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Association between Copper, Selenium and Zinc Levels and Foot Rot in Sheep: The Case of Sétif Region

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ABSTRACT

Background: Copper (Cu), Zinc (Zn) and Selenium (Se) are three trace elements whose deficiency can predispose sheep to various diseases, foot rot being the most common. This study aimed to assesses the link between Cu, Zn and Se levels and foot rot in the Sétif region of Algeria and to establish a prophylactic and therapeutic scheme to correct deficiencies.

Methods: Conducted at the "the Khababa Abdelwahab" experimental farm (Setif, Algeria), the study involved tow experiments. Experiment 1 measured serum Cu, Zn and Se in 37 Ouled Djellal sheep (15 healthy, 12 stage 2, 10 stage 3) using atomic absorption spectrophotometry. Experiment 2 evaluated *Thespophor Oligo* (oral Cu/Zn) and *Sodiferol* (injectable Se) in 15 stage 1 cases. Treatments were administrated over several days, repeated after 30 days and serum trace elements retested. Data were analyzed using t-tests, Bonferroni correction, repeated-measures ANOVA and Pearson correlation, with significance set at $P<0.05$ (adjusted to 0.0167 for multiple comparisons).

Result: In foot rot-affected sheep, severity was associated with a marked selenium and zinc decrease and a pronounced copper increase especially at stage 3. Treatment corrected selenium deficiency but not zinc deficiency or copper excess. Hypercupremia appears linked to metabolic or inflammatory disturbances and mineral imbalance.

Key words: Copper, Foot rot, Selenium, Sheep, Zinc.

INTRODUCTION

Foot diseases, particularly foot rot, represent a significant constraint to sheep production in Algeria, causing substantial economic losses, with a prevalence reaching up to 45.6% of the livestock population, especially in the Sétif region (Sissaoui, 2014). Foot rot is a complex infectious condition primarily attributed to *Dichelobacter nodosus,* whose incidence and severity are modulated by several factors, including nutritional imbalances and, more specifically, deficiencies in trace elements such as zinc, copper and selenium (Roberts *et al*., 1969; Bennett *et al*., 2011).

Inadequate levels of these trace elements compromise immune competence, impair keratinization and reduce hoof integrity, thereby predisposing animals to infections (Hall *et al*., 2009; Suttle, 2010). Previous studies have shown that zinc supplementation improves hoof quality and claw integrity in cattle (Cross *et al*., 1981; Bauer *et al*., 2018), suggesting similar benefits in sheep. However, despite these findings, information on the trace element status in sheep with foot rot in Algeria and on the efficacy of targeted pharmacological treatments remains limited.

Based on these considerations, we hypothesize that deficiencies in Cu, Zn and Se are associated with increased foot rot severity and that corrective supplementation may improve trace element status and mitigate disease outcomes. Accordingly, the present study pursued two main objectives : 1) to investigate the involvement of Cu, Zn and Se in the onset and progression of foot rot in sheep in the Setif region of Algeria; and 2) to evaluate the therapeutic effectiveness of two veterinary formulations, *Thespophor Olig* and *Sodiferol,* in correcting these deficiencies and supporting disease management.

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MATERIALS AND METHODS

Experimental protocol

The study was conducted between March and September 2023, encompassing the spring and summer seasons at the "Khababa Abdelwahab" experimental farm (Sétif region, Algeria), the Department of General, Specialized and

Operative Surgery, Saint Petersburg State University of Veterinary Medicine, Russia and Department of Agricultural Sciences, University of Ferhat Abbas (Sétif, Algeria).

Experiment 1: Assessment of trace elements in blood serum

Serum concentrations of copper, zinc and selenium were quantified. Thirty-seven Ouled Djellal sheep, aged 4±1.33 years, were divided into three groups:

- The control group comprised 15 healthy sheep.
- The second group included 12 sheep showing clinical signs of stage 2 foot rot (Fig 1).
- The third group consisted of 10 sheep with clinical signs of stage 3 foot rot (Fig 2).

Blood samples were obtained via jugular venipuncture, collected in dry Sarstedt tubes and transported under controlled refrigerated conditions to the Djilani Biochemistry Laboratory (Sétif, Algeria), where the assays were performed by qualified specialists. The blood was then centrifuged and stored at -20°C until analysis, which was conducted within one month. Serum concentrations of copper, zinc and selenium were determined using standard atomic absorption spectrophotometry, following the protocols of Bellanger and Lamand (1975) and Voronin *et al*. (2006), without modifications.

Experiment 2: Therapeutic evaluation of treatments

To evaluate the therapeutic efficacy of the *Thespophor Oligo* (oral solution) and *Sodiferol* (injectable preparation) as trace element supplementation strategies, we formed a fourth experimental group of 15 sheep aged 4.4±1.33 years, all in stage 1 foot rot (Fig 3). *Thespophor Oligo* (containing copper chloride and zinc chloride) was administered per os, whereas *Sodiferol* (containing sodium selenite) was administered intramuscularly.

Thespophor Oligo (LBVET, Algeria) was administered at 50 ml/animal diluted in 1 L of water, once daily for 5 days. *Sodiferol* (LABORATOIRE BIARD, Arques, France) was administered intramuscularly at 2 ml/10 kg body weight, once daily for 4 days, according to the manufacturer's recommendations.

After 30 days, the treatment regimen was repeated to account for the limited persistence of trace elements (Cu, Se, Zn), whose efficacy typically declines after 4-7 weeks and to ensure optimal intake before tissue stores are depleted. One month after the second treatment, blood samples were collected from the jugular vein to measure the concentrations of trace elements (Cu, Se, Zn) in blood serum and thus assess the cumulative efficacy of the regimen at 30-day intervals (Mills *et al*., 1967; Grace and Knowles, 2012; Genther-Schroeder *et al*., 2025).

Statistical analyses

IBM SPSS Statistics (version 21) was used for all statistical analyses. To evaluate differences in copper, zinc and selenium concentrations between the control group and the experimental groups (Table 1), an independent-samples Student's t-test was applied to compare the means of independent groups, with a significance threshold set at $P<0.05$.

Bonferroni correction was applied to control multiple comparisons. Since three trace elements were analyzed, the adjusted significance threshold was $P<0.0167$.



Fig 1: Sheep No. 023, 6 years old, second stage of foot rot (dermatitis with exudates).

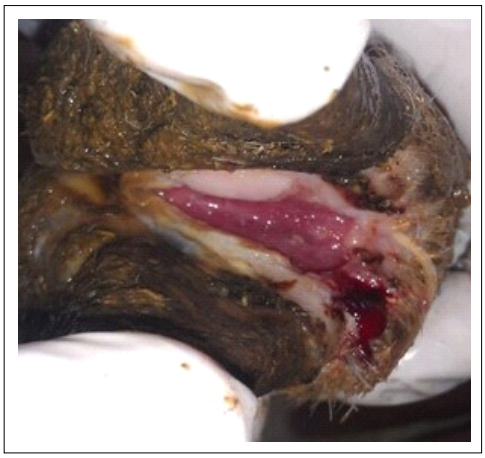


Fig 2: Sheep No. 033, 5 years old, third stage of foot rot (necrosis reached the soft horn tissue).



Fig 3: Sheep No. 002, 5 years old. First stage (mild dermatitis).

Only P-values below this threshold were considered statistically significant.

For pre- and post-treatment comparisons in the same animals (Table 2), repeated-measures ANOVA was used to analyze intra-animals variations in trace element levels. Results were considered statistically significant if $P<0.05$. Additionally, Pearson correlation analysis was conducted to examine relationships between serum levels (of selenium, copper and zinc) and the severity and prevalence of foot rot in sheep. Correlation coefficients and corresponding P-values are reported for each analyzed association.

RESULTS AND DISCUSSION

Experiment 1

The results of serum trace element concentrations in healthy sheep and those affected by foot rot are presented in Table 1. In healthy sheep (*n=15*), copper levels were within the physiological range, mean of 9.07 µmol/l/ (95% CI: 8.31-9.83). In diseased sheep, values varied according to the severity. Serum concentrations of copper, selenium and zinc in each group, by disease stage are summarized in Table 3.

In sheep with second-stage foot rot (*n=12*), copper concentration was 11.03 µmol/l (95% CI: 9.65- 12.41), within physiological limits. Student's *t*-test ($P<0.05$) showed copper in third-stage group (n=10) increased significantly to 22.91 µmol/l (95% CI: 18.99-26.83), more than twice the physiological value. Indicating a marked rise at advanced stages. These differences in serum copper concentrations by disease stage are illustrated in Fig 5.

Healthy sheep had selenium concentrations of 1.58 µmol/l (95% CI: 1.47-1.69). Stage 1 sheep showed 1.10 µmol/l (95% CI: 1.01- 1.19) dropping to 0.39 µmol/l (95% CI: 0.20-0.58) at stage 2 and 0.36 µmol/l (95% CI: 0.21-0.51) at stage 3 ($P<0.05$), indicating a progressive decline with advancing disease. These progressive reductions in serum selenium concentrations by disease stage are illustrated in Fig 7.

Zinc averaged 16.39 µmol/l (95% CI: 14.16 -18.62) in healthy sheep. Stage 2 values were 13.42 µmol/l (95% CI: 11.72-15.12) and stage 3, 13.60 µmol/l (95% CI: 11.40-15.80); both were below physiological range, with no significant differences between stages. As illustrated in Fig 6, serum zinc concentrations were consistently lower in diseased sheep compared with controls with minimal variation across disease stages.

Correlation analysis (Table 3)

Pearson correlation analysis Serum showed selenium was strongly and significantly negatively correlated with severity (r=-0.955, P=0.045). Health status as binary variable (Table 1; healthy =1, diseased = 0) yielded a positive correlation (r =0.707,

Table 1: Serum concentration of trace elements in healthy sheep and those affected by foot rot.

Groups	Cu (Physiological value: 7.9 - 11 µmoll)	Se (Physiological value: 1.00 - 1.50 µmoll)	Zn (Physiological value: 15.3 - 30.6 µmoll)
Control group (N=15)	9.07±1.38	1.58±0.19	16.39±4.03
2nd experimental group (N=12)	11.03±2.17	0.39±0.30*	13.42±2.67
3rd experimental group (N=10)	22.91±5.48*	0.36±0.21*	13.60±3.08

Values marked with an asterisk (*) indicate a statistically significant difference compared to the control group, determined by the Student's t-test (P<0.05) with a Bonferroni correction (P<0.0167). N: number of sheep in each group.

Table 2: Dynamics of trace element concentrations (µmol/l) in sheep blood serum before and after treatment with *Thespophor Oligo* and *Sodiferol* in the 4th experimental group.

Trace element	Copper	Selenium	Zinc
Before treatment (N=15)	12.70±0.8	1.10±0.15	12.56±1.08
After treatment (N=15	12.42±3.44 (↓ 2.20%)	1.40±0.22* (↑ 27.27%)	8.40±0.63* (↓ 33%)
Physiological value	7.90-11 µmol/l	1.00-1.50 µmol/l	15.3-30.6 µmol/l

P: The significance values (P≤0.05) derive from the repeated-measures ANOVA for the differences before and after treatment. N: Number of animals in the group. Values marked with an asterisk (*) indicate a statistically significant difference between the levels before and after treatment, determined by the repeated-measures ANOVA (P<0.05).

Table 3: Analysis of the variation in blood trace element concentrations according to the stages of foot rot in sheep.

Groupe	Stade du pietin	N	Cu (Physiological value: 7.9 - 11 µmol/l)	Se (Physiological value: 1.00 - 1.50 µmol/l)	Zn (Physiological value: 15.3 - 30.6 µmol/l)
Control group	Healthy animals	15	9.07±1.38	1.58±0.19	16.39±4.03
Exp. Gp. 4	Stage 1	15	12.70±0.8	1.10±0.15*	12.56±1.08
Exp. Gp. 2	Stage 2	12	11.03±2.17	0.39±0.30*	13.42±2.67
Exp. Gp. 3	Stage 3	10	22.91±5.48*	0.36±0.21*	13.60±3.08

*Significant at P 0.05, N: Number of animals in the group. Exp. Gp : Experimental group.

P=0.001). Copper correlated strongly positively with severity (*r* =0.834), mainly due to high values at advanced stages. Zinc showed a moderate, non-significant negative correlation (*r* =-0.584), remaining below consistently below physiological values at all stages, with little variation, suggesting a background or predisposing role. Overall, selenium and copper are more closely associated with course disease than zinc.

Experiment 2

This experiment evaluated the efficacy of *Thespophor Oligo* solution and the injectable *Sodiferol* for correcting deficiencies in foot rot affected sheep. The treatment stabilized vital signs: body temperature (38.80±0.38°C), heart rate (97±3.92 bpm), respiratory rate (30.80±2 cyclesmin), all *P*<0.05 (repeated measures ANOVA).

Modification of serum trace element levels following treatment

- **Selenium (Se):** Increased from 1.10 µmoll (95% CI: 1.01-1.19) to 1.40 µmoll (95% CI: 1.31-1.49; +27.27%, *P*<0.05). Pre-treatment was at the lower physiological limit (1.00-1.50 µmoll), remaining below healthy controls (1.58 µmoll; 95% CI: 1.47-1.69: P≤0.05; Table 1). Indicates treatment effectively improved selenium status.
- **Zinc (Zn):** Despite treatment, zinc levels fell from 12.56 µmoll (95% CI: 11.99-13.13) to 8.40 µmol/l ((95% CI: 8.10-8.70; -33%, *P*<0.05). This post-treatment value remained well below the physiological range (15.3-30.6 µmol/l). This indicates that oral zinc supplementation was insufficient to correct zinc deficiency.
- **Copper (Cu):** Serum copper levels decreased slightly, from 12.70 µmoll (95% CI: 12.27-13.13) to 12.42 µmoll (95% CI: 10.90-13.94; -2.20%, *P*>0,05). Levels remained above the physiological range (7.90-11/ µmol/l), indicating that the treatment was ineffective in reducing elevated copper concentrations.

Selenium status improved significantly, whereas zinc levels decreased further and copper remained slightly above physiological value. These trends are illustrated in Fig 4. These findings suggest that the treatment protocol effectively addressed selenium deficiency but was insufficient to normalize zinc concentrations or reduce excess copper. This highlights the need for a tailored supplementation plan to meet the specific requirements of affected animals and achieve an optimal trace element balance.

Observations on hypercupremia

Hypercupremia observed in sheep with stage 2 and 3 foot rot is likely attributable to copper toxicity, either from excessive intake or antagonistic interactions with molybdenum and zinc in soil (Todd *et al*., 1959; Suttle, 1977; Stogdale, 1978; Auza, 1983). Serum copper concentrations increased progressively with disease severity, peaking at stage 3 (Table 3), which may reflect impaired hepatic metabolism or systemic inflammatory response. Elevated copper thus constitutes a marker of oxidative or inflammatory stress in advanced cases. This association is further supported by the boxplot in Fig 5, which shows significantly higher serum copper concentrations in sheep with advanced disease stages. These findings reinforce the role of copper as a biomarker of disease progression and its involvement in inflammatory processes. In healthy sheep, copper concentrations remain within physiological limits (Table 1), thereby excluding inherent toxicity. Inflammation, often associated with hypozincemia, is likely to underlie hypercupremia and the progression from mild interdigital inflammation (stage 1) to necrotic lesions (stage 3) (Haumesser, 1981; Bohraman *et al*., 1986; Marin *et al*., 2012). Mineral imbalances in grazing ruminants can further alter copper-zinc metabolism, contributing to hypercupremia (Arthington *et al*., 2005).

Observations on hypozincemia

Hypozincemia primarily results from dietary hypozincemia, exacerbated by overgrazing and antagonistic interactions

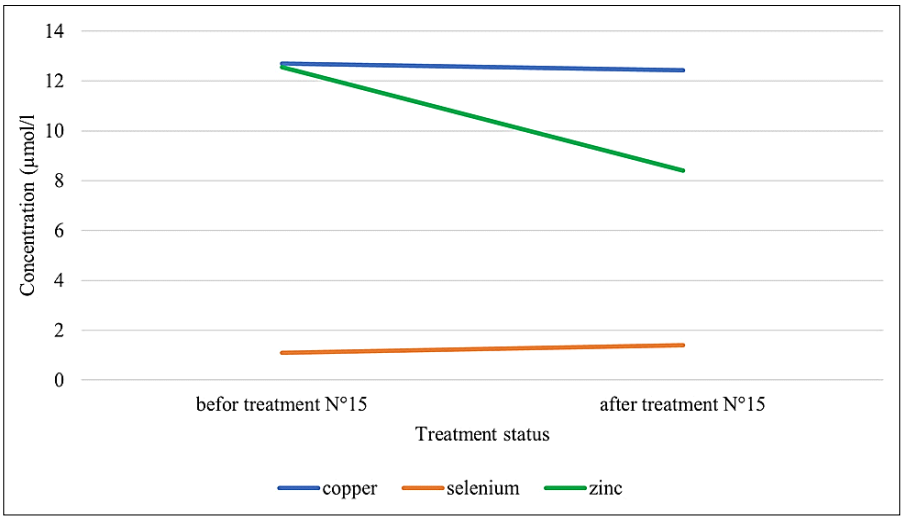


Fig 4: Dynamics of trace element concentrations (µmol/l) in sheep blood serum before and after treatment with Thespophor Oligo and Sodiferol.

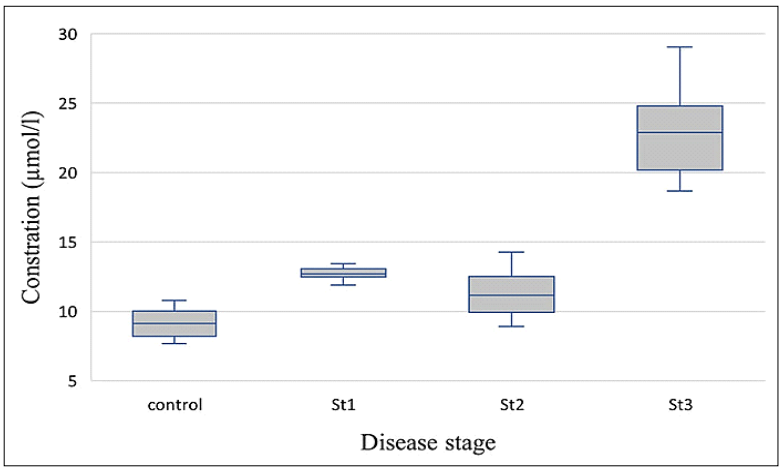


Fig 5: Serum Cu concentration(µmol/l) by disease stage.

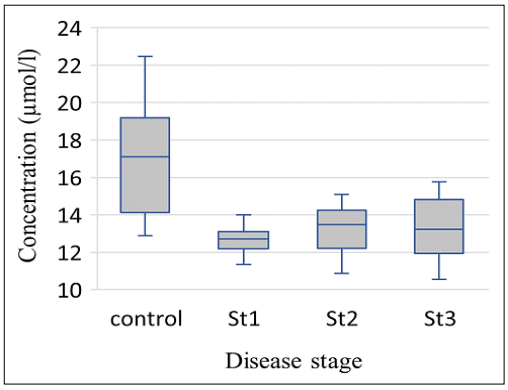


Fig 6: Serum Zn concentration(µmol/l) by disease stage.

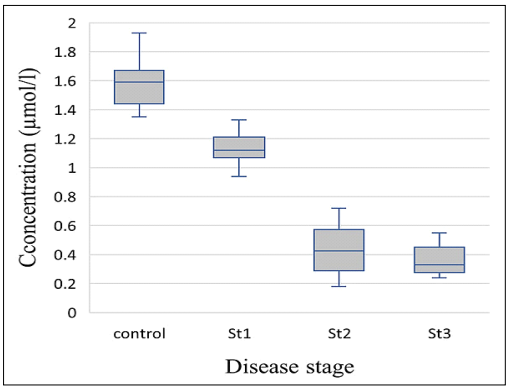


Fig 7: Serum Se concentration (µmol/l) by disease stage.

with cadmium, calcium, molybdenum and sulfur (Faye *et al*., 1990; Thornton, 2002; Marx, 2002; Mallam *et al*., 2010). Infection further depresses zinc levels below physiological thresholds, as observed in disease-affected cohorts (Marx, 2002). Clinically, hypozincemia manifests as hoof deformities and interdigital skin lesions, with absorption impaired in zinc-deficient soils (Arthington *et al*., 2005; Christian, 2014). Sheep affected by foot rot exhibited reduced serum zinc concentrations compared to healthy controls (Table 3). The steepest decline occurs at stage 1, with levels stabilizing around 13-13.6 µmol/l thereafter, indicating a persistent deficit that remains constant regardless of disease progression. This is corroborated by Fig 6, which illustrates chronically low serum zinc concentrations across all disease stages, suggesting an underlying predisposing factor in the flock and indicating the limited effectiveness of oral supplementation. Hypozincemia compromises skin and hoof integrity but to a lesser extent than hyposelenemia (Zia *et al*., 2018; Yeltekin *et al*., 2018; Manimaran *et al*., 2024). Given the low bioavailability of dietary zinc and soil imbalances, regular supplementation is essential for preventing foot disorders and reinforcing immune function (Raju *et al*., 2022; Durak *et al*., 2024; Manimaran *et al*., 2024).

Observations on hyposelenemia

Hyposelenemia progressively deteriorates with disease severity, decreasing sharply from stage 1 (Table 3), indicating a strong pathophysiological link. Selenium, as a cofactor of antioxidant enzymes like glutathione peroxidase, protects against oxidative stress and supports immune function. Its deficiency increases susceptibility to foot rot and other infections (Zia *et al*., 2018; Yeltekin *et al*., 2018; Mohammed *et al*., 2024). Hyposelenemia is common in ruminants due to low soil and forage selenium content (Dedie *et al*., 1985; Richy, 1978; Marx, 2002; Pont, 2011), impairing antioxidant defenses and immune responses, including neutrophil phagocytosis (Aziz *et al*., 1986; Stabel *et al*., 1990; Hall *et al*., 2011; Hugejiletu *et al*., 2013). Our findings align with literature reports, showing lower selenium levels in diseased compared to healthy sheep (Hall *et al*., 2011) and highlight selenium as the earliest and most prominent biochemical marker for foot rot progression, as illustrated by the pronounced decline in Fig 7. Therefore, monitoring and early intervention addressing selenium deficiency are critical for disease mitigation.

Given this context, supplementation strategies should be carefully adapted to local mineral profiles and integrated within a broader management framework, including pasture quality, overall diet composition and environmental factors, to optimize hoof health and reduce the incidence of foot rot (Raju *et al*., 2022; Mohammed *et al*., 2024).

Medication selection

Selenium and zinc deficiencies were addressed *via* injectable *Sodiferol* and oral *Thespophor Oligo* providing more controlled dosing than mineral blocks, which may induce copper toxicity (Faye *et al*., 1984). Although the effectiveness of the treatment depends on several factors (Cote, 2005), *Sodiferol* and *Thespophor Oligo* were selected due to their availability in Algeria. Parenteral administration of sodium selenite has demonstrated benefits in promoting foot rot healing (Hall *et al*., 2011). While selenium levels substantially improved following treatment, serum zinc declined and copper levels remained elevated (Fig 4). These differential responses underscore the necessity of tailored supplementation strategies that consider the distinct metabolic profiles of each trace element. Narrow confidence intervals for selenium indicate consistent intra-group responses, whereas the wider intervals for zinc and notably copper during advanced disease stages reflect individual variability and the complex nature of trace element metabolism affecting disease progression and response to therapy.

CONCLUSION

Our results demonstrate a significant positive correlation between selenium and foot rot, identifying selenium deficiency as the earliest and most prominent marker directly linked to disease progression. A sharp decline from stage 1, progressing to severe deficits at advanced stages, underscoring the need for early monitoring and supplementation. By contrast, correlations with copper and zinc were weak and statistically non-significant. Zinc deficiency, though consistent, showed little variation across stages, suggesting a predisposing rather than severity modulating role. Conversely, marked copper elevation in advanced cases indicates involvement in inflammatory processes and may serve as useful marker of progression.

Taken together, selenium decline and copper increase are strongly associated with severity, whereas zinc plays a minor role. Copper and selenium may thus serve as key biochemical indicators to classify risk levels and guide preventive strategies. The crucial role of selenium highlights the value of targeted supplementation, though confirmation in larger, more varied populations is needed.

Figures illustrate how mineral profiles influence pathophysiology and therapeutic response, with selenium emerging as the most sensitive marker. Persisting zinc deficits and copper excess emphasize the need for individualized herd management. Serial monitoring of trace elements as outlined in Fig 4, 7, could optimize both prevention and treatment. Regular monitoring of trace elements particularly selenium and copper in severe cases.

Overall, these findings clarify the biochemical changes linked to foot rot and the limits of current zinc and copper supplementation, while emphasizing selenium's central role. They provide a rationale for more comprehensive trace element management in both clinical and herd settings.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but disclaim any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for the experiments were approved by the Experimental Animal Care Committee. Care and handling techniques were approved by the University Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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