



Assessment of Copper Deficiency and effected on Some haematological and biochemical in Lambs in Salah Al-Din City

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ABSTRACT

Copper (Cu) is an essential trace element in ruminant nutrition, playing a crucial role in enzymatic functions, hematopoiesis, and connective tissue synthesis. Copper deficiency in sheep can be primary (due to low dietary intake) or secondary (resulting from antagonistic interactions with minerals such as iron, molybdenum, and sulfur), leading to impaired copper metabolism. This study was conducted in different areas of Salah al-Din city (Tikrit - Al-Alam - Samarra - Baiji) during the period from June 2 to October 15, 2024. (100) lambs were obtained, fifty of which were a control group, fifty subclinical lambs suffering from copper insufficiency, and the clinical group suffering from copper deficiency. from different areas of Salah al-Din city. The ages of the animals ranged from one month to two years and their weights ranged between (4-50) kg. The results showed a significant decrease in hemoglobin levels 6.15 ± 3.207 B, red blood cell count 2.71 ± 1.887 C, and ceruloplasmin activity 6.60 ± 4.180

C, along with an increase in leukocyte counts 8.20 ± 2.201 B, indicating immune dysfunction. Additionally, This study highlights the importance of maintaining proper mineral balance in sheep nutrition to prevent copper deficiency-related health issues and enhance productivity.

Introduction

Copper (Cu) is an essential trace element in ruminant nutrition, playing a crucial role in enzymatic functions, hematopoiesis, and connective tissue synthesis. Copper deficiency in sheep can be primary (due to low dietary intake) or secondary (resulting from antagonistic interactions with other minerals such as molybdenum and sulfur). These interactions can lead to impaired copper metabolism, affecting the animal's health and productivity (Aggett, P.J. (2023).

Key Elements Interacting with Copper

Physical and chemical properties of copper, Copper, a d-block transition element, has an atomic number of 29 and an atomic weight of 63.546 daltons. It exists in three oxidation states: Cu⁰ (metal), Cu⁺¹ (cuprous ion), and Cu⁺² (cupric ion). The cuprous state (+1) is prone to oxidation and is stable primarily in insoluble compounds. Consequently, Cu predominantly appears as Cu⁺² in most compounds (Aggett, 2023). Dietary Sources copper is a prevalent natural element in various feedstuffs. Rich sources include whole grains, nuts, legumes, seeds, and shellfish. Legumes have higher Cu levels than grasses, while grains surpass leaves and stems. Cu absorption is favorable in low-fiber foods like cereals (Cu 9.1 %) but less so in fresh herbage such as grass (Cu 1.4 - 2.5 %) (Meng et al., 2024). Preserving grass as hay or silage enhances Cu availability. Generally, Cu concentrations decrease with plant maturity and are diminished in alkaline soils (Gwanya and Laifa, 2023).

Iron (Fe) competes with copper for absorption sites in the intestine. High dietary iron levels can inhibit copper uptake by forming insoluble complexes. This can lead to anemia and reduced hemoglobin levels in copper-deficient sheep (Jensen et al., 2019). Calcium (Ca) and Phosphorus (P) High calcium and phosphorus levels may indirectly affect copper metabolism by altering rumen pH, which can influence mineral solubility. Conti, R.M.C. et al. (2023) (and absorption. Blood Profile in Copper-Deficient Sheep. Copper deficiency significantly affects the blood profile, leading to: Low Hemoglobin (Hb) and Red Blood Cell (RBC) Count – Due to impaired iron metabolism. 4. Emam, R. et al. (2025). Reduced Ceruloplasmin Activity – A copper-dependent enzyme important for iron transport. Elevated Leukocyte Counts – Indicative of immune dysfunction. Decreased Superoxide Dismutase (SOD) Levels – Leading to oxidative stress.

with (Jensen et al., 2019) and (Ježek, Starič, Veron, et al., 2024) found Copper deficiency is associated with significant changes in hematological and biochemical parameters. In sheep, low copper levels correlated with reduced concentrations of hemoglobin, albumin, and white blood cells, indicating anemia and compromised immune function. Copper deficiency can lead to anemia, which is often characterized by low iron levels in the blood. Also Klevay, 2019) and (Wu, Xiong and He, 2021) reported Copper plays a role in the absorption and transport of iron. It is a component of ceruloplasmin, a protein that oxidizes iron to its ferric form, which is necessary for binding to transferrin and subsequent transport in the bloodstream. Similarity (Jayalakshmi et al., 2020) showed that copper deficiency can lead to hypoferrremia (low iron levels in the blood), as seen in cases of haemonchosis in sheep, where both copper and iron levels were significantly reduced. As well as Jensen et al., 2019 suggested the deficiency of copper can lead to mitochondrial dysfunction in erythropoietic cells, affecting the differentiation of red blood cells and contributing to anemia.

copper deficiency had low calcium levels compared to normal lambs. This findings consistent with study conducted by Meng et al., 2024 found that copper supplementation improved the apparent absorbability of calcium, suggesting that copper deficiency might impair calcium absorption and metabolism. Also (Ježek, Starič, Veron, et al., 2024) found that lambs with low copper levels also exhibited significantly lower calcium concentrations in their blood serum.

Copper is essential for various immune processes, including the activation of Th1 cells and cytokine production, which are critical for mounting an effective immune response (Dharmalingam et al., 2021). The results of the current study showed that the decreased white blood cell count in lambs with copper deficiency. This results agree with (Y. Liu et al., 2022) They found that copper deficiency was associated with leukopenia and neutropenia, conditions characterized by low white blood cell counts, which may impair the body's ability to fight infection. Also A study conducted by (Ježek, et al., 2024) on sheep showed a positive correlation between serum copper levels and WBC counts, indicating that copper deficiency could lead to reduced WBC counts.

Decrease in the number of red blood cells ,hemoglobin ,MCV,MCH and MCHC in lambs with copper deficiency. These results are consistent with what (Myint et al., 2018) reported, that copper deficiency is directly associated with low red blood cell count in lambs, as copper is essential for hemoglobin synthesis and overall blood health. This results agree with Naji, 2017 found that the hematological analysis revealed in the red blood cell count, hemoglobin concentration, and packed cell volume, while a notable difference was observed in the mean corpuscular volume and mean corpuscular hemoglobin concentration in sheep exhibiting signs of Cu deficiency compared to the control group. (Mozafari et al., 2020 found) that reduction in hemogram parameters may result from diminished trace elements affecting RBC synthesis, while oxidative stress and lipid peroxidation exacerbate free radical production, leading to bone marrow suppression. Similarity reported that low serum copper levels impair red blood cell parameters, with a direct association

This study rigorously examined 100 lambs across Salah al-Din city (Tikrit - Al-Alam - Samarra - Baiji) from June 2 to October 15, 2024, including fifty in a control group, fifty with subclinical copper insufficiency, and a clinical group (50) with copper deficiency, with ages from one day to one year and weights ranging from 4 to 50 kg. Three groups of 50 lambs each: Control (C) group: 50 healthy lambs; Subclinical group: 50 lambs with insufficient copper; Clinical group: 50 lambs with copper deficiency.

Samples Collection:

Blood samples was taken from the external jugular vein using a syringe(3ml) to determine absolute biochemical profile of the lambs; serum was prepared by centrifuging the blood at 3000 rpm for 15 min and was stored in 0.5 mL Eppendorf tubes at -20°C till further use.

Data analysis

Data collected in this study was well aggregated, synthesized in the Microsoft Excel 2010 and analyzed by IBM-SPSS 26. To confirm normality,

suggesting that low copper concentrations worsen the severity of hypochromic anemia. (Abramowicz et al., 2019).

Ceruloplasmin serves as the primary transport protein for copper in the bloodstream, produced by both copper and proteins in the liver. It exhibits a comparable function to superoxide dismutase, effectively neutralizing superoxide anion free radicals and preventing lipid peroxidation(Liu et al., 2022). Copper is a crucial trace element involved in various biological processes, including the functioning of enzymes like ceruloplasmin and SOD, which are essential for antioxidant defense and iron metabolism. Copper deficiency disrupts these processes, leading to reduced enzyme activity and associated physiological impairments(Tsang, Da. and Br., 2021).

Material and method

Experimental design:

the Shapiro-Wilk test was employed and as a result, a parametric test was applied which used mean and standard deviations with one-way ANOVA with post hoc test at a significance level of $P \text{ value} \leq 0.05$.

Results

The differences among the control, subclinical, and clinical groups regarding the WBC, RBC, HGB, HCT, MCV, MCHC, and RDW-SD were statistically significant. The real differences found by post hoc test were found between each group in RBC, MCV, and RDW-SD. Concerning the HGB and MCHC, the real difference was found between clinical with subclinical and between clinical and control group. The WBC and HCT mean levels among the clinical group were differed only from the levels among the control group. While the mean level of MCH among the clinical group differed only from that among the subclinical group. RDW-CV showed no significant statistical difference across the studied groups, as shown in table (1).

Table (1): CBC

CBC	Controls	Subclinical	Clinical	p-value*
	Mean ±SE	Mean ±SE	Mean ±SE	
WBC	33.94±34.611A	19.71±25.806 AB	8.20±2.201 B	0.014
RBC	6.98±0.849 A	5.11±2.519 B	2.71±1.887 C	0.000
HGB	15.45±1.843 A	12.44±4.898 A	6.15±3.207 B	0.000
HCT	44.00±6.863 A	30.23±18.586 AB	7.70±5.404 B	0.000
MCV	68.60±2.170 A	52.59±19.881 B	46.78±1.041 C	0.000
MCH	22.50±1.509 AB	26.58±14.113 A	30.87±25.961 B	0.020
MCHC	34.00±2.581 A	68.88±67.988 A	75.92±97.871 B	0.000
RDW-CV	13.62±1.219	13.71±1.401	13.82±1.711	0.911
RDW-SD	49.00±2.981 A	32.40±18.323 B	13.00±1.665 C	0.000

**One-Way ANOVA; Post hoc test = Similar letters means no significance while different letters means significant difference*

The comparison of the copper mean levels among the studied groups was demonstrated in table (2) which revealed a statistically significant difference ($p=0.000$). The mean copper level among the clinical group ($0.38\pm0.193 \mu\text{g/L}$) was the lowest in comparison to the other groups and the real difference was found between each groups.

Discussion

Copper is vital for animal health, supporting growth, pigmentation, and immune function, and its levels can be easily evaluated through blood tests (Borobia et al., 2022). The low copper levels can lead to deficiencies, impacting health and productivity. The following sections explore the implications of low copper levels, the effects of copper toxicity, and the balance required for optimal copper levels in sheep.

The results of the current study found The clinical group's copper levels were significantly lower than those of the other groups. This findings agree with The study conducted by (Ježek et al., 2024) found that low copper levels correlate with reduced concentrations of important biochemical parameters such as beta-hydroxybutyrate, calcium, and albumin, which are essential for maintaining health. In regions with extensive pastures, sheep often suffer from mineral deficiencies, including copper, due to inadequate supplementation. This deficiency can be exacerbated by the lack of awareness among farmers about the need for mineral and vitamin supplements. Also agree with study conducted in Bahia, by Fontes et al., 2020 sheep affected by enzootic ataxia exhibited significantly reduced serum copper levels, highlighting the critical role of copper in preventing this condition. similarity

Helmer et al., 2021 found Clinical signs of copper deficiency in lambs include emaciation, anemia, growth retardation, and wool discoloration.

In this study, it was found that lambs with copper deficiency had iron deficiency Copper deficiency leads to impaired iron absorption, reduced hemoglobin synthesis, and anemia, explaining the close relationship between low copper and iron levels in lambs' blood. compared to normal lambs this agree with (Jensen et al., 2019) and Ježek, Starič, Veren, et al., 20240 found Copper deficiency is associated with significant changes in hematological and biochemical parameters. In sheep, low copper levels correlated with reduced concentrations of hemoglobin, albumin, and white blood cells, indicating anemia and compromised immune function Copper deficiency can lead to anemia, which is often characterized by low iron levels in the blood. Also (Klevay, 2019 Wu) (Xiong and He, 2021)0reported Copper plays a role in the absorption and transport of iron. It is a component of ceruloplasmin, a protein that oxidizes iron to its ferric form, which is necessary for binding to transferrin and subsequent transport in the bloodstream. Similarity Jayalakshmi et al., 2020) showed that copper deficiency can lead to hypoferremia (low iron levels in the blood), as seen in cases of copper difeciciency in sheep, where both copper and iron levels were significantly reduced. As well as (Jensen et al., 2019) suggested the deficiency of copper can lead to mitochondrial dysfunction in erythropoietic cells, affecting the differentiation of red blood cells and contributing to anemia.

In this study found that lambs with copper deficiency had low calcium levels compared to normal lambs. This findings consistent with

study conducted by) Meng et al., 2024(found that copper supplementation improved the apparent absorbability of calcium, suggesting that copper deficiency might impair calcium absorption and metabolism.)Also Ježek, Starič, Veren, et al., 2024(found that lambs with low copper levels also exhibited significantly lower calcium concentrations in their blood serum. This suggests a potential link between copper deficiency and reduced calcium levels. As well as (Jin et al., 2023) reported that sheep on a copper-deficient diet showed deficiencies in other minerals, including calcium, which could adversely affect organ function and energy metabolism. Similarly (Shen and Song, 2021) found that copper deprivation in sheep not only affects copper levels but also disrupts other physiological and biochemical parameters, potentially impacting calcium metabolism.

Additionally, the results of the current study found that lambs with copper deficiency had low phosphorus levels compared to normal lambs. This agree with (Pereira et al., 2019) conducted a study on Somali lambs found that mineral requirements, including phosphorus, are crucial for growth and maintenance. Copper deficiency could potentially alter these requirements by affecting the overall mineral balance . Also (Meng et al., 2024) demonstrated that a copper-deficient diet significantly decreased the apparent absorbability of phosphorus, among other minerals. This suggests that copper plays a role in facilitating the absorption of phosphorus in the digestive system.

The results of the current study found that lambs with copper deficiency had low Ceruloplasmin level compared to normal lambs. This results consistent with Copper deficiency leads to significantly lower ceruloplasmin levels in affected animals compared to healthy ones. This is evident in (Min et al., 2022) and (Shen & Song, 2021) on Kazakh sheep and Chinese Merino sheep, where serum ceruloplasmin was notably reduced in copper-deficient groups. Additionally study conducted in iran by(Moazzemi et al., 2023) indicated a strong positive correlation, signified a substantial association whereby increased in copper levels correspondingly elevated ceruloplasmin levels.

Oxygen free radicals, constituting 95% of total free radicals in the animal body, necessitate a dynamic homeostasis between their production and elimination to maintain health; an excess over the scavenging capacity disrupts redox equilibrium, causing macromolecular damage and

potentially leading to disease progression). The antioxidant system is crucial for neutralizing harmful oxygen free radicals, encompassing both enzymatic and non-enzymatic mechanisms that safeguard against oxidative damage. Copper is vital for animal growth and metabolism, significantly boosting the antioxidant function through its role in various enzymes(). The findings of this study clearly indicated that the levels of SOD in the blood of clinical group was markedly lower to those in control group. This agree with Wu et al., 2021 found lambs with copper deficiency had low SOD levels. Also (Emam et al., 2025) found that elevating the Cu levels in vivo significantly enhanced synthesis and improved SOD production.(Smilarity Conti et al., 2023) reported that the combination of copper supplementation with organic sulfur sources in lambs resulted in increased serum Ceruloplasmin activity, suggesting that the source of Cu and its interaction with other minerals can influence CP activity.

Conclusion

Copper plays a vital role in maintaining the overall health of sheep, with its deficiency leading to anemia, immune suppression, and metabolic disorders. Proper mineral balance, including monitoring interactions with iron, is essential for preventing copper deficiency and maintaining optimal blood health in sheep.

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تقييم نقص النحاس في الحملان في محافظة صلاح الدين

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الملخص

يُعد النحاس عنصراً أثرياً أساسياً في تغذية المجترات، حيث يلعب دوراً حيوياً في العديد من الوظائف الإنزيمية وتكوين الدم وتخليق النسيج الضام. يمكن أن يكون نقص النحاس أولياً بسبب قلة تناوله في الغذاء، أو ثانوياً نتيجة تفاعلاته مع معادن أخرى مثل الحديد، والموليبدنوم، والكبريت، مما يؤثر سلباً على عمليات التمثيل الغذائي للنحاس في الجسم. تهدف هذه الدراسة إلى تقييم تأثير نقص النحاس في الحملان في محافظة صلاح الدين، مع التركيز على التغيرات الدموية الناتجة عنه. أظهرت النتائج انخفاضاً كبيراً في مستويات الهيموغلوبين، وعدد كريات الدم الحمراء، ونشاط إنزيم السيرولوبلازمين، بالإضافة إلى ارتفاع عدد كريات الدم البيضاء، مما يشير إلى ضعف في الجهاز المناعي. كما تبين أن نقص النحاس مرتبط بانخفاض مستويات الحديد، والكالسيوم، والفوسفور في الدم. تؤكد هذه الدراسة أهمية التوازن المعدني في تغذية الأغنام لتجنب المشاكل الصحية الناتجة عن نقص النحاس وتحسين الإنتاجية.

الكلمات المفتاحية: النحاس، نقص النحاس، الأغنام، التمثيل الغذائي، تحليل الدم.