Is level of trace minerals important for healthy hoof in dairy cows?

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Abstract: The aim of this study was to investigate the some trace mineral values on hoof in cows with hoof disease and comparison with healthy ones. Twenty-three Holstein dairy cows (hoof disease group (HD, 18 cases); control group (C, 5 cases) were studied. Determinations of Pb, Fe, Ni, Zn, Cd, Cu and Mn in the samples were carried out by inductively coupled plasma optical emission spectroscopy. Lead, Fe, Ni, Cd, Cu and Mn values were higher in group HD than group C, but just Pb, Ni, Cd, and Cu values were statistically significant. Zn value was lower significantly in group HD than group C. Sole ulcer (7 cases, 39%) was the most prominent hoof disease in group HD. In conclusion, we found that cattle which has various hoof diseases have lower level of Zn and higher levels of Pb, Ni, Cd, and Cu than the control group. Keeping in mind of trace mineral values would be useful for protection and treatment to hoof diseases in dairy cows.

Keywords: cow, hoof, trace mineral

Süt ineklerinde tırnaklardaki iz mineral düzeyleri tırnağın sağlığı için önemli midir?

Özet: Bu çalışmanın amacı, tırnak hastalığı bulunan ineklerin tırnaklarındaki iz mineral düzeylerini belirlemek ve sağlıklı olanların değerleri ile karşılaştırmaktır. Çalışmada; 23 inek (18 tane tırnak hastalıklı, 5 tane sağlıklı) kullanıldı. Örneklerde Pb, Fe, Ni, Zn, Cd, Cu ve Mn düzeyleri hızlandırıcı eşleştirmeli plazma optik emisyon spektroskopi ile belirlendi. Kurşun, Fe, Ni, Zn, Cd, Cu ve Mn değerleri tırnak hastalıklı grupta kontrol grubundan yüksekti ama sadece Pb, Ni, Cd ve Cu değerleri istatistiki olarak önemliydi. Çinko değeri, tırnak hastalıklı grupta, kontrol grubundan istatistiki olarak düşük bulunmuştur. Taban ülseri (7 olgu, %35) tırnak hastalıklı grupta en fazla karşılaşılan hastalıktı. Sonuç olarak değişik tırnak hastalıkları bulunan ineklerin tırnaklarında sağlıklı olanlara göre düşük Zn; yüksek Pb, Ni, Cd ve Cu konsantrasyonu bulunduğu belirlenmiştir. İz element değerlerinin, süt ineklerinde tırnak hastalıklarından korunma ve sağaltımda göz önünde tutulması yararlı olacaktır.

Anahtar Kelimeler: inek, tırnak, iz mineral

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INTRODUCTION

Some trace minerals (TM) are very important in maintenance of hoof epithelial and connective tissues. Copper (Cu), zinc (Zn), and manganese (Mn) have received the most attention by investigators. These TMs have important roles in protein synthesis, vitamin metabolism, formation of connective tissue, and immune function (1,2). During the process of keratinization, epidermal cells rely upon the dermal layers for the supply of nutrients, macro and trace minerals, and vitamins. This supply must be provided entirely via diffusion from blood vessels in the underlying dermis because the epidermis is an avascular tissue (3,4). Hendry et al. 5 reported that little is known about the control mechanism for nutrient flow and rate of keratinization.

Zinc has been identified as a key mineral in the processes of keratinization (4,6). Copper is needed for activation of the thiol oxidase. Of greatest importance in the keratinizing horn cell is the activity of this enzyme (4,7). Manganese helps minimize feet problems by maintaining proper leg formation (1,4). Additionally, iron (Fe), Nickel (Ni), Cadmium (Cd), and Lead (Pb) also plays important role of some enzymes activation which is important role of keratinization (1,8).

The level of serum trace minerals concentration and effect of trace mineral source in farm animals have been studied several times and represented in detail (8-10). However, trace minerals status in hoof has been reported in a few studies (11) and according to our acknowledgement no study reported comparison to status of trace minerals between healthy and diseased hoof of cattle. The aim of this study was to investigate and comparison the some trace mineral values on hoof in healthy cows and with hoof disorders.

MATERIAL and METHODS

Experimental design and animals

Twenty-three Holstein dairy cows (18 with hoof disorders, group HD; 5 control, group C) were studied in a private dairy farm. Calving number, calving time, lactation number, and lactation stage were detected from farm recording system. The dairy herd was housed in naturally ventilated open shade straw bedded barns. Requirements of cows were calculated from the average weight and milk yield according to the National Research Council (NRC) requirements (12). The average feed composition is listed in Table 1. They were walked about 150 meters toward the milking house three times a day. Their hooves had not been trimmed till a few years. All of the cows were trimmed and checked for lameness score and hoof disease such as solea ulcer, heel erosion, white line disease etc.

Table 1. The composition of ration used in the study (as fed)

Ingredients	Daily quantity, kg / cow
Wheat straw	6.00
Alfalfa hay	4.00
Barley	4.00
Cottonseed meal	2.00
Wheat bran	1.00
Sugar beet pulp	15.00
Limestone	0.10
Salt	0.04
Vitamin-mineral premix*	0.03
Total	32.17
Composition (DM)**	
DM, %	53.19
CP, %	13.43
ME, Mcal/kg	2.32
NEL, Mcal/kg	1.44
NDF, %	50.00
ADF, %	31.00

DM: Dry Matter, CP: Crude Protein, ME: Metabolizable Energy, NEL: Net Energy Lactation, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber

*Provided per 1 kg of premix: Vit. A 15000000 IU, Vit. D3 3000000 IU, Vit. E 30000 mg, Mn 50000 mg, Zn 50000 mg, Fe 50000 mg, Cu 10000 mg, I 800 mg, Co 150 mg, Se 150 mg

** NRC (2001)

Trace minerals analyses

Hoof pieces weighing 300-400 mg were taken once for determinate of trace minerals. Examples were collected from solea ungulae and paries ungulae on the hoof. Hoof pieces of cows were clipped, scraped and then rinsed with distilled water, 99.5 % acetone, 96% ethanol and distilled water, separately (13). Then, the pieces were dried at 60^0C. Dried samples were homogenized using on agate homogenizer and stored in pre-cleaned polyethylene bottles until the analysis started. All the plastic and glassware were cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water. Each sample (0.5 g) was then digested with 10 mL of HNO_3 (65%) using a CEM-MARS 5 (CEM Corporation Mathews, NC, USA) microwave digestion system (maximum power: 1200 W, power: 100%, ramp: 25:00 min, pressure: 170 psi, temperature: 210^{o}C and hold time: 10:00 min). After digestion, the volume of each sample was adjusted to 25 ml using double deionized water. Determinations of Pb, Fe, Ni, Zn, Cd, Cu and Mn in the samples were carried out by inductively coupled plasma optical emission spectroscopy (Varian-Liberty II, ICP-OES).

Statistical analyses

The data were analyzed by SPSS (version 15.0) utilizing Spearman's correlation and Mann-Whitney U tests. Study was case control type research.

RESULTS

The average age of dairy cows was 5,0 (4,5-6,5) years. Cows were multiparous. Cows were in the middle of lactation in the study. There were no significant differences between the groups for age, calving number, calving time, lactation number, and lactation stage (data not shown).

Because the previous trimmings were not performed before, excessive overgrowth was present on claws. Lameness and all hoof lesions occurred at a higher frequency in hind than in front and analyses were performed only in hind hoof. The lameness scores ranged from 3 (lame) to 5 (extremely lame) in group HD. Lameness increased with age ($P<0.05$). The effect of lactation number on these traits was apparent ($P<0.05$). Sole ulcer (7 cases, 39%) was the most prominent hoof disease in group HD (Figure 1 and 2). There was no hoof disease and lameness in group C.

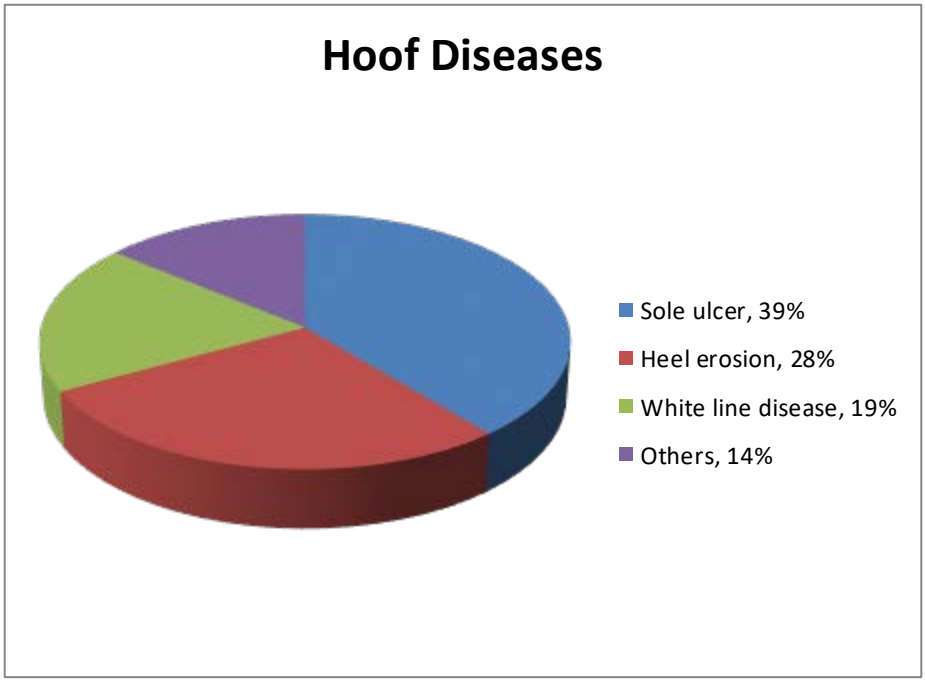

Figure 1. Distribution of hoof diseases in group HD

Figure 2. View of ulcus solea on a hoof

Lead, Ni, Cd, and Cu values were significantly higher in the group HD than group C. Nevertheless Zn value was significantly lower in the group HD than group C (Table 2). The pieces had measurable concentrations of trace minerals. Mann-Whitney U test showed highly significant differences in hoof contents of Pb (P<0.05), Ni (P<0.01), Zn (P<0,01), Cd (P<0,01), and Cu (P<0.05) among the groups.

Table 2. Distribution of some trace minerals in the hoof pieces*

Group	N	Pb	Fe	Ni	Zn	Cd	Cu	Mn
Group HD	18	7.38±2.16	0.60±0.11	0.47±0.14	0.11±0.02	0.29±0.05	0.58±0.05	0.38±0.07
Group C	5	0.10±0.01	0.50±0.11	0.03±0.01	1.35±0.17	0.01±0.00	0.19±0.10	0.28±0.25
P		< 0.05	> 0.05	< 0.01	< 0.01	< 0.01	< 0.05	> 0.05

*Part per million, HD: hoof disease group, C: control group.

Spearman's correlation coefficient showed significant positive relationships between hoof Pb and Zn, and Cu concentrations (P<0.05 r = 0.947; P<0.05 r = 0.947) respectively, Fe and Mn values (P<0.05 r = 0.900), Ni and Mn levels (P<0.05 r = 0.894), and Zn and Cu concentrations (P<0.01 r = 1.000) in group C (Table 3). There was no significant correlation between trace mineral values in group HD (Table 4).

Table 3. Correlation coefficient between parameters in group C

Parameter	Hoof Pb	Hoof Fe	Hoof Ni	Hoof Zn	Hoof Cd	Hoof Cu	Hoof Mn
Hoof Pb	1						
Hoof Fe	0.205	1					
Hoof Ni	0.344	0.783	1				
Hoof Zn	0.947*	0.359	0.459	1			
Hoof Cd	0.000	0.354	0.395	-0.181	1		
Hoof Cu	0.947*	0.359	0.459	1.000**	-0.181	1	
Hoof Mn	0.564	0.900*	0.894*	0.667	0.354	0.667	1

*: P<0.05; **: P<0.01

Table 4. Correlation coefficient between parameters in group HD

Parameter	Hoof Pb	Hoof Fe	Hoof Ni	Hoof Zn	Hoof Cd	Hoof Cu	Hoof Mn
Hoof Pb	1						
Hoof Fe	0.086	1					
Hoof Ni	0.015	-0.127	1				
Hoof Zn	-0.411	0.068	0.168	1			
Hoof Cd	0.133	0.104	-0.033	0.029	1		
Hoof Cu	-0.272	-0.193	-0.095	0.106	0.099	1	
Hoof Mn	-0.046	0.262	-0.066	-0.365	0.247	-0.173	1

DISCUSSION

Kılıç et al., (14) reported that the mean concentration of Zn in serum of lame cattle were significantly lower than control group; hence a potential interaction between lameness and serum Zn value in dairy cattle was proposed. Conversely, it was demonstrated that serum Zn and Cu concentrations of healthy, mild, moderate and severe lame cows were not significantly different (11,15). Zn value in hoof pieces was significantly lower in group HD than group C. This factor may come into play and increase these animals visibility to hoof disorders. Kellogg (9) reported that dietary ZM (zinc methionine) improved hoof quality of cows, compared with controls. In current study, hoof Cu level was significantly higher in group HD than group C.

Cases with hoof diseases had significantly lower value of Zn compared with group C. Some hypotheses may undertake with these results, displaying the need for more research to be done: (1) Hoof disease directly decreases Zn level in the hoof by increasing unquality

production; (2) as inadequate feeding as a symptom of the hoof disease, this may affect nutrients intake including Zn; and (3) these patients had some severe Zn deficiency before being affected by hoof disease and this deficiency was the likely cause of their hoof disorders.

Very few studies have investigated the actual biological values of hoof Zn and Cu (4,10,11). Based on the values presented in Table 2 and 3, a positive correlation (0.106-1.000) was determined between Zn and Cu concentrations. Contrary to this study, Sadeghi-nasab et al., (11) reported that a negative correlation between Zn and Cu concentrations ($P<0.05$ $r = -0.281$). In a study has been reported that, cows given zinc methionine produced more milk with lower somatic cell counts than cows given zinc oxide and methionine (9). Some articles reported that the harder keratin of the hoof wall contained a greater Zn concentration than the softer keratin of the heel. It is found that concentration of Zn in the hoof horn of lame cattle were lower than no lameness animals (4,10,11). In reported study, Zn values of hoof pieces were lower than another studies with 117 ± 11 μg/g and 71.03 ± 13.78 μg/g, respectively, in group HD (4,11). Hoof pieces were also softer, which is determined by palpation, in group HD than group C.

It is reported that Zn and Cu serum levels were decreased significantly in dogs with dermatologic disease. Acanthosis and parakeratosis were thought to be related to decreased Zn level, and hyperplasia to decreased Cu level (16). It is adviced that keratinized tissue such as wool and feathers can be used for determine to level of Zn (17). It is noticed that the concentrations of trace minerals at feathers and hair use by clinical researchers to point out mineral profile of body to indicate alimentare deficiencies (18). Zn and Cu levels in hoof which is also keratinized tissue were determined in this study. Cows were not any alimentare deficiency.

Sadeghi-nasab et al. (11) reported that significant negative correlation was observed between locomotion scores with hoof Cu concentration ($P<0.05$ $r = -0.323$). In group HD, cattle showed significant higher hoof Cu level ($P<0.05$) than in group C. These results are not concordant with previous studies (4,10,11). This finding can be explained by hypotheses among which review here: Metabolic disorder in hoof may result increasing Cu level at hoof epithelial cells.

The connective tissue that suspends the distal phalanx within the hoof capsule is strengthened by the Cu dependent enzyme lysyl oxidase, which forms the cross linkages between collagen fibers (2,6). Overloading the suspensory connective tissue of the distal phalanx compresses the corium, resulting in the development of hoof diseases such as white line separation, sole hemorrhages, and sole ulcers.

Manganese appears to have a lesser role in maintaining hoof integrity. Manganese plays an indirect role in the keratinization process. The Mn-dependent enzymes, galacto transferase and glycosyl transferase, are required for the formation of proteoglycans (1,2,6). In this study, Mn level in group HD and group C were 0.38 ± 0.07 ppm and 0.28 ± 0.25 ppm, respectively. These values were not demonstrated significant differentiation ($P>0.05$).

Cases with hoof diseases had demonstrated significant higher value of Pb, Ni, Cd, and Cu but no Fe and Mn. This can be discussed by different hypotheses: Hoof disorders and irritated corium may be result increased trace mineral levels in epithelial cells. Increased Pb, Ni, Cd and Cu levels can be cause softer horn production.

The results of this study are compatible with previous findings asserting that hoof Zn value in the hoof of cattle with lameness is much lower compared with healthiest. Zinc value in hoof samples of group HD was significantly lower than the group C (Table 2). Nevertheless, this

study as a pilot study was planned just to maintain an overall impression about the some trace minerals values among the above-mentioned groups an object accomplished. Comprehensive investigations with samples representative for the general populations are needed to acquire a more diverse effecting.

In conclusion, we found that cattle which has various hoof disease have lower level of Zn and higher levels of Pb, Ni, Cd, and Cu than the control group. Keeping in mind of trace mineral values would be useful for protection and treatment to hoof diseases in cows. More comprehensive and meticulously designed studies are required to research the efficacy on hoof integrity these minerals and to evaluate any possible effect of Zn supplements on the occurrence and treatment of hoof disease.

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