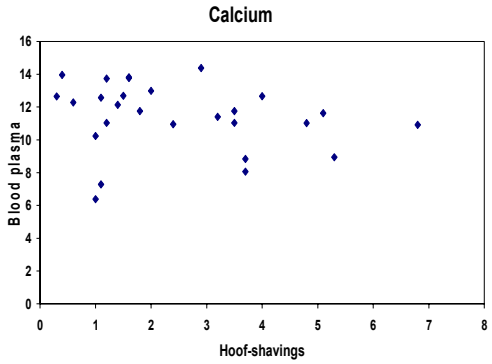

Fig .1

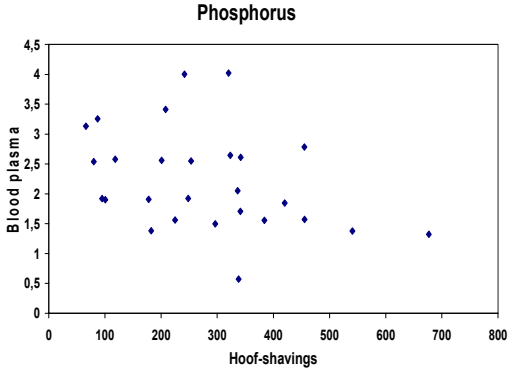

Fig.2

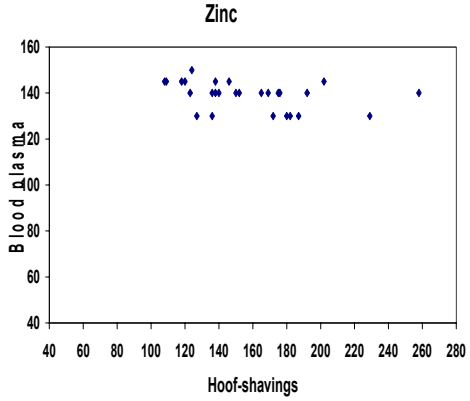

Fig.3

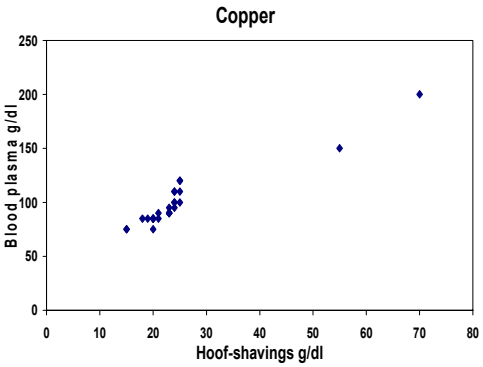

Fig.4

Fig.1-4 : Relationship of calcium, phosphorus, zinc and copper contents of hoof-shavings to plasma concentrations.

The findings, achieved in the present investigation, suggest that copper concentrations in plasma and hoof-shavings are related (Fig.4&5) and there exists a significant correlation between hoof-shaving and blood plasma concentration levels, with a correlation coefficient of 0.944158.

A linear regression was run, yielding the following trend line:

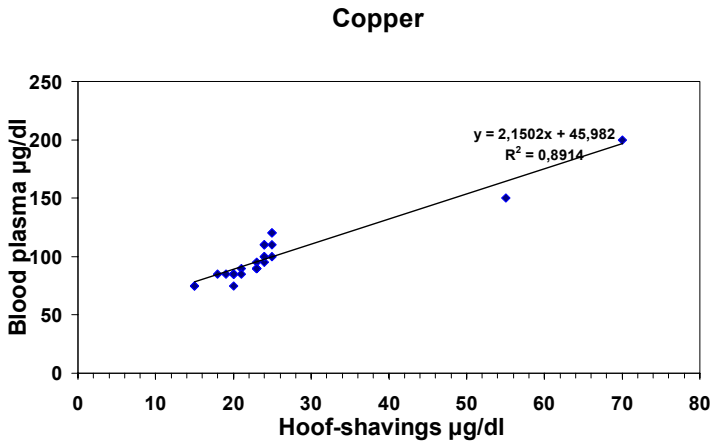

Fig.5. Correlation between hoof-shaving and blood plasma concentration levels of copper

Although this is a pure statistical measure and thus no proof of any underlying hypothesis, nevertheless it gives emphasis to the theory that copper concentration in plasma and hoof-shavings are related and that it may be possible to predict from blood findings the corresponding concentration of copper in the horse's hoof. Although proof for this hypothesis must come from much larger statistical sample, at least the hypothesis has been supported by this study's small sample.

A similar regression line for zinc showed that hoof shaving levels of zinc vary independently from the relative stable blood plasma levels.

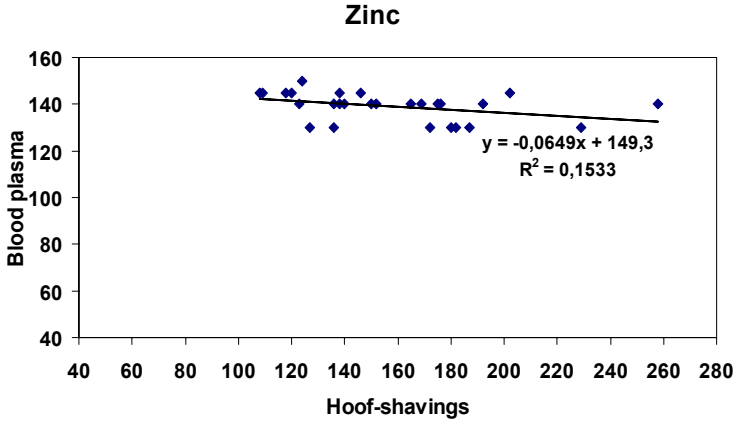

Fig. 6 Correlation between hoof-shaving and blood plasma concentration levels of zinc

Concerning the concentration of zinc it was found that the blood plasma levels were within a very limited range, while hoof concentrations differed over a wide range. As the metabolism of the trace mineral zinc underlies a strict homoeostasis, gastrointestinal absorption as well as endogen zinc-excretion manage to keep zinc levels widely constant (Weigand and Kirchgessner, 1977). Thus, zinc concentration in blood could not be taken as a good indicator of the supply situation. While the internal metabolism is very successful in keeping a constant level of zinc in the blood, the keratin build-up in the hoof utilizes different concentrations. In Spitzlei's_study, a reaction to zinc supplementation was even found in existing hoof horn (Spitzlei, 1996). Thus, the evaluation of the nutritional status of the horse concerning the supply of zinc should ignore the blood parameters and concentrate rather on the hoof.

References

1. Baggott, D.G. (1982): Hoof lameness in dairy cattle. In Practice 4, 133 - 141.
2. Suppl. Vet.Rec. 111, Nr.12.
3. Banting, A. (1978): A few notes on the probable role of zinc in abnormal foot conditions in ruminants. Report presented at the second symposium on bovine digital disease, Vet. Institute, Skara, Sweden.
4. Comben, N.; R.J.Clark and D.F.B.Sutherland (1984): Clinical observation on the response of equine hoof defects to dietary supplementation with biotin. Vet.Rec. 115, 642 - 645.
5. Davis, G. and W. Mertz (1987): Copper in: Trace elements in human and animal nutrition (W.Mertz publisher) Vol.I, 5.edition Academic Press, San Diego, 301 - 364.
6. Demertzis, P.N. (1978): Zinc deficiency as a cause of foot diseases in ruminants. In: 2.Int.Sympos. on Disorders of the Ruminant Digit, 1978, Skara, 33-37.
7. Grosenbaugh, D.A. and D.M. Hood (1992): Keratin and associated proteins of the equine hoof wall. Am.J.Vet.Res. 53, 1859 -1863.
8. Johnson, A. and L. Schugel (1994): The role of trace elements in bovine lameness. Eight international symposium on disorders of the ruminant digit and international conference on bovine lameness. June 1994, Banff, Canada.
9. Jossek, H. (1991): Hufhornveraenderungen bei Lipizzanerpferden und ein Behandlungsversuch mit Biotin.Untersuchungen des makroskopischen Hufstatus und des Hornwachstums zum Verlauf des Plasmabiotinspiegels und ueber genetische Grundlagen der Hufhornschaeden. Zuerich, vet. Fak. der Univ.Zuerich, Diss.
10. Kempson, S.A. (1991):
    The role of nutrition in the growth and structure of hoof horn. in: Laing, J.A., Prevention, management and repair of trauma in transport animals. World

Association for Transport Animal Welfare and Studies, Spring Meeting, May 15, 1991, Animal Heath Trust, New York, 51 - 59.

11. Knickel, U.; C.Wilczek and K.Jöst (1995): MemoVet - Praxis-Leifaden Tiermedizin. F.K.Schattauer Verlagsgesellschaft mbH, Stuttgart, veterinärnedizin
12. Moor, J., Gerber,H., Martig,J. and Stampfli,G. (1975): Normal serum values of calcium, magnesium and inorganic phosphorus in Simmental cattle. Archiv fuer Tierheilkunde, 117:365-381.
13. Mülling, C.; H. Bragulla, S. Reese; K.Budras and W.Steinberg (1999):
14. How structures in bovine hoof epidermis are influenced by nutritional factors. Anat.Histol.Embryol., 28:103-108.
15. Sasimowski, E.; M.Budzynski; C.Lipecka; J.Moczybroda and M.Kapron (1987):
16. Chemical composition of the hoof horn of thoroughbred and heavy draught horses entered in polish stud books. Roczniki Nauk Rolniczych.Seria B. Zootechniczna, 103, 131 -147.
17. Smart, E.; Cymbaluk, N. and Christensen, D. (1992): A review of copper status of cattle in Canada and recommendations for supplementation. Can.Vet.J., 33: 163-170.
18. Spitzlei, S. (1996): Untersuchungen zur Zusammensetzung des Hufhorns beim Pferd, deren Bedeutung für die Stabilität und Beziehungen zur Nährstoffversorgung.
    Hannover, Tierärztl. Hochsch., Diss
    Stubley, D.; C. Campbell; C. Dant and D.J. Blockmo**re** (1983): Copper and zinc levels in the wood of thoroughbreds in training in the United Kingdom. Equine Vet. J.15, (3), 253-256
19. Vermunt, J.J. (1990): Lesions and structural characteristics of the claws of dairy heifers in two management systems. MSC Thesis, University of Saskatchewan, Saskatoon.
20. Vicanova. J.; Boelsma, E.; Mommas, A.; Kempenaar, J.; Forslind, B.; Palloen, J.; Egelrud, T.; Koerten, H. and Ponec, M (1998): Normalization of epidermal calcium distribution profile in reconstructed human epidermis is related to improvement of terminal differentiation and stratum corneum barrier formation, J.Invest. Dermatol., 111: 97- 106.
21. Weigand E. and M. Kirchgessner (1977): Homeostatic adaption of Zn absorption and endogenous Zn excretion over a wide range of dietary Zn supply. In: Kirchgessner (Publ.): Trace element metabolism in man and animals 3, 106 - 109.
22. Weiser, M.; W. Stoeckl; H.Walch and G.Brenner (1965)?:
    Ueber die Verteilung von Natrium, Kalium, Kalzium, Phosphorus,Magnesium, Kupfer und Zink im Hufhorn von Pferden. Arch. Exp. Med. 19, 927- 931.

جودة الحافر : العلاقة بين مستويات الكالسيوم، الفوسفور، النحاس و الزنك في القشرة الخارجية للحافر و بلازما الدم في الخيول العربية في المملكة العربية السعودية

محمد رصين عابدين بك

قسم الدراسات الإكلينيكية بكلية الطب البيطري والثروة الحيوانية
جامعة الملك فيصل الإحساء – المملكة العربية السعودية

الملخص:

في هذه الدراسة أخذت كميات من الدم الوريدي من ٢٣ من الخيول العربية و ٤ من الخيول العربية المهجنة بالإضافة إلى ٢٧ عينة من القشرة الخارجية لحوافر نفس الحيوانات لمقارنة محتوياتها من بعض المعادن مثل الكالسيوم و الفسفور و النحاس و الزنك، مستخدمين في ذلك جهاز مقياس الضوء الطيفي الذرى. وبعد ذلك تم الحصول على المتوسط الحسابي والانحراف المعياري. توضح هذه الدراسة وجود علاقة طردية معنوية لمادة النحاس في الدم والحافر و في المقابل لم نتمكن الحصول على علاقة معنوية بين مكونات الدم والحافر من حيث الكالسيوم والفسفور. لذلك توصى هذه الدراسة أن تعيين مستوى النحاس في الدم يعطى دلالة على وجوده بنسب معقولة في الحافر. أما تركيز الزنك فهو مرتفع في الحافر عما هو عليه في الدم ولو بدرجة ضيقة.