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EFFECT OF SUPPLEMENTATION WITH ORGANIC COPPER INTO BASAL DIET ON COPPER TOXICITY, AND PHYSIOLOGICAL RESPONSE OF AWASSI LAMBS

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Abstract

This study was conducted to evaluate tolerance and deficiencies of copper (Cu) of Awassi lambs fed on different levels of organic copper. Twenty-four lambs with age eight months and an average weight of 25.5 ± 3.69 kg were used over a duration of 90 days. The animals were randomly assigned to four feeding treatments: OCC (basal diet contained on premix of minerals and vitamin), OCF (basal diet without mineral premix), OCC5 (basal diet with 0.5 g organic copper / kg of feed), and OCC1 (basal diet with 1 g organic copper / kg of feed). Throughout the trial samples were collected to examine the hematological and biochemical characteristics of the blood, as well as the levels of metals in the blood, feed, and fecal samples. The results showed a significant decrease ($P<0.01$) in platelet, platelet distribution width at day 90 and procalcitonin levels at day 45 in OCC1 compared to control treatment (OCC). During the mid of experiment, the concentration of AST enzyme, copper, and iron (Fe) significantly increased in OCC1 compared to the other treatments. At the end of the experiment ALT enzyme increased significantly in OCF compared to other treatments. There were no significant variations in several blood traits across the treatments over the duration of the study. The study's results suggest that providing Awassi lambs with a diet containing more than 0.5 g/kg of Cu for a duration of 90 days did not lead to Cu toxicity or any negative health consequences.

Keywords: Awassi sheep, Copper toxicity, Haematological, Biochemical, Mineral balance.

تأثير أضافة مستويات مختلفة من النحاس العضوي للعليقة على التسمم بالنحاس والاستجابة الفسلجية في الحملان العواسية

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الخلاصة

أجريت هذه الدراسة على الحملان العواسية لتقييم قابلية التحمل والفقد للنحاس عند إضافة مستويات مختلفة من النحاس العضوي إلى عليقة. أستخدم 24 ذكراً بعمر ثمانية أشهر وبمعدل وزن 5.25 ± 3.69 كغم ولمدة 90 يومًا. وزعت الحيوانات بشكل عشوائي على أربع معاملات علفية شملت المعاملة الاولى (عليقة أساسية احتوت على خليط معادن وفيتامينات) والمعاملة الثانية (عليقة اساسية + بدون اضافة خليط المعادن) والمعاملة الثالثة (عليقة اساسية + 0.5 غم نحاس عضوي/ كغم علف) والعليقة الرابعة (عليقة اساسية + 1 غم نحاس عضوي/ كغم علف). جمعت العينات خلال التجربة لدراسات الصفات الدمية والكيموحيوية وتركيز العناصر المعدنية في الدم وكذلك عينات العلفية والفضلات. أظهرت نتائج الدراسة أن هناك انخفاض معنوي بمستوى ($P < 0.01$) في الصفائح الدموية ومعدل توزيع الصفائح الدموية في اليوم 90 و procalcitonin عند اليوم 45 في المعاملة الرابعة مقارنةً بالمعاملة الأولى (السيطرة). أما بالنسبة للصفات الكيموحيوية فقد كان هناك ارتفاع معنوي بمستوى ($P < 0.05$) في تركيز إنزيم AST والنحاس والحديد في منتصف التجربة في معاملة النحاس الرابعة مقارنة بباقي المعاملات. كما لوحظ ان هناك زيادة معنوية في نهاية التجربة في المعاملة الثانية في تركيز إنزيم ALT وبمستوى معنوية ($P < 0.01$) مقارنة بباقي معاملات التجربة. لم يكن هناك اختلاف معنوية في معظم الصفات الدمية المدروسة خلال فترة التجربة بين المعاملات. يمكن ان نستنتج من هذه الدراسة ان تغذية الحملان العواسي على عليقة تحتوي على أكثر من 0.5 غم نحاس عضوي/ كغم علف ولمدة 90 يوما لم تسبب التسمم بالنحاس او اعراض صحية غير مرغوبة.

كلمات مفتاحية: الأغنام العواسي، التسمم بالنحاس، الصفات الدمية، الكيموحيوية، التوازن المعدني.

Introduction

The concentration of mineral elements in the bloodstream of ruminants has a significant impact on several biochemical variables and physiological alterations within the body (20). Hence, the alteration in the concentration of these minerals whether in animal feed or grazing lands, poses a nutritional challenge in numerous countries worldwide (26). Sheep in dry and semi-arid locations often experience nutritional issues due to changes in the mineral composition of the plants they graze on. However, numerous sheep breeds encounter a significant issue caused by Cu toxicity. This occurs if sheep consume excessive amounts of Cu or when Cu accumulates to dangerous levels in liver (29). By means of faecal or urine, sheep have limited capacity to rid Cu from bodies, which finally results in death. Although it acts as an antioxidant as well as a vital marker for measuring Cu levels in the bloodstream, superoxide dismutase is also a One accurate way to assess the state of sheep subjected to negative side effects is by measuring blood Cu content. Furthermore, useful indicators in this evaluation are additional blood markers both cellular and biochemical (14). According to Song et al. (34), increase Cu levels in animals caused a significant rise in the MDA enzyme level in blood. Moreover, Wumeng sheep with coarse wool had far lower levels of the catalase and superoxide dismutase enzymes. Copper reduces the action of antioxidants (2). Rising Cu levels in the bloodstream and liver caused negative effects on hemoglobin levels, packed cell volume, and red blood cell count, therefore causing severe anemia in sheep (15). Safin et al. (31) claim that sheep absent of Cu had reduced Cu levels in blood serum and less activity of Cu-containing enzymes such Ceruloplasmin and Superoxide Dismutase. Inadequate Cu in the diet can have detrimental effects on blood characteristics, therefore influencing the packed cell volume (23), hemoglobin concentration, and red blood cell count. Lack of research on tolerance or depletion of Cu explains the large distribution of Awassi sheep over the Middle East and West Asia. The purpose of this study was to find whether increasing various levels of organic Cu to Awassi sheep diets affected activities. The study focused on understanding the physiological blood response, the activity of SOD and MDA enzymes, the activity of liver enzymes, and the levels of Cu and fe in blood serum. It is interesting that there is a lack of research on the trace mineral content of wild herbs specific to the geographical areas being studied.

Materials and Methods

This study was conducted according to the experimental procedures on animals proposed by the Institutional Committee for Research Ethics at the University of Anbar (Ref. 18/2024). This experiment was carried out in the ruminant farm of the College of Agriculture, University of Anbar (coordinates 33.427117N, 43.332602E). Twenty-four lambs of the local Awassi breed, eight months old with an average weight of 25.5 ± 3.69 kg were used for a 90-day period. The animals were randomly distributed into four treatments, each containing six lambs. The lambs were placed in individual cages (1 lamb per cage) with dimensions of 150 × 100 × 110 cm. The lambs were fed four diets, including OCC (control diet; basal diet + vitamin and mineral premix), OCF (basal diet + free of mineral premix), OCC5 (basal diet + 0.5 g organic copper/kg feed), and OCC1 (basal diet + 1 g organic copper/kg feed). The basal diet contained 14.33% protein and 1.7 mcal/kg metabolized energy. These diets were similar in terms of isonitrogen and isoenergetics that was formulated according to the recommendations of the NRC (24). The animals had free access to water. The feed was computed according to average body weight and modified daily in response to feed refusal. The feed also was offered twice daily at 9:00 a.m. and 4:00 p.m. and the diets ratio contained concentrate and roughage (20:80). Copper Proteinate 15% (Buffermin, JH Biotech Inc., USA) was used as a source of organic protein consisting of chelated copper bound to amino acids and hydrolyzed protein with a crude protein content of not less than 25% and a Cu content of more than 15%. Blood samples of 10 ml were drawn from the jugular vein at 9:00 am on the first day of the experiment, day 45 (mid-experiment) and day 90 (end of experiment) of the experiment for all lambs, using syringes capacity 5 and 10 ml (China manufactured), and the blood was placed in test tubes that were containing the anticoagulant Ethylene diamine tetra acetic acid (EDTA) and other test tubes (Plain tube) for serum samples. The blood tubes were placed in an iced container and transported to the laboratory. Serum was separated by centrifugation (Model PLC-01, Taiwanese-made), for 3000 rpm. The malondialdehyde (MDA) enzyme was measured according to the method of Witte et al. (41). While, activity of the superoxide dismutase enzyme (SOD) was estimated according to Marklund and Marklund (19), and the activity of the enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST) were estimated according to the method of Tiez (37) using the Chem-SI device manufactured by the American company Genex), and the blood metals (Cu and Fe) were measured using the method of Richards (27), and then the blood characteristics of the blood were measured using the blood analyzer (CELL-DYN 3700 Abbott, USA). The calculation of intake, fecal excretion, apparent absorption, retention, and mineral balance was conducted in accordance with Wang and Fisher (40). The data were collected through the studied characteristics of the treatments, and the General Linear Model (GLM) was used for statistical analysis of the data using Statistical Analysis Software (version 9.4). Analysis of variance and Duncan's test were used to determine the differences at the probability level ($P<0.01$ and $P<0.05$) between the treatments. The differences between the treatments were estimated using the mathematical model:

$$Yij = \mu + \alpha i + eij$$

Where:

Yij: the effect of the observation that Treatment I was taken. μ: The overall mean of the trait. Ti: The effect of treatment i, where i = 1, 2.eij: The effect of the random error in observation j within treatment i, which is normally distributed with a mean of (zero) and a variance of σ2e.

Results and Discussion

It is clear from the results that supplementing organic copper to the lambs' diet on day 45 of the experiment had no significant effect on the studied blood characteristics, with exception of the platelet distribution width which decreased significantly ($P < 0.05$) in OCF and OCC1 compared to OCC groups (Table 1). A significant decrease (P

< 0.05) was also observed in the concentration of procalcitonin for the OCF compared to the OCC and OCC5, in which the concentration of procalcitonin was high. By the end of the study (day 90), there was a significant drop ($P < 0.05$) in the number of platelets in the OCF, OCC5, and OCC1 treatments (126.50, 247.0, and 283.30 × 10^9/L, respectively) compared to the OCC, which had the highest rate of 351.5 × 10^9/L (Table 2). On the other hand, there was no significant difference between the experimental treatments in the remaining blood characteristics. The results of this study has revealed that giving lambs diets supplemented with organic copper had no negative consequences for blood cell components. This finding was in line with other research showing that in certain other global breeds, such Dorper sheep (30), high Cu levels had no influence on blood properties. The results showed no significant differences in blood properties during all phases of the study. These findings revealed that the OCC showed the highest values for these indicators among all the treatments and that supplementation organic copper at various levels had little to no impact, these findings were line up with the observations (10 and 34). Nonetheless, in a recent experimental study, sheep without Cu toxicity showed hemolytic crises and that the blood traits of sheep impacted by Cu were lower than those of unaffected animals (42). Apart from platelets, all other factors did not show significant differences at the end of the experiment. These findings aligned with (15) who found that the increasing Cu levels in the blood and liver causes detrimental effects on hemoglobin levels, packed cell volume, and red blood cell count in the blood. Reduced hemoglobin levels follow from increased Cu interfering with Fe absorption and usage in hemoglobin synthesis (30). Red blood cells may be destroyed or synthesis in the bone marrow may be lowered if the packed cell volume is less than the normal rate, therefore producing severe anemia in sheep (22). Consequently, increasing the amount of copper in the feed by 0.5 to 1 g/kg may lead to a reduction in platelet count relative to the OCC, which exhibited the greatest values for these characteristics compared to other Cu-supplemented treatments. In contrast, the supplementation of organic copper in treatments may adversely affect coagulation and wound healing processes, highlighting the importance for a precise balance in supplying appropriate copper levels in sheep feed while preventing excesses that could result in health issues. This result is consistent with Cybulski et al. (7). Copper has a major role in many metabolic processes and vital enzymes, such as the formation of collagen, melanin, and hemoglobin. Although Cu has a supporting role in the formation of hemoglobin, Fe is the main, determining, and essential element for blood properties. Supplementation levels of Cu at levels of 0.5 and 1 g/kg may not be sufficient to cause noticeable effects on blood properties. Thus, levels of Cu toxicity may show these negative changes in the blood and body. High Cu concentrations may have a direct effect on the cells responsible for platelet production (18 and 34). The OCC1's reduction in platelet distribution rate and procalcitonin could point to the importance of organic copper supplementation, which improved lambs' inflammatory and immunological responses. This outcome lines up with research done by Bugdayci et al. (5).

Table 1: The effect of organic copper supplementation on the hematological characteristics of lambs at 45 days.

Variables	WBC (10 *^{9}/L)	Lymph (%)	RBC (10*^{12}/L)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (fL)	PLT (10*^{9}/L)	MPV (fL)	PDW (fL)	PCT (mL/L)
OCC	31.13	79.23	9.66	9.58	27.20	34.66	13.80	39.33	16.53	741.50	10.800	4.13 ^{a}	1.57 ^{a}
OCF	18.96	73.16	9.33	9.63	31.60	33.83	10.33	30.53	20.30	594.00	9.86	2.86 ^{b}	0.59 ^{b}
OCC5	22.72	70.56	9.81	10.13	32.56	33.26	10.30	31.10	19.33	719.66	10.70	3.60 ^{ab}	1.09 ^{a}
OCC1	18.19	63.90	10.21	10.43	33.73	33.10	10.23	30.86	18.16	733.33	11.13	3.40 ^{b}	0.82 ^{b}
SEM	2.474	3.404	0.208	0.287	1.472	0.297	0.837	2.118	1.245	66.822	0.444	0.169	0.134
p value	0.2410	0.5137	0.5503	0.7398	0.4746	0.2487	0.3896	0.4361	0.7914	0.8842	0.8272	0.0305	0.0235

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). ^{a,b} Means in the same row with different superscripts are significantly different. WBC: white blood cell, RBC: red blood cell, Lymph: lymphocytes, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: MCH concentration, RDW: RBC distribution width, PLT: platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width and PCT: Procalcitonin. $^{a, b,}$ means with different superscripts in the same column in different treatment groups are significantly different.

Table 2: The effect of organic copper supplementation on the hematological characteristics of lambs at 90 days.

Variables	WBC (10 *^{9}/L)	Lymph (%)	RBC (10*^{12}/L)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (fL)	PLT (10*^{9}/L)	MPV (fL)	PDW (fL)	PCT (mL/L)
OCC	12.55	39.00	8.60	12.56	0.25	29.30	14.63	50.16	26.46	351.50^a	3.90	2.96	1.43
OCF	18.83	55.63	8.11	12.16	0.23	28.43	14.96	52.80	22.10	126.50^b	4.33	3.96	0.71
OCC5	15.18	64.33	8.39	12.00	0.24	28.13	14.33	50.90	24.90	247.00^b	3.96	2.96	0.85
OCC1	15.44	58.26	8.44	11.76	0.22	26.30	13.93	53.00	17.56	282.30^b	3.86	2.96	1.08
SEM	1.04	3.84	0.18	0.30	0.01	0.69	0.24	0.79	1.66	29.18	0.13	0.29	0.11
p value	0.166	0.166	0.859	0.868	0.657	0.539	0.571	0.563	0.267	0.0096	0.6416	0.6123	0.1522

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). ^{a,b} Means in the same row with different superscripts are significantly different. WBC: white blood cell, RBC: red blood cell, Lymph: lymphocytes, Hb: hemoglobin, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: MCH concentration, RDW: RBC distribution width, PLT: platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width and PCT: Procalcitonin. $^{a, b,}$ means with different superscripts in the same column in different treatment groups are significantly different.

The results in Table 3, indicated that there was no significant increase in oxidation enzymes (SOD and MDA) in addition to the ALT enzyme between the experimental treatments. While a significant increase ($P<0.05$) was observed in the liver enzyme AST in the OCC1 compared to all treatments, with the highest concentration of 113.3 IU/mL, the lowest concentration of 3.50 IU/mL was recorded in the OCC5. At the end of the experiment, there was a significant increase in the ALT enzyme, with a rate of 31.55 IU/mL in the OCF, and the lowest concentration of 14.55 IU/mL in the OCC5. The results of the current study showed that there were no significant differences in each of the remaining enzymes studied among all experimental treatments (Table 4).

Table 3: Biochemical variations of local lambs fed on different levels of organic copper at day 45 of the study.

Variables	ALT (IU/mL)	AST (IU/mL)	SOD (pg/mL)	MDA (pg/mL)
OCC	17.00	74.50 ^{b}	3.34	72.64
OCF	23.33	88.00 ^{ab}	3.13	70.21
OCC5	15.66	83.50 ^{b}	3.52	70.81
OCC1	20.66	113.33^a	3.32	71.66
SEM	1.60	6.01	0.13	1.22
p value	0.3281	0.0241	0.8201	0.9307

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). [a,b] Means in the same row with different superscripts are significantly different. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, SOD: Superoxide dismutase, and MDA: Malondialdehyde.

The SOD enzyme is an antioxidant that shields against damage caused by free radicals; hence, the lack of effect of SOD and MDA by supplementation organic copper at levels that these concentrations may not be sufficient to cause observable effects on the measured oxidative indicators; hence, there were no significant differences for the SOD and MDA enzymes. The results indicate that Cu at an amount of 1 g/kg did not significantly affect SOD enzyme activity, suggesting that the sheep breed possesses effective systems to manage this level of Cu exposure (6, 12 and 32). Enzyme concentration was higher in OCC5 compared to other treatments. This suggests that growing oxidative stress resulted from rising Cu levels, this result was in accordance with the finding of (42). The level of AST enzyme in the serum of the organic copper group was found to be noticeably greater than in the control group. These findings aligned with the findings reported (22 and 34). Mostly present in the muscles and the liver, high level of AST enzyme in the blood point to damage or disturbance in the operation of certain tissues. The results showed that although supplementation organic copper 1 g/kg increase the activity of antioxidants, the duration of the feeding period and the accumulation of Cu greatly influences the activity of the enzyme, hence contributing to the increase in the level of the AST enzyme.

Table 4: Biochemical variations of local lambs fed on different levels of organic copper at day 90 of study.

Variables	ALT (IU/mL)	AST (IU/mL)	SOD (pg/mL)	MDA (pg/mL)
OCC	16.40^{b}	103.90	7.20	18.72
OCF	31.55^{a}	135.10	6.60	19.41
OCC5	14.55^{b}	104.70	2.67	19.76
OCC1	17.40^{b}	121.40	4.90	18.89
SEM	2.44	8.24	0.84	0.38
p value	0.0127	0.5439	0.1861	0.8114

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). [a,b] Means in the same row with different superscripts are significantly different. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, SOD: Superoxide dismutase, and MDA: Malondialdehyde.

This results causes liver dysfunction in lamb, this could be a sign of liver or muscle damage as well as disorders of the cardiac muscle even though in chronic liver disorders, AST also increases in mitochondria (13, 17 and 29). Some studies suggest that supplementation intended to elevate copper levels in feed may lead to the degradation of certain enzymes, including AST, hence adversely affecting the enzyme's sensitivity in assessing copper accumulation in the liver (8, 15 and 28). The present investigation revealed that at the end of the experiment notable variations in the level of the ALT enzyme in the OCF. This high level of the ALT enzyme implies the existence of damage or disturbance in liver activities (30). This could be the result of the blood serum lacking some important minerals. The increase concentration of this enzyme in the blood serum indicates the existence of damage to liver activities and its capacity to metabolize and control appropriately (9). Factors include inflammation, toxins, liver disorders, or in other tissues could be responsible for this malfunction. High Cu content caused several enzymes particularly ALT to reduce, this finding was aligned with what found in studies conducted by (22 and 28).

On the first day of the experiment, there was no significant difference in the concentration of Cu and Fe in the blood serum among the treatments. While the OCC1 showed a significant increase ($P < 0.01$) for Cu in the mid of the experiment (45 days), outperforming all treatments at a rate of 3.56 mg/dL, the OCC5 recorded the lowest concentration at a rate of 0.40 mg/dL (Table 5). While at the end of the experiment (day 90 of the experiment) indicated that there was a significant increase ($P < 0.05$) for Cu in the OCC5 over all treatments at a rate of 2.62 mg/dL, the results showed a significant increase at a significant level in the total serum Cu concentration rate, where the OCC1 and OCC5s had the highest concentration (1.99 and 1.61 mg/dL, respectively) compared to the OCF and OCCs. The current study's showed that the OCC1 had a significantly higher serum Fe concentration ($P<0.01$) than the OCC in the mid of the experiment, at a rate of 532.2 mg/dL. When the lambs were fed different amounts of organic copper, the serum Fe concentration decreased significantly at the end of the experiment compared to the control group. The results also showed that the OCC1 had the highest total Fe concentration rate compared to the rest of the treatments. The body maintains a balance between Cu and Fe.

Table 5: Effect of supplementation organic copper on copper and iron concentration in the serum of Awassi lambs.

Variables (%)	OCC	OCF	OCC5	OCC1	SEM	p value
Cu (mg\dL)						
Cu 0 day	1.49	1.52	1.52	1.60	0.036	0.8018
Cu 45 day	0.63 [b]	1.57 [b]	0.40 [b]	3.56 [a]	0.431	0.0077
Cu 90 day	0.87 [b]	1.32 [b]	2.92 [a]	0.81 [b]	0.271	0.0002
Total Cu	1.00 [b]	1.47 [b]	1.61 [a]	1.99 [a]	0.120	0.0051
Fe(mg\dL)						
Fe 0 day	39.33	39.77	37.87	39.00	0.636	0.8018
Fe 45 day	82.56 [b]	125.0 [b]	77.21 [b]	532.20 [a]	61.67	0.0008
Fe 90 day	33.00 [a]	7.46 [b]	6.110 [b]	5.93 [b]	3.475	0.0001
Total Fe	51.63 [b]	57.43 [b]	40.40 [b]	192.40 [a]	20.20	0.0010

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). [a,b] Means in the same row with different superscripts are significantly different.

Supplementing organic copper at a concentration of 1 g/kg more especially, in the mid of the experiment which led to a significant increase in serum Fe content, this result was in line with (1 and 30). Copper supplements are sometimes necessary to improve the levels of this element in the body, but an increase in plasma Cu concentrations may lead to some side effects. It is essential to monitor these levels to avoid any negative effects that might occurred during feeding sheep. It was found that there were no significant differences in Cu and Fe levels at the beginning of the experiment, indicating that the groups were initially homogeneous (30). It was proven that there is a complex relationship between Cu and Fe, and it was clearly shown that the Cu concentration was much lower than the Fe content in animals suffering from Cu deficiency, the results reflected what had already been found (33). High Cu levels lower platelet counts since Cu is crucial for Fe processing and thereby influences hemoglobin levels in the blood. Rising Cu levels might cause an imbalance in other minerals, which influences the generation of platelets. Some enzymes involved in the process of transforming Fe into a form fit for use in hemoglobin synthesis depend on Cu as well. These findings matched with Cybulski et al. (7) observed. While at the end of the experiment Cu was high in the OCC5 and the rate of supplementation Cu in the treatment was 0.5 g/kg, the outcomes of the experiment revealed that the increase in the concentration of organic copper in the OCC1 and that the rate of addition in this treatment had a role in increasing the absorption or accumulation of this metal in the middle of the experiment. Since Cu is necessary for Fe metabolism and its shortage influences the transport and use of Fe in the formation of hemoglobin and helps prevent disease and preserve the general health of the animal, increasing Cu levels may either have positive or negative effects on physiological functions. These findings suggest that rising Fe concentration in the blood could be the reason of low Cu concentration in the blood serum (3 and 33). The body increases the absorption of these vital components and more effectively distributes them to help to preserve balance (6). The demand for Fe and Cu usually increases to support growth and development processes, which leads to a temporary decrease in concentrations. These concentrations often return to normal levels with the completion of growth. Fe and Cu concentrations during growth decreased for a period with increasing body weight, these results are consistent with (43 and 44). Feeding lambs on copper rich palm oil residues significantly increased serum zinc and Fe concentrations, a finding consistent with (1 and 30). The results indicated that there was a significant increase in Fe concentration in the OCC1. It is likely that the increased serum Fe concentrations were caused by the destruction of red blood cells and not by increased Fe absorption or liver damage (35). Increased Fe absorption primarily leads to Fe deposition in the liver and sometimes in the bone marrow. Excess Cu appears to affect Fe transport, leading to increased serum Fe concentrations, this is consistent with the findings of (8). The results showed that there was a decrease in Fe levels by organic copper supplementation compared to the control group. A significant decrease in Fe levels was noted at the end of the experiment in the OCF, OCC5, and OCC1s. Copper has an effect on some factors that regulate Fe metabolism in the body, thus consuming Fe. Since there is a need for an appropriate ratio between Fe and Cu to ensure good absorption of both, it reduces the amount of excess Cu and eliminates toxicity (3, 33 and 36).

The OCC1 was significantly increased to all treatments in terms of Cu intake, excreted, retained, apparent absorption, and mineral balance compared to the OCC, with the lowest concentration of Cu intake reaching 3.68 mg/day, and in the OCF, the lowest concentration of digested Cu reached 2.38 mg/day (Table 6). Another interesting results from the study is that there was a significant difference ($P < 0.01$) in the amount of excreted Cu in the fecal. In the OCC1 group, the Cu level excreted in fecal was 12.60 mg/day, while in the control group, it was only 0.70 mg/day. The OCC1 (organic copper treatment), on the other hand, had a significant difference ($P < 0.01$) in the amount of absorbed and retained Cu at an average of 87.84 % and 95.17

mg/day respectively. While, the lowest rates for absorbed and retained Cu were 49.82% and 2.38 mg/day, respectively, in OCF group's.

Table 6: Effect of different levels of organic copper on Copper balance in lambs.

Variables	OCC	OCF	OCC5	OCC1	SEM	p value
Mineral Intake (mg/d)	3.68^{b}	4.88^{b}	57.48^{a}	107.77^{a}	13.31	0.0005
Fecal Excretion (mg/d)	0.70^{b}	2.50^{b}	7.40^{b}	12.60^{a}	1.44	0.001
Retention (mg/d)	2.98^{b}	2.38^{b}	50.08^{a}	95.17^{a}	11.93	0.0005
Apparent Absorption (%)	78.84^{ab}	49.82^{b}	87.40^{a}	87.84^{a}	4.71	0.0001
Mineral Balance (%)	56.32^{ab}	11.13^{b}	74.90^{a}	75.81^{a}	7.57	0.0001

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). ^{a,b} Means in the same row with different superscripts are significantly different.

The results showed that there were significant differences in Fe concentration in the droppings when feeding lambs with different levels of organic copper (Table 7). The amount of Fe was significant higher (P<0.01) in the lambs fed 0.5 g/kg organic copper OCC5 (82.71 mg/d), compared to OCC1, OCFs and OCC which are (76.24, 23.20, and 12.89 mg/d, respectively). The amount of Fe absorbed was significantly lower in all experimental treatments compared to the OCC (P<0.01). With 90.54 mg/day, the OCC had the highest concentration; the OCC5 had the lowest concentration, at 61.25 mg/day. In relation to the retained Fe, the experimental treatments clearly showed statistically significant variations (P<0.01). Moreover, the results showed no significant differences in Fe absorption or intake among different treatments.

Table 7: Effect of different levels of organic copper on Iron balance in lambs.

Variables	OCC	OCF	OCC5	OCC1	SEM	p value
Mineral Intake (mg/d)	152.00	210.90	208.90	204.80	19.21	0.7179
Fecal Excretion (mg/d)	12.89^{b}	23.20^{b}	82.71^{a}	76.24^{a}	10.88	0.0103
Retention (mg/d)	139.10	187.70	126.20	128.50	16.71	0.5909
Apparent Absorption (%)	90.54^{a}	89.21^{a}	61.25^{b}	61.28^{b}	4.629	0.0007
Mineral Balance (%)	80.48^{a}	80.89^{a}	22.49^{b}	23.02^{b}	8.737	0.0001

OCC: (Basal diet), OCF: (Basal diet + free of organic copper), OCC5: (Basal diet + 0.5 g organic copper / kg DM), OCC1: (Basal diet + 1 g organic copper / kg DM). ^{a,b} Means in the same row with different superscripts are significantly different.

The study's results indicated that lambs consumed diets with organic copper at concentrations of 0.5 and 1 g/kg, resulting in a significant increase in OCC1 across all treatments concerning both dietary Cu intake and the quantity of Cu ingested and present in the feed, potentially influencing various physiological functions. Copper consumption in the diet is vital for the body and is absorbed through the intestine following ingestion. Excess copper in the body is stored in the liver. The quantity of Cu expelled in the feces represents the residual Cu ingested post-digestion and absorption, contributing to Cu homeostasis. The quantity of its excretion in feces exceeds that in urine, as the principal route for excreting trace minerals is via feces (16). The substantial enhancement in the digestibility and absorption of Cu by the digestive system is influenced by various digestibility factors, including nutritional elements such as proteins, vitamins, and other minerals. Following the transport of dietary Cu across the apical membrane in the digestive tract, it attains a reduced state through mineral reductions during digestion and via the enzyme Cytochrome-B-1 reductase in the duodenum, which aids absorption. Notably, critical protein transporters, particularly the high-affinity Cu transporter, play a vital physiological role in Cu absorption. The intestine dramatically boosted the absorption of Cu, which then directly entered the bloodstream. Not all ingested copper may be assimilated, as its absorption is influenced by several circumstances, including pollutants and pharmaceuticals. This may be attributed to the function of protozoa in diminishing Cu absorption. Copper absorption in ruminants typically transpires in the proximal segment of the small intestine, predominantly within the duodenum. The jejunum and ileum may also facilitate copper absorption (9, 25 and 38). Regarding retained copper, a notable disparity was observed in OCC5, potentially attributable to the quantity of copper sequestered in bodily tissues, including the liver, muscles, and bones. A portion of the absorbed copper is reintroduced into the bloodstream, while the majority is predominantly held in the liver. Daily use of feed containing copper may lead to liver damage due to the absorption of copper beyond the body's requirements and the subsequent accumulation of excess copper in the liver to some degree.

Excessive Cu intake in the diet results in elevated copper levels in the liver, with a portion of the deposited copper being eliminated by the bile duct. The reduction in liver Fe concentration beginning in the eleventh week is attributable to the supplementation of organic copper in lambs' diets, corroborating the findings of Rodrigues et al. (28). The study's results demonstrated that lambs fed diets supplemented with organic copper at concentrations of 0.5 and 1 g/kg exhibited no significant difference in the quantity of iron consumed and retained. However, there was a reduction in iron concentration levels in the copper treatments compared to the initial and organic copper formulations in both absorbed and balanced iron. Significant differences were seen in the quantity of Fe expelled in the feces of OCC5: the majority of Fe in the feed is not absorbed; instead, excess Fe is eliminated by releasing stored Fe from the liver and spleen into the intestines, then excreted in the feces (21). The ingested iron exhibited a notable reduction in the third and OCC1s, as iron is primarily absorbed in the upper section of the small intestine (duodenum). Certain substances, like vitamin C, facilitate Fe absorption, whilst others, such as calcium, impede it. The reduction of copper absorption during iron metabolism indicates that the absorbed iron in copper treatments diminished due to the antagonistic relationship between the two elements, which facilitated iron transport in the bloodstream, associated with proteins responsible for iron transport and storage in the body, namely transferrin and ferritin. Adult animals assimilate 5–10% of the iron in natural feed; however, this percentage can increase to 15-20% when the meal has low iron concentration or during the depletion of iron stores. The majority of iron is absorbed primarily in the duodenum via two stages: absorption through the mucosal membrane and subsequent transfer to the serum (1, 36 and 39). kept iron was markedly diminished in copper treatments, as stored iron or iron kept in the body is associated with ferritin, a protein utilized for iron storage in the liver, spleen, and muscles. Retained iron can be utilized to synthesize hemoglobin, myoglobin, and various enzymes, positively influencing biochemical properties without adversely affecting the hematological parameters essential for the organism. Maintaining sufficient iron reserves in the ovine body is crucial for health and production. Inadequate iron intake may result in elevated serum copper levels, consequently diminishing iron retention in hepatic tissue. No reduction in Fe content was observed, although it is established that sheep with Cu deficiency may experience anemia linked to excessive Fe accumulation in the liver (28 and 35).

Conclusions

The results indicated that the supplementation of organic copper at levels of 0.5 to 1 g/kg in the feed did not produce significant differences in blood measures across the various treatments and did not adversely impact the hematological parameters of the lambs. This suggests that organic copper supplementation enhanced the health and biochemical condition of the lambs, resulting in a reduction of blood platelets attributed to the elevated Cu levels.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Study conception and design: Osama A. Saeed; data collection: Ahmad H. Abdan; analysis and interpretation of results: Osama A. Saeed; draft manuscript preparation: Ahmad H. Abdan. All authors reviewed the results and approved the final version of the manuscript.

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References

1. Abdan, A. H., and Saeed, O. A. (2024). Growth performance, rumen fermentation, and nutrient digestibility of awassi lambs fed different levels of organic copper in a basal diet. Anbar Journal of Agricultural Sciences, 22(2): 1542-1556. https://doi.org/10.32649/ajas.2024.184733.

2. Arthington, J. D., Corah, L. R., and Blecha, F. (1996). The effect of molybdenum-induced copper deficiency on acute-phase protein concentrations, superoxide dismutase activity, leukocyte numbers, and lymphocyte proliferation in beef heifers inoculated with bovine herpesvirus-1. Journal of Animal Science, 74(1): 211-217. https://doi.org/10.2527/1996.741211x.
3. Autukaitė, J., Juozaitienė, V., Antanaitis, R., Poškienė, I., Baumgartner, W., Žilinskas, H., and Žilaitis, V. (2021). The impact of breed, testing time and metabolic profile on the variation of copper concentration in sheep blood serum. Indian Journal of Animal Research, 55(7): 767-773. DOI: 10.18805/IJAR.B-1352.
4. Bidewell, C. A., Drew, J. R., Payne, J. H., Sayers, A. R., Higgins, R. J., and Livesey, C. T. (2013). Case study of copper poisoning in a British dairy herd. Veterinary Record Case Reports, 1(1): e100267. http://doi.org/10.1136/vr.100267.
5. Buğdayci, K. E., Oğuz, M. N., Canbay, H. S., Gümüş, H., and Oğuz, F. K. (2023). Using rose pulp silage instead of sugar beet pulp silage in lambs fed with total mixed ration. Turkish Journal of Veterinary and Animal Sciences, 47(1): 42-54. https://doi.org/10.55730/1300-0128.4267.
6. Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M. L., and Benedito, J. L. (2006). Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in veterinary Science, 80(2): 133-139. https://doi.org/10.1016/j.rvsc.2005.06.003.
7. Cybulski, W., Jarosz, L., Chalabis-Mazurek, A., Jakubczak, A., Kostro, K., and Kursa, K. (2009). Contents of zinc, copper, chromium and manganese in silver foxes according to their age and mineral supplementation. Polish Journal of Veterinary Sciences, 12(3): 339.
8. DiSilvestro, R. A., and Marten, J. T. (1990). Effects of inflammation and copper intake on rat liver and erythrocyte Cu-Zn superoxide dismutase activity levels. The Journal of nutrition, 120(10): 1223-1227. https://doi.org/10.1093/jn/120.10.1223.
9. Faixová, Z., Piešová, E., Maková, Z., Čobanová, K., and Faix, Š. (2016). Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on ruminal enzyme activities and blood chemistry in sheep. Acta Veterinaria Brno, 85(2): 185-194. https://doi.org/10.2754/avb201685020185.
10. Fenger, C. K., Hoffsis, G. F., and Kociba, G. J. (1992). ldiopathic immune-mediated hemolytic anemia in a calf. Journal of the American Veterinary Medical Association, 201(1): 97-99.
11. Goff, J. P. (2018). Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of dairy science, 101(4): 2763-2813. https://doi.org/10.3168/jds.2017-13112.
12. Han, H., Archibeque, S. L., and Engle, T. E. (2009). Characterization and identification of hepatic mRNA related to copper metabolism and homeostasis in cattle. Biological Trace Element Research, 129: 130-136. https://doi.org/10.1007/s12011-008-8293-6.
13. Hameed, A., Abdul-Rahaman, Y., and Mohammed, T. (2023). Addition of VêO® premium and Vitamin C to improve the productive and reproductive performance

of local Iraqi ewes. Tikrit Journal for Agricultural Sciences, 23(2): 171-181. https://doi.org/10.25130/tjas.23.2.14.

14. Joshp, P. K., Bose, M., and Harish, D. (2002). Changes in certain haematological parameters in a siluroid cat fish Clarias batrachus (Linn) exposed to cadmium chloride. Pollution Research, 21(2): 129-131.
15. Khdhr, D. M., and Karim, K. J. (2024). Cytogenetic study of the Hamdani sheep breed of the Iraqi Kurdistan Region. Anbar Journal of Agricultural Sciences, 22(2): 913-924. DOI: 10.32649/ajas.2024.147549.1160.
16. Kwak, W. S., Kim, Y. I., Choi, D. Y., and Lee, Y. H. (2016). Effect of feeding mixed microbial culture fortified with trace minerals on ruminal fermentation, nutrient digestibility, nitrogen and trace mineral balance in Sheep. Journal of Animal Science and Technology, 58: 1-8. https://doi.org/10.1186/s40781-016-0102-8.
17. Li, D., and Fu, J. (2024). The gene IFIT1 is associated with dietary copper-induced yellow fat disease in sheep. Animal Research and One Health, 2(3): 273-284. https://doi.org/10.1002/aro2.66.
18. López-Alonso, M., and Miranda, M. (2020). Copper supplementation, a challenge in cattle. Animals, 10(10): 1890. https://doi.org/10.3390/ani10101890.
19. Marklund, S., and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47(3): 469-474.
20. Massanyi, P., Stawarz, R., Halo, M., Formicki, G., Lukac, N., Cupka, P., Schwarcz, P., Kovacik, A., Tusimova, E., and Kovacik, J. (2014). Blood concentration of copper, cadmium, zinc and lead in horses and its relation to hematological and biochemical parameters. Journal of Environmental Science and Health, Part A, 49(8): 973-979. https://doi.org/10.1080/10934529.2014.894322.
21. McDowell, L. R. (2003). Minerals in animal and human nutrition. 2nd ed. Amsterdam: Elsevier Science. P. 644.
22. Min, X., Yang, Q., and Zhou, P. (2022). Effects of nano-copper oxide on antioxidant function of copper-deficient Kazakh sheep. Biological Trace Element Research, 200(8): 3630-3637. https://doi.org/10.1007/s12011-021-02975-w.
23. Naji, H. A. (2017). The effect of zinc and copper deficiency on hematological parameters, oxidative stress and antioxidants levels in the sheep. Basrah Journal of Veterinary Research, 16(2): 344-355. https://doi.org/ 10.33762/bvetr.2017.143554
24. NRC. (2007). Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. The National Academies Press. Washington, DC.
25. NRC. (2005). Mineral tolerance of animals. Book. National Academies Press.
26. Qudoos, A., Khan, M. N., Sajid, M. S., and Muhammad, G. (2017). Correlation of Trace Mineral Profiles with Gastrointestinal Worm Burden in Rangeland Sheep of Chakwal District, Punjab, Pakistan. International Journal of Agriculture and Biology, 19(1): 140-144.
27. Richards, L. A. (1968). Diagnosis and Improvement of Saline and Alkaline Soils. 1st Ed. Agri. Handbook No. 60. IBH Pub. Co. New Delhi, India.

28. Rodrigues, F. A. M. L., Sousa, R. D. S., Minervino, A. H. H., Oliveira, F. L. C. D., de Sousa, I. K. F. D., Araújo, C. A. S. C., Mori, C. S., Antonelli, A. C., Junior, R. A. B., and Ortolani, E. L. (2023). Effects of sodium monensin on copper metabolism of Brazilian Santa Inês Sheep submitted to different dietary copper. Biological Trace Element Research, 201(1): 196-203. https://doi.org/10.1007/s12011-022-03132-7.
29. Saeed, O. A., Kee, L. T., Sazili, A. Q., Akit, H., Jahromi, M. F., Alimon, A. R., and Samsudin, A. A. (2019). Effects of corn supplementation on the antioxidant activity, selected minerals, and gene expression of selenoprotein and metallothionein in serum, liver, and kidney of sheep-fed palm kernel cake: urea-treated rice straw diets. 3 Biotech, 9: 1-10. https://doi.org/10.1007/s13205-019-1681-0.
30. Saeed, O. A., Sazili, A. Q., Akit, H., Alimon, A. R., and Samsudin, A. A. (2019). Effects of corn supplementation into PKC-urea treated rice straw basal diet on hematological, biochemical indices and serum mineral level in Lambs. Animals, 9(10): 781. https://doi.org/10.3390/ani9100781.
31. Safin, M., Ibragimov, D., and Ibragimova, F. (2024). Discussion of the biochemical mechanisms of copper deficiency in Karakul sheep bred in the Hungry Steppe and its prevention. In BIO Web of Conferences, 95: p. 01048. https://doi.org/10.1051/bioconf/20249501048.
32. Sansinanea, A. S., Cerone, S. I., Quiroga, M., and Auza, N. (1993). Antioxidant capacity of erythrocytes from sheep chronically poisoned by copper. Nutrition Research, 13(8): 891-899. https://doi.org/10.1016/S0271-5317(05)80592-5.
33. Shen, X., Song, C., and Wu, T. (2021). Effects of nano-copper on antioxidant function in copper-deprived Guizhou black goats. Biological Trace Element Research, 199: 2201-2207. https://doi.org/10.1007/s12011-020-02342-1.
34. Song, C., Gan, S., and Shen, X. (2020). Effects of nano-copper poisoning on immune and antioxidant function in the Wumeng semi-fine wool sheep. Biological Trace Element Research, 198: 515-520. https://doi.org/10.1007/s12011-020-02085-z.
35. Sousa, I. K. F. D., Minervino, A. H. H., Sousa, R. D. S., Chaves, D. F., Soares, H. S., Barros, I. D. O., de Araujo, C. A. S. C., Junior, R. A. B., and Ortolani, E. L. (2012). Copper deficiency in sheep with high liver iron accumulation. Veterinary Medicine International, 2012(1): 207950. https://doi.org/10.1155/2012/207950.
36. Suttle, N. F. (2010). The mineral nutrition of livestock-4-th ed. Wallingford, Oxfordshire: CABI Publishing.
37. Tiez, N. W. (1995). Clinical Guide to Laboratory Tests, Philadelphia; W. B. Saunders. P. 22-23.
38. Turner, J. C., Shanks, V., Osborn, P. J., and Gower, S. M. (1987). Copper absorption in sheep. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 86(1): 147-150. https://doi.org/10.1016/0742-8413(87)90157-5.
39. Vashchenko, G., and MacGillivray, R. T. (2013). Multi-copper oxidases and human iron metabolism. Nutrients, 5(7): 2289-2313. https://doi.org/10.3390/nu5072289.

40. Wang, W. X., and Fisher, N. S. (1999). Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. Environmental Toxicology and Chemistry, 18(9): 2034-2045. https://doi.org/10.1002/etc.5620180923.
41. Witte, V. C., Krause, G. F., and Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science, 35(5): 582-585. https://doi.org/10.1111/j.1365-2621.1970.tb04815.x.
42. Wu, T., and Shen, X. (2020). Response of Wumeng Semi-Fine Wool Sheep to Copper-Contaminated Environment. Polish Journal of Environmental Studies, 29(4): 2917-2924. https://doi.org/10.15244/pjoes/111881.
43. Zhang, H., Nie, H. T., Wang, Q., Wang, Z. Y., Zhang, Y. L., Guo, R. H., and Wang, F. (2015). Trace element concentrations and distributions in the main body tissues and the net requirements for maintenance and growth of Dorper× Hu lambs. Journal of Animal Science, 93(5): 2471-2481. https://doi.org/10.2527/jas.2014-8306.
44. Zhang, H., Nie, H., Wang, Z., and Wang, F. (2018). The net iron, manganese, copper, and zinc requirements for maintenance and growth of Dorper× Hu ewe lambs. Italian Journal of Animal Science, 17(4): 941-949. https://doi.org/10.1080/1828051X.2018.1431964.