HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Investigation of Serum Copper and Zinc Levels in Sheep with Foot Rot in Şanlıurfa Region

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Abstract: This study aimed to determine the serum copper and zinc levels in healthy and footrot-affected sheep in the Şanlıurfa region and investigate the relationship between these parameters and the presence of the disease. Footrot is an infectious foot disease that develops in the interdigital region of the foot and affects the skin and corium ungulae. It is characterized by necrosis, inflammation, and contagious properties. The study material consisted of 10 Akkaraman and 10 Awassi sheep clinically diagnosed with footrot (footrot group), as well as an equal number of clinically healthy animals from the same breeds, which served as the control group. Serum copper and zinc levels were measured and recorded from blood samples collected from all sheep. When the data were analyzed independently of breed, serum copper and zinc levels were significantly lower in the footrot group compared to the control group (P<0.001). When analyzed independently of disease status, no significant difference was observed in serum copper and zinc levels between Awassi and Akkaraman sheep (P>0.05). When all variables were evaluated, serum copper and zinc levels in footrot-affected Awassi and Akkaraman sheep were significantly lower than in healthy individuals (P<0.001). These results indicate a significant relationship between footrot and deficiencies in serum zinc and copper levels. Regardless of disease status, trace element levels did not significantly differ between breeds. Overall, serum copper and zinc levels were markedly lower in footrot-affected Awassi and Akkaraman sheep in the Şanlıurfa region.

Keywords: *Akkaraman, Awassi, Copper, Foot rot, Sheep, Zinc.*

Introduction

Footrot is an infectious, necrotic, inflammatory, and contagious disease that develops in the interdigital region of the foot, affecting both the skin and corium ungulae (Alkan, 2023; Başa et al., 2020; Bennett et al., 2011; Green and George, 2008; Zanolari et al., 2021). This disease, frequently reported in countries with significant sheep production, is considered one of the most important causes of lameness in sheep, goats, and other farm animals in Türkiye (Alkan, 2023; Başa et al., 2020; Rezazadeh et al., 2012). Footrot typically begins sporadically but can rapidly become enzootic, resulting in significant losses in meat, milk, and fleece yield, particularly in adult sheep, depending on the severity of clinical symptoms (Alkan, 2023; Başa et al., 2020). In addition to physical factors such as unsanitary housing conditions and uneven flooring, nutritional deficiencies (specifically of copper, zinc, calcium, phosphorus, and selenium) within the feed ration are known to predispose animals to footrot (Belge et al., 1996; Çamaş et al., 1997; Duffy et al., 2023; Rezazadeh et al., 2012; Sertkaya and Şındak, 2004; Yıldız and Gençcelep, 2021).

Zinc deficiency in sheep may lead to general health deterioration, growth and developmental delays, incoordination, skin thickening, reduced quantity and quality of fleece, fissures, cracks in the margo coronarius and interdigital region, joint swelling. Similarly, copper deficiency has been reported to cause systemic deterioration, diarrhea, delayed growth and development, poor hair and fleece quality, depigmentation, bone deformities, and reduced osteoblastic activity. Furthermore, copper deficiency adversely affects hoof health by impairing the growth of keratinized tissues such as the horn and claw, and by disrupting the cross-linking of keratin molecules (Baran et al., 2015; Başa et al., 2020; Belge et al., 1996; Çamaş et al., 1997; Dufy et al., 2023; Konig et al., 2011; Glynn, 1993; Wani and Samanta, 2006; Whittier and Umberger, 2009; Zanolari et al., 2021).

The present study aimed to determine serum copper and zinc levels in clinically healthy sheep raised in the Şanlıurfa region, where sheep production is widespread, and to investigate the association between these trace elements and footrot.

Materials and Methods

Before the initiation of the study, official authorization was obtained from the Republic of Türkiye, Governorship of Şanlıurfa, Provincial Directorate of Agriculture and Forestry (Dated:23.03.2021 Approval No: E-59855012-280.01.01-936647). Subsequently, an application was submitted to the Harran University Local Ethics Committee for Animal Experiments. Following the evaluation, the committee determined that the study fell within the scope of “non-experimental agricultural and clinical veterinary practices.” Thus, the official authorization was deemed sufficient, and no additional ethical approval was required (Decision No: 2021/ 004/03).

The material of this study consisted of a total of 40 sheep, including 10 Akkaraman (n=10) and 10 Awassi (n=10) sheep that were examined and clinically diagnosed with footrot, as well as an equal number of clinically healthy Akkaraman (n=10) and Awassi (n=10) sheep, which formed the control group. All animals were selected from flocks from farms in the Şanlıurfa region.

Initially, breeders were interviewed to determine whether animals in the flock exhibited lameness, particularly during grazing, and to identify specific individuals showing clinical signs. Animals suspected of lameness underwent clinical examination, and the severity of lameness was recorded. Lameness in sheep was evaluated using the four-point numerical scoring system (Table 1) adapted from modified system for small ruminants, as described by Kaler and Green (2008). Each animal was observed individually while walking on a flat, non-slippery surface at its normal pace. Scoring was based on gait symmetry, stride length, and weight-bearing behaviour.

Table 1. Lameness Scoring System for Sheep (Kaler and Green, 2008).

Score	Description
0	Normal gait, bearing weight evenly on all limbs.
1	Mild unevenness, gait slightly irregular but difficult to detect at a glance.
2	Mild lameness, uneven gait is noticeable with shortened stride on one limb.
3	Moderate lameness, obvious head nodding or limping, and clear asymmetry in movement.
4	Severe lameness, animal frequently avoids bearing weight on the affected limb or is reluctant to move.

In this study, we used this system to categorize animals according to lameness severity. Sheep with a lameness score of 2 or higher were included in the footrot group, representing individuals with mild to severe clinical lameness. Animals with a score below 2 were considered clinically sound and assigned to the healthy control group

(Kaler et al., 2009; Kaler et al., 2011; Whittier and Umberger, 2009). This classification allowed us to distinguish footrot-associated lameness from normal locomotion patterns, as the presence and severity of lameness are closely correlated with the degree of footrot lesions. The severity of lameness and Once the affected limb(s) or hoof/hooves were identified, mechanical cleaning was performed, followed by a detailed inspection of the interdigital space and hoof structures (Figure 1). Clinical findings such as interdigital hyperkeratosis, hyperemia, and exudation were commonly observed, indicating inflammatory processes within the interdigital space. In more advanced cases, alopecia around the affected area, necrotic foci, and ulcerative lesions were evident, often accompanied by a foul odor and serous to purulent discharge (Figure 1). Fistula formation and erosions of the hoof horn and adjacent soft tissues were also documented, suggesting chronic infection and tissue destruction associated with the progression of the disease. Foot-rot score was assessed using a 5-point numerical scoring system as described by the Swiss Scoring System (Stewart and Claxton, 1993), where 0 indicates normal foot and 5 represents severe foot-rot (Table 2). The foot-rot group consisted of animals with foot-rot scores of 3 and above (Figure 1-3), while the healthy group scored 0 (Stewart and Claxton, 1993, Zanolari et al., 2021).

Table 2. Swiss scoring system for footrot adapted from Stewart and Claxton (1993).

Score	Clinical findings
0	Healthy, dry foot
1	Moist and inflamed interdigital space with some hair loss
2	Extensive skin inflammation, damaged horn in the interdigital space
3	Detachment of the axial horn wall, underrunning of the horn towards the sole
4	Underrunning (including separation) expands to the outer horn wall (abaxial), pododerma heavily affected
5	Removal of the hoof capsule (complete separation), extended pododerma lacerations

From both footrot-affected and healthy animals, 10 mL of blood was collected from the jugular vein using vacuum blood collection needles into red-topped tubes without anticoagulant. The blood samples were allowed to coagulate at room temperature and centrifuged at 3,000 rpm for 15 minutes. The resulting serum was transferred into Eppendorf tubes and stored at -20 °C until biochemical analysis (Sağlıyan et al., 2003; Serpek, 1983). At the time of analysis, 1 mL of each serum sample was diluted with distilled water to a final volume of 10 mL. Standard solutions at 0.5, 1, and 2 ppm concentrations were prepared from commercial stock solutions (Zinc-1000 and Copper-1000, Mercury-Merck) for calibration. The atomic absorption spectrophotometer was calibrated before analysis, and serum copper and zinc concentrations were measured using the atomic absorption spectrometry (AAS) method (Sağlıyan et al., 2003).

Figure 1. Apperance of the hoof of sheep with foot-rot (Score 3).

Figure 2. Apperance of the hoof of sheep with foot-rot (Score 4).

Figure 3. Apperance of the hoof of sheep with foot-rot (Score 5).

The statistical analyses were performed using SPSS version 25 software. Histograms, probability plots, and the Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of variable distributions. Descriptive statistics for normally distributed variables were expressed as mean ± standard deviation. Since the serum copper and zinc levels, and their association with the disease in healthy and footrot-affected sheep, were found to follow a normal distribution, comparisons between groups were conducted using Student's t-test. A p-value of less than 0.05 was considered statistically significant.

Results

When the data were analyzed, regardless of breed, serum copper and zinc levels in the footrot group were significantly lower than in the control group (Table 3; *P*<0.001).

When the data were assessed independently of disease status (i.e., without considering footrot), no statistically significant difference was observed between the serum copper and zinc concentrations of Akkaraman and Awassi sheep breeds (Table 4; *P*>0.05).

When breed and disease status were analyzed in combination, serum copper and zinc levels were significantly lower in the footrot group compared to the respective control groups within each breed (Table 3; *P*<0.001). However, when copper and zinc levels were compared between the footrot groups of Akkaraman and Awassi breeds and between the control groups of both breeds, the differences were not statistically significant (Table 5; *P*>0.05).

Table 3. Statistical comparison of foot-rot and healthy sheep according to serum zinc and copper levels.

Parametres	Foot-rot Group (n=20)	Control Group (n=20)	P -Value
Zinc (µg/dl)	56,98 ±7.61	93,52 ± 6.29	***
Copper (µg/dl)	53,02 ± 6.01	79,17 ± 6.28	***
***** *P*<0,001**			

Table 4. Statistical comparison of Akkaraman and Awassi sheep breeds according to serum zinc and copper levels.

Races	Zinc (µg/dl)	Copper (µg/dl)
Awassi (n=20)	74.01±20.01	65.76±16.26
Akkaraman (n=20)	76.49±19.79	66.42±13.06
P-value	*0,697	*0,089
* *P*> 0,05		

Table 5. Statistical evaluation of breed and disease status variables according to serum zinc and copper level parameters.

Races	Zinc (µg/dl)		P-value	Copper (µg/dl)		P-value
	Foot-Rot Group (n=10)	Control Group (n=10)		Foot-Rot Group (n=10)	Control Group (n=10)	
Awassi	55.84±8.37	92.19±7.16	0.000***	50.73±5.25	80.80±5.34	0.000***
Akkaraman	58.12±7.03	94.85±6.47	0.000***	55.31±6.08	77.53±6.98	0.000***
P-value	*0,646	*0,910		* 0,657	*0,874	

Discussion and Conclusion

Footrot is a common infectious disease in sheep production that causes significant economic losses. It is characterized by varying degrees of detachment of the capsula ungulae from the underlying tissues and necrosis of the corium ungulae. The disease typically begins with mild inflammation of the interdigital skin, corium, and associated soft tissues, initially presenting as a warm, painful, and firm swelling, which later progresses to purulent and necrotic lesions (Avki et al., 2004; Başa et al., 2020; Yurdakul, 2018; Zanolari et al., 2021).

Footrot is most frequently observed in the forelimbs, particularly during spring (April to June) and autumn (September to October). Environmental conditions such as temperate climates, rainy weather, stony ground, and poor pen hygiene significantly contribute to the development of the disease (Belge et al., 1996; Sağlıyan et al., 2003; Yıldız and Gençcelep, 2021). In most inspected holdings, inadequate flooring and hygiene problems were clearly evident. Additionally, the neglect of routine hoof care was associated with damaged and deformed claw structures. Wet flooring during winter softens the horn tissue, while hot weather in summer causes cracks in the claws, both of which predispose animals to infection (Sağlıyan et al., 2003).

A field study on footrot prevalence reported a high incidence of the disease during prolonged rainfall, attributing this to increased ground moisture (Basa et al., 2020; Yıldız and Gençcelep, 2021). If left untreated or diagnosed late, footrot can quickly spread to other animals within the flock. Pasture-based small ruminant production facilitates transmission due to contamination of grazing areas. (Yıldız and Gençcelep, 2021). Although mineral deficiencies are common in ruminants, they are often difficult to detect early without clinical signs. Zinc and copper levels in organs and tissues are influenced by factors such as breed, age, sex, dietary composition, and interactions with other elements, including calcium (Ca), phosphorus (P), molybdenum (Mo), iron (Fe), and selenium (Se). Zinc and copper concentrations may vary even among morphologically similar plant species growing in the same soil (Rezazadeh et al., 2012). Inadequate intake of trace elements such as zinc, selenium, calcium, and copper, essential for maintaining hoof hardness, can weaken claw structure and increase susceptibility to hoof and foot diseases (Berzeski et al., 1990). Mineral deficiency or imbalance compromises the structural integrity of the claw, facilitating the penetration of pathogens into the underlying tissues and leading to conditions such as hoof breakage, cracking, deformities, and footrot (Başa et al., 2020; Berzeski et al., 1990; Sağlıyan et al., 2003; Yıldız and Gençcelep, 2021). In the present study, inadequate mineral supplementation in rations was observed in many of the examined flocks, corroborating the findings of previous research (Avki et al., 2004; Başa et al., 2020; Berzeski et al., 1990; Rezazadeh et al., 2012; Sağlıyan et al., 2003; Yıldız and Gençcelep, 2021). Reported serum zinc levels in healthy sheep include 81.91 ± 8.09 to 124.29 ± 11.30 µg/dL (Alkan and Yavru, 2000), and 80-117 µg/dL (Altıntaş and Fidancı, 1993). Serum copper levels have been reported as 113.09 ± 7.52 to 142.85 ± 12.19 µg/dL, and 58-160 µg/dL, respectively. In healthy sheep in the Elâzığ region, zinc and copper concentrations were reported as 62-124 µg/dL and 66.89 ± 1.45 µg/dL, respectively (Sağlıyan et al., 2003). In a doctoral thesis investigating serum trace elements in 210 healthy sheep across five districts in İzmir, serum copper and zinc levels were reported as 109.31 µg/dL and 184.22 µg/dL, respectively, with reference ranges of 58-160 µg/dL for copper and 76-140 µg/dL for zinc (Doğanay,1996). In our study, serum zinc and copper levels in healthy sheep were 93.52 ± 6.29 µg/dL and 79.17 ± 6.28 µg/dL, respectively. Although these values were slightly lower than those reported by some researchers (Alkan and Yavru, 2000; Altıntaş and Fidancı, 1993; Sağlıyan et al., 2003), they remained within the accepted reference ranges. Since serum copper levels in healthy sheep exceeded 50 µg/dL (Doğanay,1996; Serpek, 1983), there was no indication of secondary copper deficiency among sheep in the Şanlıurfa region.Reported serum zinc levels in footrot-affected sheep include 68.07 µg/dL (Alkan and Yavru, 2000) and 23.10 µg/dL (Belge et al.,1996). In a study conducted in the Elâzığ region, sheep with footrot had serum copper levels of 60.64 ± 1.25 µg/dL and zinc levels of 46.77 ± 2.50 µg/dL (Sağlıyan et al., 2003). In the current study, sheep with footrot had mean serum zinc and copper levels of 56.98 ± 7.61 µg/dL and 53.02 ± 6.01 µg/dL, respectively. One study reported that serum zinc levels below 40 µg/dL are associated with clinical zinc deficiency (Serpek, 1983). No secondary zinc deficiency was suspected since the values observed in the present study were above this threshold. Nevertheless, the serum zinc (56.98 ± 7.61 µg/dL) and copper (53.02 ± 6.01 µg/dL) levels in the footrot group were significantly lower than those in healthy animals (zinc: 93.52 ± 6.29 µg/dL; copper: 79.17 ± 6.28 µg/dL), and this difference was statistically significant. These findings support the association between footrot and deficiencies in copper and zinc, as previously reported in the literature (Alkan and Yavru, 2000; Baran et al., 2015; Başa et al., 2020; Belge et al., 1996; Green and George, 2008; Zanolari et al., 2021).

The animals used in this study were of the Awassi and Akkaraman breeds, raised in Şanlıurfa province. Trace element levels were also evaluated by breed. In Awassi sheep, the mean serum copper and zinc levels were 65.76 ± 16.26 µg/dL and 74.01 ± 20.01 µg/dL, respectively.

In Akkaraman sheep, the corresponding values were 66.42 ± 13.06 µg/dL and 76.49 ± 19.79 µg/dL. No statistically significant differences were found between the two breeds regarding serum copper and zinc levels. Similarly, comparisons between the footrot (Figure 1) and control groups within each breed revealed no statistically significant intra-breed differences. However, when breed and disease status were assessed in combination, serum copper and zinc levels were significantly lower in footrot groups than in healthy controls across both breeds. These findings are consistent with existing literature highlighting the role of zinc and copper deficiency in the etiopathogenesis of footrot (Alkan, 2023; Avki et al., 2004; Baran et al., 2025; Bennett and Hickford, 2011; Doğanay, 1996; Zanolari et al., 2021). The results also suggest that breed does not play a determining role in susceptibility to footrot, and that environmental and nutritional factors (particularly inadequate housing, poor hygiene, and trace mineral deficiencies due to soil or diet) are likely contributors to the development of the disease.

This study demonstrates that footrot in sheep is not associated with breed predisposition and may occur independently of genetic background. Instead, environmental and nutritional factors (particularly deficiencies in zinc and copper) appear to play a more prominent role in the development and progression of the disease.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

Before the initiation of the study, official authorization was obtained from the Republic of Türkiye, Governorship of Şanlıurfa, Provincial Directorate of Agriculture and Forestry (Dated:23.03.2021 Approval No: E-59855012-280.01.01-936647). Subsequently, an application was submitted to the Harran University Local Ethics Committee for Animal Experiments. Following evaluation, the committee determined that the study fell within the scope of "non-experimental agricultural and clinical veterinary practices." Thus, the official authorization was deemed sufficient, and no additional ethical approval was required (Decision No: 2021/ 004/03).

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Similarity Rate

We declare that the similarity rate of the article is 10 % as stated in the report uploaded to the system.

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Explanation

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Author Contributions

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Analysis and / or Interpretation: KK, PFPD
Literature Review: AB, KK
Writing the Article: AB, KK
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