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Copper and zinc dynamics in ovine pneumonia: a comparative analysis of treatment regimens

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Abstract

Pneumonia remains a significant economic burden on the small ruminant industry. Excessive inflammation, oxidative stress, and alterations in copper and zinc can accompany pneumonia. As these micro-nutrients play crucial roles in immune function and anti-oxidant defence, modulating their levels may influence the disease progression. This study aimed to investigate the effects of different therapeutic regimens on copper and zinc status in lambs with pneumonia. Twenty lambs with pneumonia were randomly assigned to four treatment groups: oxytetracycline and tylosin (OT), OT plus vitamin B1 (OTVB1), OT plus vitamin C (OTVC), and OT plus vitamin B1 and vitamin C (OTVB1C). A control group received only distilled water. Blood samples were collected on days 1, 3, 6, and 14 for subsequent assessment of plasma copper and zinc concentrations. While the control group maintained stable levels, the pneumonic groups exhibited varying degrees of changes. Plasma copper concentrations increased significantly in all pneumonic groups compared to the control group throughout the study. The OT and OTVB1C groups had the highest number of lambs with increased copper level. Plasma zinc concentrations decreased significantly in the OT and OTVB1 groups, with the lowest levels in the OTVB1 group on day 3. The OTVC group mirrored the control group with stable levels. The OTVB1C group, compared to the other groups, showed a more persistent reduction. These findings suggest that the effects of the various treatment regimens on plasma copper and zinc levels may be complex and time-dependent.

Introduction

Ovine pneumonia is a common respiratory disease in livestock, causing significant economic losses in the small ruminant industry. These losses result from mortality, prolonged illness, reduced feed efficiency, slower growth, decreased productivity, and increased treatment costs, along with carcass downgrading and slaughterhouse wastage.[1-3] Various infectious agents, in conjunction with triggering factors, such as environmental conditions, stressful situations (*e.g.*, transportation), poor nutrition, and impaired immune function, predispose animals to pneumonia. A severe and uncontrolled inflammatory response coupled with the excessive release of free radicals during pneumonia can cause significant damage to lung tissues. This destructive process targets the pulmonary epithelium and its supporting structures, leading to the destruction of airways and alveoli. This damage can have profound consequences for respiratory function. To prevent further lesions, it is crucial to control inflammatory processes and enhance anti-oxidant enzyme activity. However, the regulation and extent of anti-oxidant enzyme activity in response to oxidative stress are not solely dependent on the levels but are closely linked to the availability of trace elements, such as zinc and copper.[4]

Copper is a key cofactor for anti-oxidant enzymes, like superoxide dismutase and ceruloplasmin, which protect cells from oxidative damage. It also influences prostaglandin synthesis and inflammation regulation.[5-7] Low copper levels can impair immune function, leading to increased susceptibility and severity of infections.[8]

Zinc is another essential micro-nutrient that helps prevent lipid peroxidation by neutralizing free radicals and maintaining adequate glutathione levels.[6,9] It is a cofactor for over 300 enzymes, including superoxide dismutase, and is involved in various metabolic processes, like carbohydrate metabolism, tissue growth and repair, immune function, hormonal regulation, and apoptosis.[10]

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Changes in zinc concentrations within immune cells can significantly impact the overall immune system.[11,12]

Antibiotics constitute the primary treatment regimen for infectious pneumonia, with the specific choice of antimicrobial agent determined by factors, such as diagnosis, veterinary expertise, and susceptibility testing.[13] Oxytetracyclines and tylosin are commonly recommended for respiratory diseases.

To facilitate recovery, adjunctive therapies, including ascorbic acid (vitamin C) and thiamine (vitamin B1), are frequently employed due to their immune-enhancing and anti-inflammatory properties.[6,14] A reduction in vitamin C levels can adversely affect neutrophil function in pneumonia cases.[15] Moreover, both vitamin B1 and vitamin C exhibit anti-inflammatory effects, contributing to diminished apoptosis and endothelial damage.[6,8,16]

Pneumonia can be accompanied by excessive inflammation, oxidative stress, and alterations in the micro-nutrient's status, particularly copper and zinc. Given their crucial roles in immune function, enzyme activity, and anti-oxidant defence, modulating these trace elements may influence the disease progression and outcome. This study investigated the effects of various treatment interventions on copper and zinc status in lambs with pneumonia, conducted in a region where deficiencies in certain trace elements are relatively common. In addition to oxytetracycline and tylosin, commonly used antibiotics for treating pneumonia, vitamin C and vitamin B1 were administered to explore their potential interactions with copper and zinc in lambs affected by pneumonia.

Materials and Methods

Animals. All procedures in this study were approved by the Animal Ethics Committee of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran (IR-UU-AEC-3/70). The study was conducted in the northwest of Iran, a region noted for its prevalence of both sub-clinical and clinical deficiencies in specific trace elements, particularly copper and zinc. A cohort of 20 male lambs, aged eight to 12 months and diagnosed with pneumonia, was utilized for this study. The pneumonic lambs exhibited clinical signs, including anorexia, depression, fever, nasal discharge, dyspnea, tachypnea, coughing, and abnormal lung sounds. Additionally, their leukogram displayed an inflammatory profile characterized by leukocytosis, neutrophilia, and the presence of toxic neutrophils. These pneumonic lambs were randomly assigned into four groups, each comprising five lambs, as follows: OT (oxytetracycline and tylosin) group: Received intra-muscular injections of 11.00 mg kg^{-1} oxytetracycline (Razak, Karaj, Iran) and 18.00 mg kg^{-1} tylosin (Razak) for 5 consecutive days.[13] OTVB1 (OT plus vitamin B1) group: Received the same antibiotic regimen as the OT group alongside daily intravenous injections of vitamin B1 hydrochloride (Nasr, Fariman, Iran) at a dose of 20.00 mg kg^{-1} for 5 days.[13] OTVC (OT plus vitamin C) group: Received the same antibiotic regimen as the OT group alongside daily intra-venous injections of vitamin C (Darou Pakhsh, Tehran, Iran) at a dose of 2.50 g *per* lamb for 5 days. OTVB1C (OTVB1 plus vitamin C) group: Received the combined treatment of antibiotics, vitamin B1, and vitamin C as in the aforementioned groups. In addition, a control group consisted of five clinically healthy, age-matched male lambs with normal leukocyte profiles, showing no signs of pneumonia or any other illness, randomly selected from the flock. This group received only intra-venous distilled water at the same volume administered to the pneumonic groups.

Sample collection and laboratory measurements. Jugular vein blood samples from each lamb were collected into heparinized tubes on days 1, 3, 6, and 14. The samples were centrifuged at 1,000 *g* for 10 min, and the plasma was removed for mineral analysis. Copper and zinc concentrations were measured using an automated biochemistry analyzer (BT1500; Biotechnica, Rome, Italy) with commercially available kits from Biorexfars Innovative Diagnostics (Shiraz, Iran), following the manufacturer's protocols. In brief, zinc concentrations were quantified using a direct colorimetric method based on the 5-bromo-2-pyridylazo-5′-diethylaminophenol reaction. In this assay, zinc ions react with 5-bromo-2-pyridylazo-5′-diethylaminophenol in an alkaline, buffered medium, forming a stable, colored complex. The intensity of the resulting color is directly proportional to the zinc concentration in the sample. The absorbance of the zinc-complex is measured at 546 nm. Similarly, the copper assay utilises the 3,5-dibromo-2-pyridylazo-N-ethyl-N-(3-sulfopropyl) aniline chromogenic method. In this method, copper ions, primarily bound in the ceruloplasmin-copper complex, are released in a weakly acidic buffer (pH: 4.70). The free copper ions subsequently react with the 3,5-dibromo-2-pyridylazo-N-ethyl-N-(3-sulfopropyl) aniline chromogen to form a blue-colored chelate. The intensity of the blue complex exhibits a direct proportionality to the copper concentration in the sample. This intensity is measured at a wavelength of 578 nm.

Statistical analysis. Statistical analysis was performed using SPSS Software (version 27.0; IBM Corp., Armonk, USA). Data were visually inspected for outliers and extreme values. The normality of the data distribution and homogeneity of variance were assessed using the Shapiro-Wilk test and Levene's test, respectively. To evaluate the impact of treatments on the plasma concentrations of copper and zinc over time, a repeated measures ANOVA was conducted. The sphericity assumption was verified using Mauchly's test, and multiple comparisons were adjusted using Bonferroni method. Statistical significance was determined at a *p* value threshold of 0.05. Results are presented as mean ± standard error. The observed statistical power for all analyses of the main effects of time, group, and their interaction was greater than 0.80, indicating that the sample size was sufficient to detect statistical significance.

Results

Plasma copper concentrations. Statistical analysis of copper concentrations in plasma revealed significant main effects of time ($p < 0.001$), treatment ($p < 0.001$), and their interaction ($p < 0.001$).

Within-group comparisons across time. Throughout the study, plasma copper concentrations remained stable in the control group. In the pneumonic groups, there was a non-significant decrease on day 3, followed by significant increases (Fig. 1). Increases were observed in the OT and OTVB1 groups from day 1 to 14, day 3 to 6, and day 3 to 14 ($p < 0.05$). The OTVC group showed significant increases from day 1 to 6, day 1 to 14, day 3 to 6, and day 3 to 14 ($p < 0.05$). Significant increases in the OTVB1C group were noted from day 1 to 6, day 1 to 14, and day 3 to 6 and 14 ($p < 0.01$). No other significant changes were observed.

Between-group comparisons within time. On day 1, all pneumonic groups had significantly higher plasma copper concentrations than the control group ($p < 0.05$; Table 1). This trend persisted on day 3, with the OT and OTVC groups remaining significantly elevated compared to the control group ($p < 0.01$). On day 6, all pneumonic groups maintained significantly higher levels than the control group ($p < 0.001$). On day 14, all pneumonic groups had significantly lower levels than the control group ($p < 0.001$). No significant differences were observed among the pneumonic groups at any time point (Table 1). Overall, all pneumonic groups consistently exhibited elevated plasma copper concentrations compared to the control group. While significant differences occurred between the control and specific pneumonic groups at certain time points, no significant differences were observed among the pneumonic groups.

Comparisons of plasma copper concentrations to normal reference ranges. The normal plasma copper range for sheep is 63.00 - 127.00 μg dL^{-1} (10.00 - 20.00 μmol L^{-1}).[13] Initially, all lambs exhibited normal copper levels. However, by days 6 and 14, some individuals in the OT, OTVB1, OTVC, and OTVB1C groups developed elevated copper levels. The OT and OTVB1C groups had the highest incidence of elevated copper levels, with maximum levels reaching 139.10 μg dL^{-1} in the OTVB1C group. While exceeding the normal range, this increase was relatively modest.

Plasma zinc concentrations. Statistical analysis revealed significant effects of time ($p < 0.001$), treatment ($p < 0.001$), and their interaction ($p < 0.001$) on plasma zinc concentrations.

Within-group comparisons across time. Plasma zinc concentrations remained stable in the control group (Fig. 2). In contrast, the OT and OTVB1 groups showed biphasic patterns, with significant decreases from day 1 to 3 ($p < 0.01$) and increases from day 3 to 14 ($p < 0.05$). The OTVC group had no significant changes, while the OTVB1C group showed significant increases from day 1 to 3, 6, and 14 ($p < 0.05$). The lowest zinc level was observed in the OTVB1 group on day 3. Overall, the OT, OTVB1, and OTVB1C groups displayed initial decreases followed by increases in plasma zinc levels, but these levels remained lower than the control group at subsequent time points. These findings suggest the treatments effects on plasma zinc levels are complex and time-dependent.

Between-group comparisons within time. On day 1, there were no significant differences between the control group and the pneumonic groups, or among the pneumonic groups themselves (Table 1). By day 3, plasma zinc concentrations had significantly dropped in the OT, OTVB1, OTVC, and OTVB1C groups compared to the control group ($p < 0.05$). Notably, the OTVB1 group displayed significantly ($p < 0.01$) lower levels than the OT and OTVC groups, and lower but non-significant levels compared to the OTVB1C group. Additionally, the OTVB1C group had significantly reduced levels in comparison

Table 1. Mean plasma concentration of copper and zinc in the different study groups.

Variables	Groups						Standard error	*p*-value		
	Days	Control	OT	OTVB1	OTVC	OTVB1C		Days	Groups	Days × Groups
Copper (μg dL^{-1})	1	81.70 ^{b}	105.20 ^{a}	99.10 ^{a}	96.80 ^{a}	91.60 ^{a}	4.07	< 0.001	< 0.001	< 0.001
	3	74.80 ^{b}	95.90 ^{a}	87.50 ^{ab}	95.60 ^{a}	85.00 ^{ab}	3.60			
	6	70.90 ^{b}	116.00 ^{a}	106.80 ^{a}	111.60 ^{a}	111.20 ^{a}	3.80			
	14	79.50 ^{b}	126.60 ^{a}	119.20 ^{a}	121.40 ^{a}	124.30 ^{a}	3.90			
Zinc (μg dL^{-1})	1	46.10 ^{a}	49.30 ^{a}	50.00 ^{a}	42.70 ^{a}	42.70 ^{a}	2.20	< 0.001	< 0.001	< 0.001
	3	52.40 ^{a}	29.50 ^{bc}	13.40 ^{d}	38.90 ^{b}	24.90 ^{cd}	2.90			
	6	49.40 ^{a}	34.00 ^{b}	33.70 ^{b}	39.90 ^{ab}	30.70 ^{b}	2.60			
	14	46.30 ^{a}	41.30 ^{ab}	33.90 ^{ab}	41.50 ^{ab}	28.80 ^{b}	2.90			

Control: clinically healthy lambs received distilled water, OT: Pneumonic lambs received antibiotics (oxytetracycline and tylosin) alone, OTVB1: Pneumonic lambs received antibiotics with vitamin B1, OTVC: Pneumonic lambs received antibiotics with vitamin C, OTVB1C: Pneumonic lambs received antibiotics with both thiamine and vitamin C.

^{abc} Within each day, mean values that do not share a lowercase letter are significantly different between groups ($p < 0.05$).

with the OTVC ($p < 0.05$). On day 6, the control group maintained significantly higher levels than the OT, OTVB1, and OTVB1C groups ($p < 0.001$), with no significant differences among the pneumonic groups. By day 14, the control group continued to exhibit significantly higher levels than the OTVB1C group ($p < 0.01$), while no significant differences were noted between the control group and the other pneumonic groups or among the pneumonic groups themselves. In summary, the pneumonic groups experienced varying degrees of reduction in plasma zinc concentrations, with the most pronounced decreases observed on day 3, particularly in the OTVB1 group.

Comparisons of plasma zinc concentrations to normal reference ranges. The normal plasma zinc concentration range for sheep is 80.00 – 120.00 µg dL^{-1} (12.20 - 18.20 µmol L^{-1}), with levels below 39.00 µg dL^{-1} indicating zinc deficiency.[13] In this study, all lambs, including both pneumonic and clinically healthy individuals, exhibited plasma zinc concentrations indicative of sub-clinical or marginal zinc deficiency, without overt clinical signs. While the control group maintained marginal zinc levels throughout the study, some pneumonic lambs developed plasma zinc concentrations corresponding to deficiency (< 39.00 µg dL^{-1}) from day 3 onwards. On day 3, all lambs in the OT, OTVB1, and OTVB1C groups, as well as three lambs in the OTVC group, had zinc concentrations indicative of deficiency. On day 6, all lambs in the OT, OTVB1, OTVC, and OTVB1C groups exhibited plasma zinc concentrations within the deficiency range. This trend persisted through day 14, with all lambs in the OT, OTVB1, OTVC, and OTVB1C groups maintaining zinc concentrations below the deficiency threshold.

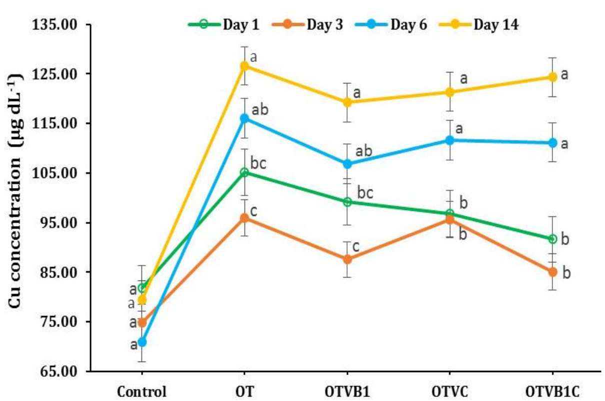

Fig. 1. Plasma copper (Cu) concentrations in clinically healthy lambs (control group; received distilled water) and pneumonia-affected lambs treated with antibiotics (oxytetracycline and tylosin) alone (OT), or antibiotics co-administered with vitamin B1 (OTVB1), vitamin C (OTVC), or both vitamins (OTVB1C).

[abc] Within each group, means without a shared letter differ significantly between days ($p < 0.05$).

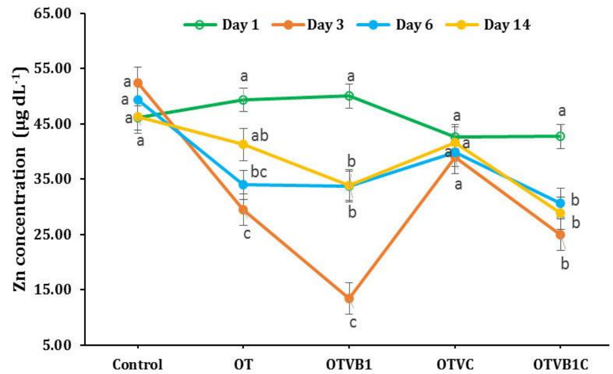

Fig. 2. Plasma zinc (Zn) concentrations in clinically healthy lambs (control group; received distilled water) and pneumonia-affected lambs treated with antibiotics (oxytetracycline and tylosin) alone (OT), or antibiotics co-administered with vitamin B1 (OTVB1), vitamin C (OTVC), or both vitamins (OTVB1C). Comparisons were made vertically along the y-axis.

[abc] Within each group, mean values not sharing a lowercase letter are significantly different between days ($p < 0.05$).

Discussion

The present study revealed a significant elevation in copper concentrations over time in all pneumonic groups, irrespective of the treatment regimen, compared to the control group, particularly from day 3 onwards. Notably, this increase was apparent in the pneumonic groups even prior to the initiation of treatment. However, subtle differences were observed between the OTVB1 and OTVB1C groups and the other pneumonic groups. Specifically, while all other pneumonic groups consistently displayed higher copper concentrations compared to the control group on all days, the OTVB1 and OTVB1C groups exhibited a trend similar to the control group on day 3, albeit with higher absolute values. Overall, although no discernible differences were observed among the pneumonic groups, the observed increases in plasma copper concentrations suggest a potential role for this trace element in the pathogenesis of pneumonia. Regarding zinc concentrations, the findings indicate a differential response among the pneumonic groups. The significant decreases in plasma zinc concentrations observed in the OT, OTVB1, and OTVB1C groups, in contrast to the stable levels in the control and OTVC groups, highlight the potential involvement of zinc in the pathophysiology of pneumonia and its modulation by therapeutic interventions. Notably, the OTVB1 group experienced the lowest zinc levels on day 3.

Vitamin C has been demonstrated to protect against apoptosis by enhancing immune cells activity and reducing the release of pro-inflammatory mediators.[17] It plays a crucial role in maintaining redox homeostasis within cells, protecting against reactive oxygen species and reactive nitrogen species during oxidative burst, re-generating other essential anti-oxidants, such as glutathione and vitamin E, and modulating cytokine production. Additionally, vitamin C possesses anti-microbial effects, with elevated levels enhancing anti-microbial activity, increasing plasma levels of complement proteins, and participating in interferon production.[7] Notably, leukocyte vitamin C levels are significantly reduced following respiratory infections.[17] Given their predominantly dry forage and straw diet, sheep may be susceptible to vitamin C deficiency, especially when grazing periods are limited.[13] Furthermore, infections can increase metabolic demands and vitamin B1 utilization, leading to potential vitamin B1 deficiency, which can adversely affect immune response, metabolic function, and the body ability to combat infectious agents.[18] Therefore, the administration of vitamins B1 and C offers significant therapeutic benefits in mitigating oxidative stress and inflammatory responses associated with respiratory infections.[16,19-21]

Trace elements, such as copper and zinc, possessing anti-oxidant properties, not only regulate host immune responses but also influence microbial genome modification.[22] Copper and zinc are essential minerals playing crucial roles in immune system function, enzyme activity, and anti-oxidant defence. During infections, the demand for these trace elements increases due to their involvement in immune responses and oxidative stress modulation. Consequently, deficiencies or imbalances in copper and zinc can impair immune function and prolong recovery from infections.[23-26]

While several nutrient metals are reduced or restricted during infections, a gradual increase in plasma copper is a common characteristic, regardless of the causative agent. This elevation in copper is primarily attributed to cuproprotein ceruloplasmin, constituting approximately 95.00% of the plasma copper content. In line with its role during infections, ceruloplasmin expression is significantly up-regulated, leading to a marked increase in protein levels. It is plausible that the increased abundance of ceruloplasmin facilitates the delivery of copper to the site of infection, as monocytes, granulocytes, and lymphocytes possess ceruloplasmin receptors.[27] Ceruloplasmin activity rises during oxidative stress to facilitate the synthesis of anti-oxidant enzymes, like superoxide dismutase and ceruloplasmin, which neutralize free radicals.[13] The elevated plasma copper levels observed in this study prior to treatment initiation in the affected groups may be attributed to this mechanism, consistent with findings in calves and lambs with pneumonia.[5,28,29] A significant correlation between copper deficiency and occurrence of pneumonia has been reported; copper deficiency disrupts the synthesis of enzymes, such as superoxide dismutase, compromising the animal immune system and increasing susceptibility to pneumonia.[30] The observed decrease in plasma copper concentrations on the third day of treatment may be attributed to anorexia, fever, impaired tissue oxidation, and accumulation of albumin-copper complexes within the red blood cells of affected animals. The subsequent increase in plasma levels on the 6th and 14th days following treatment may be attributed to the recovery of the animals and improved nutritional status.[31-32] However, the changes in copper concentrations appear to be a long-term effect in pneumonic lambs. Even after complete clinical recovery at the end of the study (day 14), the level of copper remained significantly higher than the control group. The underlying cause and significance of such persistent elevation in copper concentrations following clinical recovery remain unclear. However, a potential contributing factor may be the continuation of a sub-clinical inflammatory process. Importantly, not only copper levels approached higher levels than the control group, but they also exceeded the upper limit of the normal reference range, particularly in the OTVB1C group. The underlying mechanisms for this phenomenon, particularly when incorporating a combination of vitamins B1 and C, require further investigation through detailed studies.

A decrease in blood zinc concentration can be caused by factors unrelated to zinc status, such as the acute phase response. Inflammation leads to rapid zinc redistribution from plasma to organs, particularly the liver, resulting in decreased plasma zinc levels. This decline may be an adaptive response to deprive invading pathogens of zinc. Additionally, macrophages increase intra-cellular zinc to intoxicate phagocytosed microorganisms. Chelation of zinc by calprotectin, released by leukocytes, can also contribute to hypozincemia. Calprotectin suppresses bacterial and fungal reproductions, while increased intra-cellular zinc plays roles in energy metabolism, anti-oxidant defence, and protein synthesis. Therefore, zinc redistribution during inflammation serves multiple purposes.[33] Plasma zinc levels decrease during infections and severe inflammatory diseases due to the increased demand by immune cells.[34] In pneumonic groups, the reduction in plasma zinc concentration may be attributed to the reduced feed intake, its utilization for the anti-oxidant enzymes synthesis, and decreased availability to prevent the replication and growth of infectious agents.[35,36] Zinc deficiency can lead to carbonic anhydrase dysfunction, impairing the body ability to eliminate CO_2 from the lungs and exacerbating pneumonia symptoms.[29] Human studies have also identified low zinc levels as a risk factor for pneumonia.[37] Furthermore, combining zinc with antibiotic treatment in children with pneumonia has been shown to shorten the duration of treatment.[38] In livestock, zinc supplementation in the diet reduces fever.[13]

From day 3 onwards, a notable number of lambs exhibited zinc concentrations lower than 39.00 μg dL^{-1}, indicating a concentration corresponding to the deficiency level which could have implications for health and productivity. The lowest level of zinc was observed in the OTVB1 group with a mean value of 13.40 μg dL^{-1}, occurred on day 3. However, the group with the greatest zinc reduction was the OTVB1C group, consistently exhibited reduced zinc levels throughout the study period. On day 3, all lambs in this group had blood zinc concentrations within the deficiency range (20.90 - 37.30 µg dL^{-1}). This trend continued on day 6 and day 14, with all lambs in the OTVB1C group remaining at deficient levels (20.90 - 37.60 µg dL^{-1}). This indicates a persistent and severe zinc reduction in the OTVB1C group compared to the other groups. In contrast to the OTVB1 and OTVB1C groups, the OTVC group exhibited non-significant fluctuations in plasma zinc levels, suggesting that the use of vitamin C may prevent severe alterations in this element during pneumonia. The significant variations in the OTVB1 and OTVB1C groups across different days may indicate the reducing effects of vitamin B1 on plasma zinc levels. Reduced zinc availability is a defence mechanism to prevent bacterial proliferation.[13] This effect of vitamin B1 may have beneficial therapeutic implications for infectious pneumonia. However, severe reductions in zinc levels may also adversely affect immune system competence and lung function. Further research is necessary to elucidate the effects of vitamin B1 and vitamin C in the context of infectious processes, such as pneumonia.

In the area of this study, sub-clinical or clinical deficiencies of certain trace elements, primarily copper and zinc, are common. Despite exhibiting blood zinc concentrations at deficiency levels, as evidenced by plasma zinc concentrations below 39.00 µg dL^{-1}, none of the lambs displayed overt clinical signs of zinc deficiency during the 14-day study period. This may be attributed to the relatively short duration of the study, which may not have been sufficient to manifest clinical signs. Additionally, the absence of clinical signs during follow-up suggests that the decreased blood zinc levels may have been temporary. However, such deficiencies may create some adverse complications, which may not be easily discernible, such as reduced immune competence, increased rates of microbial infections and parasitic infestations, or reduced feed conversion rates. These issues need to be addressed to ensure optimal health and productivity in sheep.

It is noteworthy that not all studies have reported consistent results, particularly regarding copper concentrations in various disorders. In studies examining parasitic diseases in sheep caused by *Babesia ovis*, *Dicrocoelium dendriticum*, and hydatid cysts, plasma copper and zinc concentrations in infected animals were found to be lower than the control group.[35,39-41] Similarly, research on camel pneumonia and chronic airway disease in horses demonstrated that plasma copper and zinc levels in affected animals were lower than the control group.[4,9,42] While these findings align with the present study regarding zinc, they differ for copper, potentially due to the specific disease-causing factors, animal species involved, and the duration of the study.

One limitation of this study is the relatively small sample size. Additionally, the absence of inflammatory cytokine assessment and absence of a group receiving anti-inflammatory drugs hindered a more comprehensive understanding of the underlying mechanisms involved in copper and zinc fluctuations during infectious conditions. Furthermore, including a group with normal zinc levels would facilitate a clearer elucidation of the dynamics of zinc in zinc-sufficient and zinc-deficient animals.

Overall, this study demonstrated that plasma copper and zinc concentrations in lambs with pneumonia were significantly influenced by both the treatment regimen and time. Plasma copper concentrations consistently increased in all pneumonic groups compared to the control group throughout the study period. In contrast, plasma zinc concentrations significantly decreased in the OT, OTVB1, and OTVB1C groups, with the OTVB1 group exhibiting the lowest levels on day 3. The OTVC group mirrored the control group with stable zinc levels. These findings suggest that the effects of the treatments on plasma copper and zinc levels are complex and time-dependent. The biphasic patterns observed in the OT, OTVB1, and OTVB1C groups highlight the dynamic nature of these changes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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