



## The Relationship Between the Histological Quality of the Newly Formed Hoof Tissue and the Levels of Trace Elements in Blood Serum and Hoof Tissues During the Recovery Period of Some Hoof Diseases in Dairy Cows

İbrahim Akın\*, Osman Sacit Görgül, Murat Sarierler

Adnan Menderes University Veterinary Faculty, Department of Surgery, 09100, Isikli/Aydin, Turkey, TR.

### ABSTRACT

**Background:** Lameness is considered in most important problems in dairy industry and caused to poor animal welfare and economic loses. White line disease, solea ulcer and heel erosion are common foot diseases among the dairy cows. **Aim:** The present study aimed to determine the levels of some trace elements in the blood serum and liver and hoof tissues in healthy dairy cows as well as in the blood serum and hoof tissues of those being in the diagnostic and recovery period of sole ulcer, heel erosion and white line diseases. The changes of hoof tissues during healing processe were also evaluated histopathologically. The treatment groups (sole ulcer, heel erosion and white line diseases) were consisted of 18 Holstein dairy cows. The blood and hoof tissue samples were taken at the beginning of the treatments (day 0 and 15<sup>th</sup>, 30<sup>th</sup>, and 45<sup>th</sup> days of the treatments). Meanwhile, blood samples were collected before slaughtering procedure, and hoof and liver tissues were collected after slaughtering procedure from the six clinically healthy Holstein dairy cows (control group) in a slaughterhouse. **Result and Conclusion:** The mean concentrations of zinc, copper, iron and manganese in the blood serum of healthy animals were 28.1±3.3 µg/dl, 36.6±4.8 µg/dl, 31.8±3.5 µg/dl, and 2.6±0.1 µg/dl, respectively. The levels of these trace elements in the liver were also 205±12 ppm, 27±2 ppm, 541±274 ppm, and 2±1 ppm, respectively. Significant differences in the mean concentrations of zinc, copper, iron and manganese between control animals and treatment groups were found at the beginning (day 0) and the 15th, 30th and 45th days of treatments. Furthermore, histopathological evaluations suggested that hoof tissue samples had increased cellularity and decreased keratinization during the treatment period. It was concluded that, the hoof diseases caused significant changes in the levels of trace elements in hoof tissues, and observed high levels of some trace elements would not inevitable indicate the healthy hoof tissue in cows. Besides, although animals are seen as clinically healed, the histological quality of the hoof tissue might be far below the expected levels.

*Keywords: histopathology, cow, hoof, sole ulcer, white line diseases, heel erosion, trace minerals*

## Süt Sığırlarında Bazı Tırnak Hastalıklarının İyileşme Sürecinde Kan Serum ve Tırnak Dokusu İz Element Düzeyleri ile Yeni Oluşan Tırnak Dokusunun Histolojik Kalitesi Arasındaki İlişki

### ÖZET

**Öz bilgi:** Topallık süt sığırcılığı endüstrisinde en önemli problemler arasında değerlendirilir; hayvan refahında düşmeye ve ekonomik kayıplara yol açar. Beyaz çizgi hastalığı, solea ülseri ve ökçe erozyonu süt sığırlarında sık karşılaşılan ayak hastalıklarındandır. **Amaç:** Sunulan çalışmada; sağlıklı hayvanların kan serumu, karaciğer dokusu ve tırnağın taban, ökçe ve beyaz çizgi bölgelerinde bulunan bazı iz element düzeyleri ve taban ülseri, ökçe erozyonu ve beyaz çizgi hastalıklarının tanı ve iyileşme süreçlerinde kan serumu ve tırnak dokusu iz element düzeyleri ile bu süreçte tırnak dokusundaki değişimlerin histopatolojik olarak ortaya konulması amaçlanmıştır. Tedavi gruplarını (taban ülseri, ökçe erozyonu ve beyaz çizgi hastalığı) oluşturan, toplam 18 baş Holstein ırkı sığırdan, tedavi öncesinde (0. gün) ve tedavi sürecinde (15, 30 ve 45. günler) kan ve lezyonlu bölgeden tırnak örnekleri, aynı zamanda kontrol grubunu oluşturmak amacı ile de mezbahada kesilen 6 baş sağlıklı Holstein ırkı sığırdan kesim öncesi kan, kesim sonrası tırnak ve karaciğer doku örnekleri alınmıştır. **Bulgular ve Sonuç:** Sağlıklı sığırlarda serum çinko 28,1±3,3 µg/dl, bakır 36,6±4,8 µg/dl, demir 31,8±3,5 µg/dl, manganez 2,6±0,1 µg/dl olarak; karaciğer çinko 205±12 ppm, bakır 27±2 ppm, demir 541±274 ppm, manganez 2±1 ppm olarak belirlenmiştir. Hastalık grupları kan ve tırnak dokusu çinko, bakır, demir ve manganezin 0, 15, 30 ve 45. gün düzeyleri ile kontrol grubu düzeyleri arasında istatistiki olarak önemli değişimler saptanmıştır. Histopatolojik değerlendirmelerde tırnak dokusundan alınan örneklerde iyileşme sürecine paralel olarak selüleritenin arttığı, keratinizasyonun ise azaldığı belirlenmiştir. Sonuç olarak, sığırlarda ayak hastalıklarına ilgili olarak tırnak dokusu iz element düzeylerinde önemli değişimler meydana geldiği, bazı iz element düzeylerinin yüksek bulunmasının tırnak dokusunun sağlıklı olduğuna işaret etmediği, klinik olarak iyileştğine karar verilen tırnakların henüz yeterli histolojik kaliteye ulaşmadığı kanısına varılmıştır.

*Anahtar kelimeler: histopatoloji, sığır, tırnak, solea ülseri, beyaz çizgi hastalığı, ökçe erozyonu, iz elementler.*

**Correspondence to:** İbrahim AKIN, Adnan Menderes University Veterinary Faculty, Department of Surgery, 09100, Isikli/Aydin, Turkey. E-mail : ibraak@adu.edu.tr, ibraak@hotmail.com

## Introduction

Lameness is considered in the most important problems in dairy industry and caused to poor animal welfare and economic loses. White line disease (WLD), solea ulcer (SU) and heel erosion (HE) are common foot diseases among the dairy cows (Sogstad et al., 2011; Huxley 2012). Although, there are studies on etiology, classification and treatment of foot diseases (Harris et al., 1988; Hedges et al., 2001; Nocek et al., 2006, Akin et al., 2013), today it is still being counted among the most economically harmful diseases and still protects its severity in contemporary world.

Trace minerals play role in quality of epidermal structure and physiological keratinisation; these two events have an effect on hoof health and quality (Akin 2004). The relationship between trace minerals and hoof health is examined in two subjects: First, trace minerals for quality of hoof horn (in production, keratinisation); this is examined in three titles: intracellular

collected at the days zeroth (0, beginning day of the treatment), 15<sup>th</sup>, 30<sup>th</sup>, and 45<sup>th</sup> of the treatments from the healing hooves of treatment groups. The time of treatment was determined as 45 days for treatment groups. If the treatment period exceeded 45 days, the cow was excluded from the treatment group. All lesions of the treatments group cows were bandaged at day 0, and changed and clinically inspected weekly. Treatment group cows were separated in a different pen with free-stall barns to supply more dry area. Feeding method and ration were not changed throughout the recovery period. Hay was provided ad libitum and fed a concentrate ration (7-9kg per cow) and conserved forage (grass and maize silage, 15-20kg per cow). There was no regular hoof-trimming programme. The cows were milked two times daily in milking parlours. Serum Zn, Cu and Fe analyses and hoof Zn, Fe analyses and liver Zn and Fe analyses were done with flame atomic absorption spectrophotometry (Perkin Elmer® Model AAS 700). Serum hoof and liver Mn, hoof and liver Cu analyses were done with graphite furnace atomic absorption spectrophotometry (Perkin

**Table 1:** Criteria for histopathological examinations.

Cellularity	Degre
All area comprised from keratin	no cellularity (0)
Occasional cells are eligibile in one or two area	weak (+)
All areas 3-5 cell morphology is eligibile	middle (++)
More than 5 cell morphology are eligibile in all areas	advanced (+++)
Keratinization	
Keratinization and cell morphology can eligibile	weak (+)
Keratinization and occasionally cell morphology can differantiate	middle (++)
All areas comprised from keratin tissue	advanced (+++)
Staining of tissue	
All areas staining grayish pink	weak (+)
Grayish colour with homogen eosinophilic areas	middle (++)
Normal eosinophilic staining	advanced (+++)

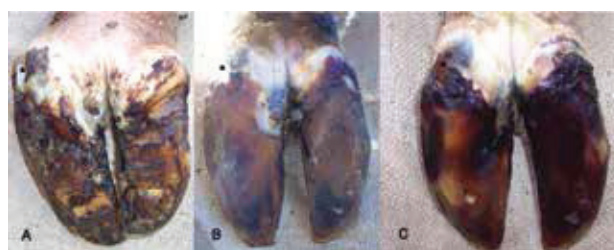
factors, extracellular factors and architecture. Second, trace mineral requirement for wound healing, amino acids and protein production, nutrient and oxygen requirement, and stabilisation of cellular walls. The healthy hoof horn, which consists of cornified dead epidermal cells and design-structure resembles of a brick wall. Epidermal cells in the healthy hoof horn are connected by an intercellular cementing substance, which resembles cement mortar (Lischer et al., 1998; Mulling et al., 1999; Tomlinson et al., 2004).

There are studies about the relation between the general animal health, hoof health and trace minerals, which is added into the ration of dairy cattle (Weaver 1987; Reiling et al., 1992; McDowell 1992; Spain et al., 1993; Uchiada et al., 2001; Nocek et al., 2000; Ballantine et al., 2002; Kincaid et al., 2003; Nocek et al., 2006; Sciliana-Jones et al., 2008). This study was conducted in Bursa, TR; and aimed to research the variation of the levels of copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) during the healing period of dairy cattle's solea ulcer, heel erosion, white line disease, and histopathologic quality of newly constructed hoof tissue in mentioned foot diseases.

## Materials and Method

A control [clinically healthy Holstein dairy cows (n:6)] group and three treatment groups [solea ulcer group (SU, n:6), heel erosion group (HE, n:6), white line disease group (WLD, n:6)] were composed by Holstein dairy cows, similar ages (4-6) and weights (500-600 kg). Blood samples were collected before slaughtering procedure, and hoof and liver tissues were collected after slaughtering procedure from the control cows in a slaughterhouse. The blood and hoof tissue samples were

Elmer® Model AAS 700). Histopathological examinations were evaluated, based on the cellularity, keratinisation and staining of tissue in all collected tissues from hooves (Figure 1\*). The benchmarks of histopathological examinations are shown in the Table 1. All statistical analyzes were performed using SPSS 11.5 software package by the computer.



**Figure 1.** Different stages of treatment from heel erosion (A- Day 0, B- day 30, C- day 45).

Şekil 1. Ökçe erozyon tedavisindeki dönemler (A-gün 0, B-gün 30, C-gün 45).

## Results

Results are presented in three subtitles: Clinical, biochemical-labaratuary and, histopathological findings.

### Clinical findings

Clinical findings of treatment cows were evaluated in three groups [SU (n:6), HE (n:6) and WLD (n:6)], and weekly during 45 days. All lesions were on the lateral hoof of the hind limb.

**Table 2.** The week after treatment when all cows showed clinical signs of epitelization, keratinization, no lesion pain and lameness score 0.

Clinical sign	SU	HE	WLD
Epitelization	3 <sup>rd</sup> week	-	3 <sup>rd</sup> week
Keratinization	4 <sup>th</sup> week	-	5 <sup>th</sup> week
No lesion pain	5 <sup>th</sup> week	4 <sup>th</sup> week	6 <sup>th</sup> week
Lameness score 0	-	3 <sup>rd</sup> week	6 <sup>th</sup> week

The clinical signs (epitelization, keratinization, lesion pain and lameness score) are presented in Table 2.

Sole ulcer group (SU); Lameness did not evaluate in this group because of applied easy block (easy bloc<sup>®</sup>Demotec). Newly formed hoof tissues could be seen in carefully inspection at the edges of sole ulcer lesions at second week controls. At fourth week, all lesions covered with keratinised hoof tissue. There were no pain and sensibility at direct palpation, but in three cases there were sensibility at indirect palpation. In fifth week, all ulser lesions covered horny hoof, but had not enough thickness. Easy blocks were taken off at sixth week. There is no lameness, pain or sensibility in all cases. In this condition all cases evaluated as clinically healed.

Heel erosion group (HE); On the day 0, cracks on the heel of lame foot and digital dermatitis lesions in all cases (Fig. 1a) and severe pain in indirect palpation of heel region. In second week controls, all cases had mild lameness, cracks on the heel began to disappear, and digital dermatitis lesion in 5 cows healed. At the 3<sup>th</sup> week lameness nearly recovery in 5 cows and heel cracks' level could not be notised easily. Sensibility of the heel region disappeared in palpations, and cracks were almost closed in fourth week (Fig. 1b). A week later, there were no local lesions, sensibility and lameness, and animals were evaluated as clinically healed. Although they were accepted as healed, requirement of the hoof samples from that regions at the sixth week-45th day (Figure 1c), foot were bandaged against to contamination.

White line group (WLD); Five cows had typical separations on the white line and one had not, but it had pain in the percussion and indirect palpation of hoof. In this case's lesion was revealed after the hoof trimming. There was severe pain while indirect palpation of all cases at the beginning. In second week, slightly cornification could be seen in carefully inspection at the edge's of lesion but not on the surface of the entire lesions. It was noticed in the third week that cornified hoof tissue began to move towards the center of the lesions, from the edges of the lesions. Two cows were still slightly lame at the fifth week but significant horny tissue could be seen on the lesions. In the sixth week, keratinised hoof tissue covered all lesions in cases and no pain on indirect palpation and all cases could walk normally. All cases evaluated as clinically healed.

The zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) levels in hoof regions of control group are shown in Table 3. In healthy hooves Zinc levels were significantly different ( $P<0.001$ ) across the hoof regions (Sole, Heel and White line).

During the healing period, Zn, Cu, Fe and Mn levels of the healing hooves from treatment groups are offered in Table 4. Significances were found on Fe levels ( $P<0.05$ ) and Mn levels ( $P<0.001$ ) of sole ulcer group, Zn and Mn levels ( $P<0.05$ ) of heel erosion group.

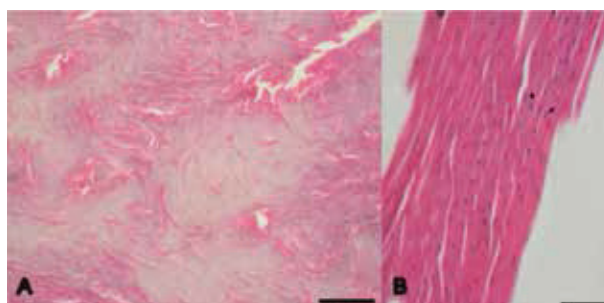
Comparison of trace mineral levels of blood and hoof tissues between the control and treatment groups during recovery period, and significant differences between the hoof and blood

samples of the control and treatment groups are presented in Table 5. Zn Hoof levels (ppm) were significantly higher ( $P<0.001$ ) than healthy hooves. Mn blood levels (ppm) were significantly lower in cows with healing hooves than in cows with healthy hooves. Mn hoof levels (ppm) were significantly higher in cows with healing hooves than healthy hooves.

#### Histopathological Findings

According to histopathological findings, there were no histopathologically significant differences among heel, solea and white line tissues. Marked differences were observed in (recovery period between) the hoof tissue's cellularity, keratinisation and staining of tissues at 15, 30 and 45th days than beginning day (Table 6).

On the beginning day (Day 0) cell morphology and cell borders were not determined. The hoof tissues included just keratinisation (Figure 2A). However on the 15<sup>th</sup> and 30<sup>th</sup> days of the study, keratinocytes with thin and long nucleus could be observed. On the 45<sup>th</sup> days of studies, keratinocytes morphology was recognizable. This process may be a sign of development of architectural structure of hoof horn (Figure 2B).



**Figure 2:** Solea, Day 0. Keratinocyte morphology could not found and all area stained with pale pink HE. Bar: 50 µm. (A); Solea, 45th.day. Keratinocytes are distinct (arrows) and eosinophilic staining. HE. Bar: 50 µm. (B)

**Şekil 2.** Solea, 0. gün. A. Soluk pembe boyalı sahalarda keratinosit morfolojisi görülmedi, Solea 0. gün. B. Keratinosit morfolojisi belirgin, Solea 45. gün. HE. Bar: 50 µm.

#### Discussion and Conclusion

Absorbtion and distribution of trace elements in animals can differ due to the breed, age, gender of the animal, physiologic condition and form (organic/inorganic) of the trace element in the feed (Kovacs and Szilagyi 1973a; Kovacs and Szilagyi 1973b; McDowel 1992; Reiling et al., 1992; Underwood 1977; Hedges et al., 2001; Ballantine et al., 2002; Kincaidet al., 2003; Tomlinson et al., 2004; Nocek et al., 2006; Sciliana Jones et al., 2008). While, it was the main criteria that the animals included the study group should not have lack of trace mineral (Kelly 1974; Underwood 1977; Blood et al., 1979; McDowel 1992), we paid as much as attention to constitute the groups from animals in the same breed (Holstein), closer ages (4-6)

**Table 3:** Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn) Levels in Hoof Regions in Control Cows:

Hoof region	n	Zn X±S <sub>x</sub>	Cu X±S <sub>x</sub>	Fe X±S <sub>x</sub>	Mn X±S <sub>x</sub>
Solea (ppm)	6	95±2 <sup>b</sup>	1,1±0,3	196±16	0,3±0,1
Heel (ppm)	6	75±9 <sup>c</sup>	1,0±0,2	224±17	0,5±0,1
White line (ppm)	6	131±3 <sup>a</sup>	1,1±0,3	155±23	0,3±0,1
P		***	NS	NS	NS

a, b, c: Different superscripts indicate statistically significant differences in the same column (\*\*\*: P<0.001, NS: Non-significant), (SPSS 11.5, Anova, Duncan's).

**Table 4:** Zn, Cu, Fe and Mn Levels of Healing Hooves of Treatment Groups During Recovery Period.

Treatment Groups	Elements	n	Day 0 X±S <sub>x</sub>	15 <sup>th</sup> day X±S <sub>x</sub>	30 <sup>th</sup> day X±S <sub>x</sub>	45 <sup>th</sup> day X±S <sub>x</sub>	P
Sole Ulcer (ppm)	Zn	6	267±26	286±16	247±19	272±11	NS
	Cu	6	3,4±0,6	6,2±2,6	4,2±0,1	5,0±0,1	NS
	Fe	6	265±66 <sup>a</sup>	136±12 <sup>b</sup>	137±3 <sup>b</sup>	141±5 <sup>b</sup>	*
	Mn	6	0,7±0,2 <sup>c</sup>	2,5±0,1 <sup>b</sup>	2,3±0,1 <sup>b</sup>	3,9±0,3 <sup>a</sup>	***
Heel Erosion (ppm)	Zn	6	163±8 <sup>b</sup>	201±15 <sup>a</sup>	0,231±12 <sup>a</sup>	212±5 <sup>a</sup>	*
	Cu	6	3,6±1,3	2,5±0,6	2,0±0,4	3,3±0,6	NS
	Fe	6	85±16	1552±1036	1328±573	164±29	NS
	Mn	6	1,7±0,5 <sup>b</sup>	3,2±0,3 <sup>a</sup>	1,5±0,2 <sup>b</sup>	1,4±0,4 <sup>b</sup>	*
White Line Disease (ppm)	Zn	6	241±21	275±8	277±1	280±4	NS
	Cu	6	9,0±2,6	12,3±2,7	10,0±4,3	18,2±3,8	NS
	Fe	6	223±26	2263±1919	477±236	527±77	NS
	Mn	6	3,3±1,2	5,9±1,0	3,2±0,8	4,4±0,6	NS

a, b, c: Different superscripts indicate statistically significant differences in the same line (\*: P<0.05, \*\*\*: P<0.001, NS: Non-significant), (SPSS 11.5, General linear model, Duncan's).

**Table 5:** Comparison of trace minerals between the control (n:6) and treatment groups (n:18) during recovery period

Treatment Groups	Sample	Trace element	Control (n:6) X±S <sub>x</sub>	0. day X±S <sub>x</sub>	15. day X±S <sub>x</sub>	30. day X±S <sub>x</sub>	45. day X±S <sub>x</sub>
Sole ulcer (n:6)	Hoof (ppm)	Zn	95±2	267±26 <sup>iii</sup>	286±16 <sup>iii</sup>	247±19 <sup>iii</sup>	272±11 <sup>iii</sup>
		Cu	1,1±0,3	3,4±0,6	6,2±2,6 <sup>i</sup>	4,2±0,1	5,0±0,1
		Fe	196±16	265±66	136±12	137±3	141±5
		Mn	0,3±0,1	0,7±0,2	2,5±0,1 <sup>iii</sup>	2,3±0,1 <sup>iii</sup>	3,9±0,3 <sup>iii</sup>
	Blood (µg/dl)	Zn	28,1±3,3	37,5±5,1	46,3±3,7 <sup>i</sup>	51,1±5,7 <sup>ii</sup>	42,8±2,4
		Cu	36,6±4,8	34,2±1,3	35,8±2,2	36,4±2,4	34,9±2,5
		Fe	31,8±3,5	31,6±7,3	27,4±6,5	42,2±17,2	33,4±9,0
		Mn	2,6±0,1	0,3±0,1 <sup>iii</sup>	0,2±0,1 <sup>iii</sup>	0,2±0,1 <sup>iii</sup>	0,2±0,1 <sup>iii</sup>
Heel erosion (n:6)	Hoof (ppm)	Zn	75±9	163±8 <sup>iii</sup>	201±15 <sup>iii</sup>	231±12 <sup>iii</sup>	212±5 <sup>iii</sup>
		Cu	1,0±0,2	3,6±1,3	2,5±0,6	2,0±0,4	3,3±0,6
		Fe	224±17	85±16	1552±1036	1328±573	164±29
		Mn	0,5±0,1	1,7±0,5	3,2±0,3 <sup>iii</sup>	1,5±0,2	1,4±0,4
	Blood (µg/dl)	Zn	28,1±3,3	47,5±4,9	44,3±7,3	45,1±6,2	43,1±4,2
		Cu	36,6±4,8	29,5±2,8	32,1±4,5	31,3±2,8	31,1±1,9
		Fe	31,8±3,5	35,5±8,1	39,4±5,2	49,8±12,0	35,2±7,6
		Mn	2,6±0,1	0,9±0,4 <sup>ii</sup>	1,1±0,4 <sup>i</sup>	0,6±0,2 <sup>iii</sup>	0,5±0,2 <sup>iii</sup>
White line diseases (n:6)	Hoof (ppm)	Zn	131±3	241±21 <sup>iii</sup>	275±8 <sup>iii</sup>	277±1 <sup>iii</sup>	280±4 <sup>iii</sup>
		Cu	1,1±0,3	9,0±2,6	12,3±2,7	10,0±4,3	18,2±3,8 <sup>ii</sup>
		Fe	155±23	223±26	2263±1919	477±236	527±77
		Mn	0,3±0,1	3,3±1,2	5,9±0,1 <sup>iii</sup>	3,2±0,8	4,4±0,6 <sup>i</sup>
	Blood (µg/dl)	Zn	28,1±3,3	56,8±4,0 <sup>ii</sup>	60,9±5,7 <sup>iii</sup>	50,5±5,1 <sup>i</sup>	56,4±6,1 <sup>ii</sup>
		Cu	36,6±4,8	24,0±1,7 <sup>i</sup>	26,0±1,1	26,5±1,9	24,8±1,3 <sup>i</sup>
		Fe	31,8±3,5	27,9±8,5	27,3±5,1	52,0±13,9	22,3±4,8
		Mn	2,6±0,1	0,4±0,1 <sup>iii</sup>	0,4±0,2 <sup>iii</sup>	1,2±0,5 <sup>ii</sup>	0,6±0,1 <sup>iii</sup>

Differences between the Control (n: 6) and Treatment groups (n:18) in recovery period. Significant, i: P<0.05, ii: P<0.01, iii: P<0.001 (SPSS 11.5, General linear model, Tukey).



**Table 6.** Histopathological evaluation of the healing hooves from the treatment groups during healing period.

Case no	Hoof Region (Treatment Group)	0 Day			15 <sup>th</sup> Day			30 <sup>th</sup> Day			45 <sup>th</sup> Day		
		Cel	Ker	Sta	Cel	Ker	Sta	Cel	Ker	Sta	Cel	Ker	Sta
1	HE	0	+++	+	+	++	+	+	+	++	++	+	++
	SU	+	+++	+	+	++	+	++	++	+++	+++	+	+++
	WLD	0	+++	+	+	++	+	++	++	++	+++	+	+++
2	HE	0	+++	+	+	++	+	++	+	++	+++	+	+++
	SU	0	+++	+	+	++	++	++	++	++	++	+	++
	WLD	0	+++	+	+	+++	+	++	++	++	++	++	++
3	HE	+	+++	+	+	++	++	++	+	+++	+++	+	+++
	SU	+	+++	+	+	++	++	++	++	++	++	+	++
	WLD	0	+++	+	+	+	+	+	+	++	++	++	++
4	HE	0	+++	+	+	+	+	+	++	++	+++	+	+++
	SU	+	+++	+	+	+	+	+	++	++	+++	+	+++
	WLD	+	++	+	+	+++	+	+	+	++	++	+	++
5	HE	0	+++	+	+	++	+	++	+	+++	+++	+	+++
	SU	0	+++	+	+	+	+	+	++	++	++	+	++
	WLD	+	+++	+	+	++	+	++	+	+++	+++	+	+++
6	HE	0	+++	+	0	+++	+	+	++	++	++	+	++
	SU	0	+++	+	0	+++	+	+	++	++	++	+++	++
	WLD	0	+++	+	0	+++	+	+	++	++	++	++	++

Cel: Cellularity, Ker: Keratinisation, Sta: Staining of tissue, HE: Heel erosion, SU: Solea ulcer, WLD: White Line Disease, 0: No alteration, +: Weak, ++: Mild, +++: Severe.

and similar weights (500-600 kg). Feding method and ration was not changed throughout the study. Thus, the effects of environmental factors on the results of study were minimized.

Iron levels at the 15<sup>th</sup> and 30<sup>th</sup> day in the heel erosion group, and at the 15<sup>th</sup> day in the white line group had very higher than the control and disease groups. As the reason higher levels of iron in mentioned days there might be a contamination during the preparations of samples before the analysis; even it was very sensitive work during the study. This high level of iron existence in that four samples which is increasing the average, supports this opinion. In order to not provoke the hoof lesions and not prolonged the treatment period, limited amount of hoof samples collected from the each foot lesion. Therefore, repetition of the analyses was not possible. However, if the samples susceped of contamination due to the iron analysis were excluded, out of our consideration, average level of iron, for the hoof eroded ones at 15<sup>th</sup> and 30<sup>th</sup> day, is respectively 523±160 ppm, 445±100 ppm. For the white line group, at the 15<sup>th</sup> day, iron level is 346±118 ppm. When we compare these values with the other days' values, all values seem similar to each other.

During the examination of the trace mineral levels of hoof tissue in healthy animals, only zinc has difference and it is found mostly in the white line region.

The addition of the trace minerals into the animals ration is useful. However, it is mandatory to keep these levels at the optimum levels for both animal's health and environmental health. Abondancy of the trace mineral in the blood and hoof does not mean always that the animal is healthy. In the proposed study, it is found that trace mineral level is higher

in the treatment groups than the control group. This result is probably caused by the accumulation of the trace minerals around the lesion so as to support the hoof construction. At this point, as it is mentioned in the literatures (Ugel 1975; Kempson and Logue 1993; Mulling et al., 1999; Winkler et al., 2001; Tomlinson et al., 2004; Budras et al., 2005;), the fact of that trace mineral is in which protein's structure comes forward more than the density of the trace minerals; since a single trace mineral's relationship with a single structural factor is hard to proof, it is concluded that we need more studies to reveal trace mineral's importance in the epidermal structures, cellular activities and even to specify their molecular levels.

Distribution of the white lines, sole ulcer and histopatologic structure of the new hoof tissue created during the healing process of the heel erosion were investigated, and it is found that cellularity has increased and the ceratination decreased in this lesionic area during this 45-days healing period in which the sick animals were kept under surveillance. At the end of this term, although they were accepted healed according to clinical findings, histopathological findings showed that they need longer time for fully recovery. So we suggested that treatment should be continued for a longer time after clinically recovery. But there were need to more advanced studies for determined the real healing time of hoof.

There is a few data about both levels of trace minerals and variation of these levels in healthy and disease hooves in dairy cattle. Therefore, it was hard to interpret the obtained datas, which is not uniform in this study. Kovacs and Szilagy (1973a) examining the zinc, copper and iron level in the hoof of cattles; but, it is not shown that from which part of the hoof these samples are taken and there was no any information about

Mn. Randhawa et al., (2014) reported that, higher Zn and Cu levels and less Fe and Mn levels are present in the wall than sole. They also concluded that lower levels of Zn and Cu are determined in the diseased hooves. In our study, only Zn levels were significantly different among the hoof regions in control cows (Table 3), and higher levels of Zn, Cu and Mn were found in treatment groups (Table 4). Randhawa et al., (2014) were used different farms in India, as mentioned before, trace mineral levels would be affected by many reasons. Perhaps, this is why the differences. The given results in the proposed study were from only one farm from Turkiye, TR; and in our knowledge, it is the first time that, about the variation in the trace minerals levels in both healthy and foot diseased animals, which were evaluated during the recovery period of foot diseases, blood and three part of the hoof.

Thus, this study is shed light the pathway for the future studies. We carried out this study in a small group of animals because of economical problems, and possibly this resulted in an inhibitory effect on the results, preventing to reach clear cut conclusions. As a result, the hoof diseases cause significant changes in the levels of trace elements in hoof tissues. Observed high levels of some trace elements in the present study would not indicate quality hoof in cows. Although animals are seen as clinically healed, the histological results suggest that they need more time for real healing. Lame cows should be treated for longer time than clinically healing time.

## Acknowledgements

This study is summarized from the PhD thesis of Dr. Ibrahim Akin, Bursa, TR, 2008.

Autors would like to thank to, Dr. Onur Tatli, Adnan Menderes University Faculty of Veterinary Department of Animal Nutrition and Nutritional Diseases, Adnan Menderes University Faculty of Veterinary Department of Pathology, Pamukkale University Faculty of Science and Letters Department of Chemistry.

## References

- Akin I, Belge A, Bardakcioglu H, Sarierler M, Kilic N (2013). Milk yield in dairy cows before and after treatment for foot diseases. *In proceedings: International lameness Conference on Lameness in Ruminants*, Bristol, UK, Pp 128-129.
- Akin İ (2004). İz Elementler ve Sığır Tırnak Hastalıkları. *Veteriner Cerrahi Dergisi*, 10(3-4), 54-61.
- Ballantine HT, Socha MT, Acan DPL, Tomlinson DJ, Johnson AB, Field AS, Shearer JK, Amstel SR (2002). Effects of Feeding Complexed Zinc, Manganese, Copper and Cobalt to Late Gestation and Lactating Dairy Cows on Claw Integrity, Reproduction and Lactating Performance. *The Professional Animal Scientist*, 18, 211-218.
- Blood DC, Hederson JA, Rodostitis OM (1979). *Veterinary Medicine. A Textbook of the Disorders of Cattle, Sheep, Pigs and Horses*. Fifth Edition, Bailliere Tindall, London.
- Budras KD, Mülling C, Anthauer K (2005). Membrane-coating Granules and the Intercellular Cementing Substance (Membrane-coating Material) in the Epidermis in Different Regions of the Equine Hoof. *Anatomia, Histologia, Embryologia*, 34, 298-306.
- Harris CD, Hilburt GA, Anderson GA (1988). The Incidence, Cost and Factors Associated with Foot Lameness in Dairy Cattle in South-Western Victoria. *Australian Veterinary Journal* 65, 171-176.
- Hedges J, Blowey RW, Packington AJ, Callaghan CJO, Green LE (2001). A Longitudinal Field Trial Effect of Biotin on Lameness in Dairy Cows. *Journal of Dairy Science*, 84, 1969-1975.
- Huxley JN (2012). Lameness in cattle: An ongoing concern. *The Veterinary Journal* 193 (2012) 610–611.
- Kelly WR (1974). *Veterinary Clinical Diagnosis*. Second Edition, Bailliere Tindall, London.
- Kempson SA, Logue DN (1993). Ultrastructural observations of hoof horn from dairy cows: the structure of the white line. *Veterinary Record* 132(20), 499-502.
- Kincaid RL, Lefebvre LE, Cronrath JD, Socha MT, Johnson AB (2003). Effect of Dietary Cobalt Supplementation on Cobalt Metabolism and Performance of Dairy Cattle. *Journal of Dairy Science*, 86, 1405-1414.
- Kovacs AB, Szilagyi M (1973a). Data on Mineral Components of The Horny Part of The Foot of Cattle, Sheep and Swine. *Acta Veterinaria Academia Scientiarum Hungaricae*, 23, 187-192.
- Kovacs AB, Szilagyi M (1973b). Mineral Contents of The Horn of The Foot of The Swine of Different Breed and Ages. *Acta Veterinaria Academia Scientiarum Hungaricae*, 23, 241-246.
- Lischer C, Geyer H, Ossent P, Friedli K, Naf I (1998). *Handbuch zur Pflege und Behandlung der Klauen beim Rind*. Landwirtschaftliche Lehrmittelzentrale, Länggasse 79, CH-3052 Zollikofen. ISBN: 3-906679-62-4.
- McDowell LR (1992). *Minerals in Animal and Human Nutrition*. Academic Press, Inc. New York, USA. ISBN: 0-12-483369-1
- Mulling KW, Bragulla HH, Reese S, Budras KD, Steinberg W (1999). How Structures in Bovine Hoof Epidermis are Influenced by Nutritional Factors. *Anatomia, Histologia, Embryologia*, 28, 103-108.
- Nocek JE, Johnson AB, Socha MT (2000). Digital Characteristics in Commercial Dairy Herds Fed metal-Specific Amino Acid Complexes. *Journal of Dairy Science*, 83, 1553-1572.
- Nocek JE, Socha MT, Tomlinson DJ (2006). The Effect of Trace Mineral Fortification Level and Source on Performance of Dairy Cattle. *Journal of Dairy Science*, 89, 2679-2693.
- Randhawa SS, Dua, K, Singh ST, Randhawa CS (2014). Trace Mineral Status of Wall and Sole Portions of Healthy and Diseased hoofs in Cattle and Buffaloes. *Indian Journal of Animal Science*, 84(4), 417-421.
- Reiling BA, Berger LL, Riskowski GL, Rompata RE (1992). Effects of Zinc Proteinate on Hoof Durability in Feedlot Heifers. *Journal of Animal Science*, 70 (Suppl.1), 313.
- Siciliano-Jones JL, Socha MT, Tomlinson DJ, DeFrain JM (2008). Effect of Trace Mineral Source on Lactation Performance, Claw Integrity, and Fertility of Dairy Cattle. *Journal of Dairy Science*, 91, 1985-1995.
- Sogstad AM, Fjeldaas T, Osteras O (2011). Association of claw disorders with claw horn colour in norwegian red cattle – a cross-sectional study of 2607 cows from 112 herds. *Acta Veterinaria Scandinavica*, 53-59.
- Spain JN, Hardin D, Stevens B, Torne J (1993). Effect of Organic Zinc Supplementation on Milk Somatic Cell Count and Incidence of Mammary Glands Infections of Lactating Dairy Cows. *Journal of Dairy Science*, 76 (Suppl), 265 (Abstr.).
- Tomlinson DJ, Mülling CH, Fakler TM (2004). Formation of Keratins in the Bovine Claw: Roles of Hormones, Minerals, and Vitamins in Functional Claw Integrity. *Journal of Dairy Science*, 87, 797–809.
- Ugel AR (1975). Bovine Keratohyaline: Anatomical, Histochemical, Ultrastructural, Immunologic and Biochemical Studies. *Journal of Investigative Dermatology*, 65(1), 118-26.
- Uchiada K, Mardebvu P, Ballard CS, Sniffen CJ, Carter MP (2001). Effect of Feding a Combination of Zinc, Manganese and Copper Aminoacid Complexes and Cobalt Glucoheptonate on Performance of Early Lactation High Producing Dairy Cows. *Animal Feed Science and Technology*, 93, 193-203.
- Underwood EJ (1977). *Trace Elements in Human and Animal Nutrition*. Academic Pres, New York.
- Winkler B, Margerison JK, Brennan C (2001). The Use of Texture Analysis to Asses the Structural Strenght of Hoof Horn of Dairy Cows. Department of Agriculture Food and Land Use, Seale – Hayne Faculty, Plymouth University, Newton Abbot, Devon, TQ12 6NQ. U.K. <http://www.bsas.org.uk/wp-content/themes/bsas/proceedings/Pdf2001/171.pdf> Erişim: 13 Mart 2015.
- Weaver AD (1987). Influence of Feeding on The Health of The Bovine Foot. The Second Symposium on "Bovine Digital Diseases" 25-28<sup>th</sup> September, Skora, SE.