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EFFECT OF ZINC ON INTEGRITY OF HORSE HOOF

Zuhair Noormohammady[1*], Muhammad Chamani[1], Hamid Reza Khodae[2]

[1]Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Animal Science, Islamic Azad University, Golpayegan Branch, Golpayegan, Iran

Abstract. In this research, the effect of zinc presence on hoof quality of 24 KWPN horses was investigated. This study was done in Azmoon horse club in Tehran, Iran which uses alfalfa and concentrate as diet of every horse. In this research, eight pieces each of strong hooves, weak hooves and hooves with medium strength were chosen and zinc content of them was measured. Sampling occurred in winter of 2015 and lab operations were done in spring of 2015. Analyzing lab data showed no meaningful relationship between zinc content and quality of hoof. Significance level in Pearson's coefficient was 0.437 which is not meaningful. Duncan test indicated that average zinc content in hooves with medium strength was 115.00 p.p.m, in strong hooves was 119. 38 p.p.m and in weak hooves was 129.38 p.p.m. Significance level in this test was 0.521 which shows there is no significant correlation between zinc content of the three groups of hooves. It is concluded that zinc has no role in structural integrity of hoof horn. It may improve keratinization trough taking part in function of enzyme in the living part of hoof, leading to production of more qualified horn. But its presence in the dead structure of hoof horn is not a determining factor.

Keywords: *zinc, hoof, integrity, KWPN, horse.*

Corresponding Author: *Zuhair Noormohammady, Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: znoormohammady@yahoo.com*

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1. Introduction

Zinc is a key mineral in the process of keratinization. Keratin cells in the upper living part of the hoof, lose their nucleus and organs and run downward. During this process the cells die and form horny structure of the hoof (Mulling, 2000). Zinc is the most abundant intercellular trace element. It is component of over 200 enzyme systems like RNA Nocleotide transferase, RNA polymerase, Thiol oxidase and Cu/Zn super oxidase dismutase (Mulling *et al.*, 1999). As an example, Cu/Zn super oxidase dismutase is responsible for prevention of lipid peroxidation (Mulling *et al.*, 1999). Protection of the intercellular lipids are critical in the maintenance of structural integrity since they are an integral part of cementing substance responsible for cell-to-cell adhesion (Mulling & Budras, 1998). In addition, they repel water from horn and keep it qualified (Ott and Johnson, 2002). In Cu/Zn super oxidase dismutase, Cu functions as catalytic site, whereas Zn has a role in 3-D structure of the enzyme (Cousins, 1996). This study aims to investigate the relationship between horn integrity and its zinc content.

2. Materials and methods

Hooves of 24 jumping KWPN horses between the ages of eight to twelve years were gathered. Samples were picked at trimming time and all of them were taken from left hand, wall part of the hoof. The quality of every hoof was determined by the club farrier and with his judgment eight hoof samples of each strong, medium and weak quality were chosen. An approximate amount of 0.5 grams was cut from each sample by pincer. Every horn piece was washed with distilled water and ethanol. The samples were transferred to German-made lab oven "Memmert" to lose water and get ready to be weighed. Every sample piece was weighed with a scale of three decimal places. All samples had a weight of 0.45 to 0.55 grams.

The samples were digested in two stages. In the first stage every sample was digested in a solution containing five cc of nitric acid 37% and two cc of hydrogen peroxide 20%. After 72 hours of digestion in the solution, the samples were transferred to the American-made microwave machine "Milestone SX10" to digest completely. All samples were reached to a volume of 45 cc and zinc content was determined with Atomic Absorption Spectrometer "Varian Spectra AA 200". The results were analyzed with Pearson's Correlation Test and Duncan Test through IBM SPSS statistical software version 16.

3. Results

The following table shows the score of hoof quality and zinc content of every horse.

Table 1. Score, quality and zinc content of every sample

Row	Horse	Given Score	Hoof Quality	Zinc content (p.p.m)
1	Walis	3	Strong	133
2	Take Well	3	Strong	126
3	Kronj	3	Strong	139
4	Vancouver	3	Strong	142
5	Aicardo	3	Strong	131
6	Vanilia	3	Strong	63
7	West Virginia	3	Strong	124
8	The Gilding	3	Strong	97
9	Unique	2	Medium	146
10	Waluta	2	Medium	124
11	Black Beauty	2	Medium	96
12	Bernard	2	Medium	106
13	Axcel	2	Medium	146
14	Karisoo	2	Medium	145
15	Cleopatra	2	Medium	98
16	Feliciano	2	Medium	59
17	Wonderful	1	Weak	161

18	Furan	1	Weak	139
19	Elin	1	Weak	120
20	Zakintoos	1	Weak	124
21	Ellegance	1	Weak	133
22	Julia	1	Weak	128
23	Constantin	1	Weak	105
24	Atlantic	1	Weak	125

Kolmogrov-Smirnov test was used to determine if the data distribution was normal and we can use parametric tests.

Table 2. Kolmogrov- Smirnov

	Kolmogorov-Smirnove			Shapiro-Wilk		
	Statistic	df	sig	Statstic	df	sig
Hoof Quality	0.218	24	0.005	0.797	24	0
Zn	0.210	24	0.007	0.906	24	0.029

In Kolmogrov-Smirnov test, significance level is zero for hoof quality and 0.029 for zinc content. It shows that data distribution is normal and we can use parametric tests.

To measure correlation between hoof quality and zinc content, Pearson's correlation coefficient was used. The test shows no significant correlation between the two variables.

Table 3. Pearson's- Correlation Coefficient

		Hoof Quality	Zn
Hoof Quality	Pearson Correlation	1	-0.166
	Sig. (2-tailed)		0.437
	N	24	24
Zn	Pearson Correlation	-.0166	1
	Sig. (2-tailed)	0.437	
	N	24	24

Significance level in Pearson's correlation coefficient test is 0.437 that shows there is no meaningful relationship between hoof quality and zinc content.
Duncan test was used to compare average amount of zinc in three groups.

Table 4 shows that average amount of zinc in strong hoof samples is 119.38 p.p.m, in weak hoof samples 129.38 and in horn samples with medium strength it is 115.00 p.p.m.

Table 4. Amount of zinc content in three hoof groups in Duncan test

Hoof Quality	N	Subset for alpha = 0.05
		1
2	8	115.00
3	8	119.38
1	8	129.38
Sig.		0.297

Table 5. Significance level in different groups in ANOVA

Between Groups	868.750	2	434.375	0.672	0.521
Within Groups	13569.750	21	646.179		
Total	14438.500	23			

Significance level in Duncan test is 0.521 that indicates no meaningful correlation among the three groups.

4. Discussion

In this study no meaningful relationship was observed between zinc content and quality of hoof. This result approves some research studies and disapproves some other. Roberto *et al.* (2015) compared hoof quality of Anglo-Arabian horses and Monterufoli Ponies and reported that Anglo-Arabian horses that had stronger hooves tended to accumulate more zinc in their horn. Coenen and Spitzlei (1997) showed that 25 horses with poor horn quality had lower blood and hoof zinc than 38 horses with normal feet. Baggot *et al* (1988) reported finding of lower Zn concentration in claw of lame cows than those with no history of lameness. Sargentini *et al.* (2012) studied donkey's hoof and found a negative relationship between hoof strength and zinc content.

Some researchers didn't find any relationship between hoof integrity and zinc. Huntington believes that current thinking on the relationship of diet and hoof integrity puts too much emphasis on zinc. Sheep were fed rations supplemented with $ZnSO_4$ for up to 6 months but did not show a reduction in claw problems (Cross & Parker, 1981). Butler and Hints (1977) showed that the hoof wall growth was 50% greater in growing ponies in positive energy balance than in ponies on restricted diets. But restriction in energy, protein and minerals including zinc didn't reduce hoof wall strength.

The above mentioned studies have investigated lower part of hoof that is dead and contains no enzymes. Some researchers have studied effect of adding zinc to the diet. Most of these research studies show that supplementing the animal food with zinc increases the hoof quality after a few months or a year. Hepworth (2004) said that by keeping an animal well fed with proper nutrients such as zinc and biotin, it is much more likely that they will produce good-quality horn hoof and have stronger feet. At dairies, having a high incidence of foot problems, cows were fed two to three gram per day of $ZnSO_4$ for 70 days and showed fewer claw problems in comparison with cows not receiving supplemental Zn (Demertizis, 1973). Ott and Johnson (2001) examined the effect of mineral source on hoof development of yearling horses. Fifteen yearlings

were fed one of organic or inorganic diets for 112 days. The increase in hoof growth due to the proteinated minerals was about 4%. Breaking strength of the hoof was greater for Quarter horses than Thoroughbreds but was not influenced by feeding. Siciliano *et al.* (2008) studied effect of zinc on 250 cows in two organic and inorganic treatments. Cows fed by organic zinc showed less sole ulcer, interdigital dermatitis, heel erosion and white Line disease. Of beef cattle receiving, 216 mg/d of Zn from complexed Zn, 2.45% had foot rot, whereas 5.38% of cattle not receiving complexed Zn had foot rot (Brazle *et al*., 1993). In a year-long study conducted at Illinois State University, cows fed an additional 200 mg/d of Zn from zinc-methionine had fewer cases of foot rot, heel cracks, interdigital dermatitis, and laminitis than cows not fed Zinc-Methionine (Moore *et al*., 1989). Nocek *et al.* (2000) found out that cows fed by organic zinc, copper, manganese and cobalt had less white line disease, sole hemorrhage and double soles. In another study 300 cows in a dairy in Florida were fed a combination of complexed Zn, Mn, Cu, and Co. Those fed complexed trace minerals had fewer cases of claw disorders than the ones fed inorganic minerals 75 days after calving (23.6 vs. 17.7%). Also feeding complexed trace minerals reduced incident of white line disease 75 days after calving (9.5 vs. 14.6%) and 250 days after calving (4.9 vs. 8.8%). Feeding complexed trace minerals during the late dry period and during early lactation tended to improve claw lesion scores and thus was associated with improved claw health and integrity (Ballantine *et al*., 2002). It seems that zinc and other trace minerals have greater effect through organic compounds. But it has to be mentioned that none of these research projects studied zinc content of hoof in the lower dead part of foot. They added zinc to the diet and observed improvement in hoof quality. It means that zinc can have improved integrity of hoof through taking part in enzyme systems and keratinization in the living part, not necessarily getting involved in structure of hoof.

Most researchers have mentioned catalytic role of zinc and a few have talked about structural function of it. Those who claim that Zn strengthens the keratin structure believe zinc finger compounds are responsible for it. It is supposed that cysteine residues can display high affinity toward zinc ions (Zn^{2+}) and resulting Zn^{2+}-cysteine complexes (Pace, 2014). But it looks unlikely that these weak non-covalent bonds can have important effect on integrity of hoof. If we accept that adding zinc to the diet increases hoof quality but its presence has no effect in lower dead part of hoof then we are led to the result that this microelement must impact hoof integrity in hematology and in structure of enzymes. Participating in structure of some enzymes like super oxidase dismutase, RNA nucleotide transferase, RNA polymerase, alkaline phosphatase, carboxypeptidase, alcohol dehydrogenase and carbonic anhydrase, zinc affects keratinization in upper living part of hoof (Cousins, 1996). These catalytic enzymes depend on zinc as an activator and thus an integral component in differentiation of keratinization.

Microelements like zinc, copper, manganese and cobalt have their effect on keratinization in the living part of hoof. A very good example can be thiol oxidase enzyme that is very important in keratinization process. Thiol oxidase enzyme is responsible for formation of the disulfide bonds between Cysteine residues of keratin filaments (O'Dell, 1990). Obviously enzymes like this only can be functional in upper bloody part of hoof and cannot have any role in lower dead part. So, it is concluded that zinc doesn't have a determining effect in structure of hoof horn. It is likely that the trace mineral improves keratinization and helps to produce a more qualified keratin.

A very important point that must be considered in future studies is the fact that researchers must differentiate the issue of hoof growth rate and integrity. As Ott and Jonhson (2001) stated in their study supplementing the animal diet with zinc can increase the growth rate of hoof but not necessarily its integrity. Hoof integrity is probably affected widely by race of the animal. Ott and Johnson (2001) found that Quarter horses had more breaking strength in their hoofs in comparison with Thorobred. It looks that more researches regarding to role of zinc and copper in animal blood will reveal interesting new facts. In addition, most of the former studies have been done on cows and research studies in equines like horse and donkey can uncover new information about the role of microelements in these animals.

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References

Axelsson, M., Holm, S., Nilsson, S. (1989). Flow dynamics of the crocodilian heart. *The American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, 256, R875–R879.

Baggot, D.G., Bunch, K.J., Gill, K.R. (1988). Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. *British Veterinary journal*, 144, 234-542.

Ballantine, H.T., Socha, M.T., Tomlinson, D.J., Johnson, A.B., Fielding, A.S., Shearer, J.K., Van Amstel, S.R. (2002). Effect of feeding completed zinc, Manganese, copper and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction and lactation performance. *The Professional Animal Scientist*, 18, 211-218.

Brazle, F.K., Lusby, K.S., Simms, S.H. (1993). The effect of zinc methionine in a mineral mixture on gain and incidences of footrot on steers grazing native grass pastures. *The Professional Animal Scientist*, 10, 169-171.

Butler, K.D., Hintz, H.F. (1977). Effects of level of feed intake and gelatin supplementation on growth and quality of hoofs of ponies. *Journal of Animal Science*, 44, 257-261.

Coenen, M., Spitzlei S. (1997). The composition of equine hoof horn with regard to its quality (hardness) and nutrient supply of horses. *Proc. 15th Equine Nutrition. Physiology. Symposium*, 209-212.

Cousins, R.J. (1996). Zinc. *Pages 293-306 in Present Knowledge in Nutrtion. 7th ed. E.E. Ziergler and L. J. Filer, Jr., ed. ILSI Press Washington, DC.*

Cross, R.F., Parker, C.F. (1981). Oral administration of zinc sulfate for control of ovine foot rot. *Journal of the American Veterinary Medical Association*,178, 704-705.

Demertizis, P.N. (1973). Oral zinc therapy in the control of infectious pododermatitis in young bulls. *Veterinary Rec.*, 93, 219-222.

Hepworth, K.M., Neary, M., Kenyon, S. (2004). Kenyon. Hoof Anatomy, Care and Management in Livestock. *Purdue Univesrity Cooperative Extension Service, West Lafayette, IN 47907* 1-9.

Huntington, P., Pollitt, H. (2005). Nutrition and the Equine Foot. *Kentucky Equine Research Australia and Australian Equine Laminitis Research, Queendsland, Australia*, 23-36.

Moor, C.L., Walker, P.M., Winter, J.R., Jones, M.A., Webb, J.M. (1989). Zinc methionine supplementation for dairy cows. *Transactions of the Illinois State Academy of Science,* 82, 99-108.

Mulling, C. (2000). The use of nutritional factors in prevention of claw diseases-Biotin as an example for nutritional influences on formation and quality of hoof horn.*11th international Symposium on Disorders of the Ruminant Digit. C. M. Mortellaro, L, De Vecchis, and A. Brizzi, eds. Parma, Italy,* Pages 78-80.

Mulling, C., Budras, K.D. (1998). Influence of environmental factors on horn quality of the bovine hoof. *In: Lischer CJ Ed., Proc 1th Int. Symp. on Lamness in Ruminant.*

Mulling, K.W.C., Bragulla, H.H., Rees, S., Budras, K.D., Seinberg, W. (1999). How structure in bovine hoof epidermis are influenced by nutritional factors. *Anatomia Histologia Embryologia,* 28, 103-108.

Nocek, J.E., Johnson, A.B., Soch, M.T. (2000). Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. *Journal of Dairy Science,* 83, 1553–1572.

O Dell, B.L. (1990). Copper. *Pages 261-26 in Present Knowledge in Nutrition. 6th ed. M. L. Brown, ed. ILSI Press, Washington, DC.*

Ott, E.A., Johnson, E.L. (2001). Nutritional factors influencing hoofdevelopment in horses. *In: Proc. Bluegrass Laminitis Symposium.*

Ott, E.A., Johnson, E.L. (2002). Nutritional factors influencing hoof development in horses. *In: Proc. Bluegrass Laminitis Symposium.*

Pace, N.J., Weerapana, E. (2014). Zinc-binding cysteines: diverse functions and structural motifs. *Biomolecules*, 17, 419-434.

Sargentini, C., Tocci, R., Andrenelli, L.,Giorgetti, A. (2012). Preliminary studies on hoof chracteristics in Amiata donkey. *Italian Journal of Animal Science,* 11, 122-127.

Sicilliano-Jones, J.L., Soch, M.T., Tomlinson, D.J., DeFrain, J.M. (2008). Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. *Journal of Dairy Science,* 91, 1985-1995.

Tocci, R., Sargentini, C., Martini, A., Giorgetti, A. (2015). Qualitative hoof characteristics in Anglo-Arabian Horses and Monterufoli Ponies reared in the same farm. *Global Journal of Animal Scientific Research,* 3, 20-29.