

## EFFECT OF DIETARY IRON IN PRESENCE OF SULPHUR ON SOME LIVER MINERAL CONCENTRATIONS AND PERFORMANCE OF GROWING LAMBS

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### ABSTRACT

The effect of two levels of dietary iron (Fe) in presence of three levels of sulphur (S) on Cu status and performance of growing lambs was examined using 37 weaned Texel-cross over the period of 12 weeks. The basal diet (L:L) contained no Fe or S supplement; H:M diet supplemented with 800 mg Fe/kg DM and 1.5 g S/kg DM, and H:H diet was supplemented with 800 mg Fe/kg DM and 3.5 g S/kg DM. The concentrate diet was based on dried grass nuts and barely designed for lambs to grow at the rate of 200 g d<sup>-1</sup>. The results revealed that dietary treatments had no effects on DMI, DWG, final weight, total weight gain, and FCE of lambs. The lambs fed L:L diet trended (P=0.057) to have a higher plasma Cu concentrations (12.11 μmol l<sup>-1</sup>) than those fed H:M (10.78 μmol l<sup>-1</sup>) or H:H (10.38 μmol/l) diets at week 12. Dietary treatments had no effect on hepatic Cu, Mn, Mo, and Zn concentration at week 12. However, the lambs fed H:M or H:H had higher hepatic Fe (P=0.05) concentrations than those fed L:L diet. In conclusion, supplemental Fe and S had no effects on lamb performance, but decreased plasma Cu concentrations and increased plasma Mo concentrations of growing lambs. Supplemental Fe and S reduced hepatic Cu concentration of lambs compared with those fed no Fe and S supplements but the difference lacked significance.

**Keywords:** Copper, microminerals, copper deficiency, mineral storage, plasma, sheep.

سيفدين

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تأثير الحديد مع تواجد الكبريت في العليقة على تركيز بعض المعادن في الكبد واداء الحملان

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مدرس

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المستخلص

تم دراسته تأثير مستويين من الحديد (Fe) مع ثلاثة مستويات من الكبريت (S) في حالة النحاس واداء الحملان باستخدام 37 حمل Texel مضرب مقطومة عند عمر 12 أسبوعاً. لم تحتوي العليقة الأساسية (L: L) على أي مكمل Fe أو S؛ عليقه (H:M) تحتوي على 800 ملغم حديد و 1.5 غم كبريت لكل كغم ماده جافه؛ عليقه (H:H) تحتوي على 800 ملغم حديد و 3.5 غم كبريت لكل كغم ماده جافه. اعتمدت العليقة المركزة على بلوكات الحشيش المجففة والشعير و تم تصميمه لنمو الحملان بمعدل 200غم / يوم. أوضحت النتائج أن المعاملات الغذائية لم يكن لها أي تأثير معنوي في معدل الماده الجافه المستهلكه و الزيادات الوزنيه اليوميه والوزن النهائي ومجموع الوزن الكلي و التحويل الغذائي للحملان. كان هنالك اتجاها لدى الحملان التي استهلكت العليقة L: L للحصول على تركيز أعلى (P=0.057) من النحاس في البلازما (12.11 μmol l<sup>-1</sup>) من الحملات التي تم تغذيتها على عليقه M:H (10.78 μmol l<sup>-1</sup>) أو عليقه H:H (10.38 μmol l<sup>-1</sup>) في الأسبوع 12. لم يكن للمعاملات الغذائية تأثير على تركيز النحاس والكبد والمنغنيز وال Mo و Zn في الأسبوع 12. كما حققت الحملان التي تتغذى على علائق H: M أو H: H تركيز اعلى (P=0.05) للحديد في الكبد من تلك التي تم تغذيتها على عليقه L:L. يمكن الاستنتاج بعدم تأثير معنوي لكل من Fe و S المضافان للعليقة على أداء الحمل لكنه ادى الى انخفاض تركيز النحاس في البلازما وزيادة تركيز Mo في البلازما للحملان. كما ادى اضافة Fe و S الى تقليل تركيز النحاس في كبد الحملان مقارنة مع تلك التي لم تتغذى على مكملات Fe و S ولكن الفرق لم يكن معنوياً.

الكلمات المفتاحية: النحاس، المعادن النادرة، نقص النحاس، خزن المعادن، بلازما، الاغنام.

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## INTRODUCTION

Copper (Cu) absorption is considerably lower in ruminants, especially in sheep, than in simple stomach animals (41, 36), which is mainly due to the complex interactions that occur in the functional rumen between Cu, sulphur (S), molybdenum (Mo) and iron (Fe) (24, 14). Copper as an essential micromineral is a part of many important proteins such as ceruloplasmin which is essential for absorption and transport of Fe required for haemoglobin synthesis. Copper is also important in the structure of many enzymes including cytochrome oxidase, lysyl oxidase, tyrosinase and superoxide dismutase which are necessary for electron transport during aerobic respiration, catalyses formation of desmosine cross-links in collagen and elastin that is necessary for strong bone and connective tissues; production of melanin pigment from tyrosine; protection of cells from the toxic effects of oxygen metabolites and is particularly important in phagocytic cell function, respectively (41, 10, 22). It is known that Cu deficiency in sheep and goat reduces production and causes enzootic ataxia in new born lambs and kids and subsequently death (11, 41). Although a lot of work has been done on the effect of Mo and S on Cu deficiency in sheep so far, but there are few studies on the effect of Fe on Cu deficiency in sheep so far (31). Iron is reported to be a potent Cu antagonist that decreases body Cu stores in sheep and cows (6, 7, 43). However, the mechanism behind this effect is not yet been elucidated (33). Sheep as a grazing animal get most of required Fe from feedstuff and ingested soil, which is rich in Fe, while grazing in the pasture (1, 18), or from contamination of harvested forages (31, 41). Dietary supplementation of Fe of as low as 250 to 500 mg kg<sup>-1</sup> DM has been linked to Cu deficiency in sheep and cattle (6, 12, 17, 20, 28, 30, 43). However, the associated increases in S levels in the diet may contribute to the reduction the liver Cu stores (20). Suttle et al. (40) found that feeding diet supplemented with high Fe soils develop Cu deficiency in sheep on high S content, but not in those fed low S content diet and also in the group given supplemental Fe as FeSO<sub>4</sub>. In Iraq few studies have also been done on the concentration of

heavy metals in the blood of sheep, goat, and cows (25) or the effect of blood parasite type on production of Awassi sheep (42). Due to the lack of information about the effect of dietary Fe with S on Cu status of sheep and to fill the gap the current experiment was conducted to investigate and enhance the knowledge behind the antagonistic effect of Fe and S on liver Cu status and performance of growing sheep.

## MATERIALS AND METHODS

### Animals and experimental design

Thirty-seven Texel crossbred weaned lambs (18 castrates and 19 females) with an average live weight (LW) of 26.44 ±4.2 kg were used in a completely randomised block design experiment. The lambs were blocked by LW and sex. To determine the initial liver minerals concentration of the lambs; 10 random lambs were chosen and slaughtered prior the initiation of the experiment (Table 1). The remaining twenty-seven lambs were randomly assigned to one of three dietary treatments and fed individually for 12 weeks.

**Table 1. Average liver mineral content of control lambs (mg kg<sup>-1</sup> DM; n:10)**

Mn	Fe	Cu	Zn	Mo
14.00	501.07	348.04	124.00	3.11

### Diet formulation

The raw feed ingredients used in the current experiment were chosen on the basis of published (23) and analysed low Cu content. The basal diet was formulated to supply 10.24 MJ/kg DM metabolizable energy (ME) and 86.17 g kg<sup>-1</sup> DM metabolizable protein (MP), and rationed for lambs weighing 30 kg to grow at rate of 200 g per day (2). The basal diet was predicted to supply 95.55 g kg<sup>-1</sup> ERDP and 8.92 MJ/kg FME. Lambs were assigned to one of three dietary treatments as given in Table (2).

**Table 2. Experimental treatments details**

T. Code	L:L	H:M	H:H
Iron (mg kg <sup>-1</sup> DM)	0	800	800
Sulphur (g kg <sup>-1</sup> DM)	0	1.5	3.5

LL= low Fe and low S (basal ration), H:M= high Fe and medium S (basal diet supplemented with 800 mg Fe/kg DM and 1.5 g S/kg DM), and H:H= high Fe and high S (basal diet supplemented with 800 mg Fe/kg DM and 3.5 g S/kg DM).

The raw material and chemical composition of the experimental diets are presented in Table (3). Supplemental S was added as reagent grade ammonium sulphate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (Alfa

Aesar., Ward Hill, USA) and Fe as iron sulphate  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (Fisher Scientific., Leicester, UK). The S coming from supplemental  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was accounted for and  $(\text{NH}_4)_2\text{SO}_4$  levels altered accordingly prior to mixing the diets. All diets were balanced for N using feed grade urea (Trouw Nutrition, Northwich, Cheshire, UK).

#### **Experimental routine**

Feed was allocated individually into clean plastic buckets to avoid mineral contamination twice a day. Feed samples (about 1 kg) were collected weekly and stored at  $-20^\circ\text{C}$  for the subsequent chemical analyses. Water was available *ad-libitum*. Feed refusals were recorded twice weekly. The LW of the lambs was recorded and was used to adjust daily feed offered on the following week.

#### **Tissue sample collection and analytical procedures**

At the end of week 12, all lambs were slaughtered and whole livers were collected immediately after slaughter. Livers weight were recorded and samples were taken (~50 g) from the same location per liver and stored at  $-20^\circ\text{C}$  for further analysis. Weekly concentrate samples were bulked within month and analysed according to AOAC, (5) methods for DM (934.01) and CP (988.05), whereas NDF was determined according to Van Soest et al. (35). NDF determination was conducted without sodium sulphite, with  $\alpha$ -amylase, and corrected for ash. Dietary Cu, Fe, S, Zn, Mn, and Mo were extracted using the DigiPREP digestion system (QMX Laboratories Ltd., Essex, UK) and analysed by inductively coupled plasma-mass spectrometry (ICP-MS;

Thermo Fisher Scientific Inc., Hemel Hempstead, UK) following dilution in 2%  $\text{HNO}_3$ , 1% methanol, and 0.1% Triton X-100 (Sigma-Aldrich, Dorset, UK), as described by Cope et al. (8). Plasma samples were analysed for Cu, Fe, Zn, Mn, and Mo by ICP-MS by diluting 1:20 in 0.5%  $\text{HNO}_3$  (8). Liver samples were analysed for Cu, Mo, Zn, Mn, and Fe by ICP-MS after digestion overnight at  $60^\circ\text{C}$  in concentrated nitric acid. Samples were made up to 50 ml in a DigiPREP tube (QMX Laboratories Ltd.) and diluted (1:20) in 2%  $\text{HNO}_3$ , 1% methanol, and 0.1% Triton X-100 and analysed by ICP-MS.

**Statistical analysis:** Performance, blood and plasma minerals were analysed by repeated-measures analysis of variance using GenStat 17<sup>th</sup> edition (VSN Int. Ltd., Hemstead. UK). All data were analysed by a two-way ANOVA as completely randomised design, with the main effects being Fe and S. DLWG was determined by regression analysis and analysed by ANOVA. Standard error of the mean;  $P < 0.05$  was used as the significant threshold and a trend was considered at  $P < 0.1$ . Differences between means were determined using protected least significant difference (LSD) (34).

$$Y_{ij} = \mu + A_i + B_j + e_{ij}$$

$Y_{ij}$  = observational value

$\mu$  = overall mean

$A_i$  = effect of  $i^{\text{th}}$  treatment (supplemented Fe and S)

$B_j$  = Effect of blocks

$e_{ij}$  = experimental error assumed to be NID with  $(0, \sigma^2_e)$ .

**Table 3. Raw material and chemical composition of experimental diets (Basel diet)**

Course mix			
<i>Raw material composition (g kg<sup>-1</sup>)</i>			
Dried grass nuts	500		
Barley	200		
Sugar beet pulp	85		
Soya bean meal	110		
Molasses	50		
Megalac	30		
Mins/vits <sup>1</sup> premix <sup>1</sup>	25		
Total	1000		
<i>Chemical composition (g kg<sup>-1</sup> DM)</i>	Dietary Treatments		
	L:L	H:M	H:H
DM (g/kg fresh)	897.4	888.5	889.1
CP%	20.4	19.7	17.7
NDF	291.8	311.8	342.1
EE%	2.23	2.57	2.25
Ash (g/kg DM)	102.6	111.5	112.3
<i>Mineral concentration of the experimental diets (mg kg<sup>-1</sup> DM)</i>			
Cu	10.81	12.33	10.17
Fe	509.57	905.42	893.4
Mo	2.54	2.30	2.16
S (g/kg)	4.56	6.22	7.18
Mn	152.6	157.74	126.24
Zn	163.93	158.04	139.61

<sup>1</sup> Mineral premix (25 kg/ ton) (RUMENCO LTD., Burton upon Trent, UK) containing 320,000 IU/kg Vit A, 100,000 IU/kg Vit D3, 2,000 IU/kg Vit E, 18.5% calcium, 2.0% phosphorous, 1.0% magnesium, 12.0% sodium, 25% chloride, ammonia trace, sulphur trace, potassium trace, 20 mg/kg selenium, 90 mg/kg cobalt, 150 mg/kg iodine, 3000 mg/kg manganese, and 3000 mg/kg zinc.

## RESULTS AND DISCUSSION

### Diet analysis, intake, and animal performance

The three diets used in the current study had almost similar DM, CP, NDF, EE, and ash content, with the mean values of 891, 19.3, 315.2, 2.4, and 108.8 g/kg DM, respectively (Table 3). Similarly, concentrations of Cu, Mo, Mn, and Zn were similar across all three diets, with the mean value of 11.1, 2.3, 145.5, and 153 mg kg<sup>-1</sup> DM, respectively. In contrast, the concentration of Fe was higher in diets H:M and H:H (mean value 899.4 mg kg<sup>-1</sup> DM)

competed with L:L diet (value of 509.57 mg kg<sup>-1</sup> DM). Concentration of Dietary S was also higher in H:M and H:H diets (with the mean values of 6.22 and 7.18 g kg<sup>-1</sup> DM, respectively) than S concentration of L:L diet (mean value of 4.56 g kg<sup>-1</sup> DM). Dietary treatments showed no effects ( $P>0.05$ ) on final wt., total gain, total DM intake (TDMI), average daily gain (ADG), and feed conversion efficiency (FCE). In addition, dietary treatments had no effects ( $P>0.05$ ) on rumen pH (Table 4).

**Table 4. Effect of iron in presence of sulphur on performance characteristics of growing lambs (kg)**

Mineral	Dietary treatments			s.e.d.	P Value
	L:L	H:M	H:H		
Initial weight	26.4± 1.2	26.3±1.5	26.6±1.9	1.33	0.96
Final weight	40.8± 1.3	38.2±1.8	38.8±2.0	1.60	0.28
Total WG	13.3± 0.2	11.9±0.8	12.2±0.9	1.30	0.55
TDMI	81.6± 2.1	76.3±2.5	79.7±2.3	3.49	0.34
ADG (kg/d)	0.17±0.02	0.15±0.04	0.16±0.08	0.01	0.28
FCE	0.17± 0.03	0.15±0.04	0.15±0.06	0.01	0.67
pH	5.7±0.21	5.9±0.40	5.8±0.39	0.14	0.44

L:L= Low Fe: Low S; H:M= High Fe: Medium S; H:H= High Fe: High S.

### Plasma mineral profile

The results revealed non-significant effect of dietary treatments on plasma Cu concentration from week 0 to week 8. However, at week 12 the lambs receiving L:L diet tended to have higher ( $P=0.057$ ) plasma Cu concentration compared with those given H:M or H:H diets (Table 5). Dietary treatments had no effects on plasma Fe and Mn concentration throughout the experimental period. However, the lambs receiving H:M or H:H diets had numerically higher plasma Fe concentration than those given L:L diet. The lambs fed H:M or H:H had significantly higher plasma Mo concentration ( $P=0.001$ ) at week 2 compared with those fed L:L diet. At week 8 and 12 the lambs fed H:H diet continue to have higher ( $P<0.001$  and  $P=0.001$ , respectively) plasma Mo concentration than the lambs in group L:L or H:M. The lambs given L:L diet had higher ( $P<0.05$ ) plasma Zn concentration than the

lambs fed H:H diet in week 2, 8, and 12. However, the differences in plasma Zn concentration between the lambs fed L:L and H:M diet lacked significance.

### Liver minerals concentration

The mean hepatic concentrations of Cu, Fe, Mn, Mo, and Zn at the beginning of the study was 348.04, 501.07, 14.0, 3.11, and 124.0 mg  $\text{kg}^{-1}$  DM, respectively (Table 1). Hepatic Cu concentrations at week 12 were slightly lower ( $P>0.05$ ) and even lower ( $P>0.05$ ) in lamb groups fed diet supplemented with Fe and S (Table 6). The lambs receiving L:L diet lost less liver Cu concentration ( $-38.0\pm 11.34$  mg  $\text{kg}^{-1}$  DM) than the lambs receiving H:M ( $-85.0\pm 18.46$  mg  $\text{kg}^{-1}$  DM) or H:H ( $-187.0\pm 21.65$  mg  $\text{kg}^{-1}$  DM) diets by week 12. The lambs receiving supplemental Fe had higher ( $P=0.05$ ) hepatic Fe concentration than the lambs given no Fe supplements.

**Table 5. Effect of dietary treatments on plasma Cu concentration of growing lambs ( $\mu\text{mol l}^{-1}$ )**

ID	WK	Treatments			s.e.d.	P value
		L:L	H:M	H:H		
Cu	0	16.67±0.81	16.13±1.05	15.14±1.45	1.743	0.68
	2	15.64±0.92	15.18±0.91	15.45±1.89	1.473	0.95
	8	12.62±0.67	12.78±1.08	11.62±2.03	0.809	0.33
	12	12.11±0.72	10.78±0.89	10.38±1.89	0.691	0.057
Fe	0	37.1±1.23	38.9±2.14	40.9±2.45	5.29	0.78
	2	27.3±1.37	34.0±1.78	31.7±2.11	3.66	0.21
	8	37.1±2.11	52.8±1.89	48.4±2.78	8.83	0.22
	12	44.3±2.08	47.9±2.67	53.1±1.88	4.01	0.12
Mn	0	0.365±0.01	0.373±0.06	0.421±0.02	0.1342	0.90
	2	1.000±0.02	0.670±0.05	1.160±0.07	0.3310	0.35
	8	0.636±0.01	0.603±0.03	0.334±0.02	0.2397	0.41
	12	0.521±0.01	0.547±0.04	0.501±0.03	0.1577	0.96
Mo	0	0.086±0.002	0.093±0.009	0.080±0.005	0.0225	0.84
	2	0.155±0.01 <sup>a</sup>	0.220±0.03 <sup>b</sup>	0.254±0.03 <sup>b</sup>	0.0222	0.001
	8	0.132±0.03 <sup>a</sup>	0.188±0.03 <sup>a</sup>	0.286±0.04 <sup>b</sup>	0.0281	<0.001
	12	0.167±0.004 <sup>a</sup>	0.217±0.05 <sup>a</sup>	0.296±0.04 <sup>b</sup>	0.0278	0.001
Zn	0	8.60±0.42	8.29±0.51	7.65±0.56	0.432	0.11
	2	10.78±0.52 <sup>a</sup>	9.56±0.66 <sup>ab</sup>	9.15±0.89 <sup>b</sup>	0.577	0.03
	8	11.37±0.75 <sup>a</sup>	10.61±0.78 <sup>ab</sup>	10.17±0.86 <sup>b</sup>	0.408	0.03
	12	12.37±0.71 <sup>a</sup>	11.07±0.89 <sup>ab</sup>	10.90±0.99 <sup>b</sup>	0.562	0.04

L:L= Low Fe: Low S; H:M= High Fe: Medium S; H:H= High Fe: High S.

Dietary treatments had no effects ( $P>0.05$ ) on hepatic Mn, Mo, or Zn concentration at the end of the study. Non-significant effect of dietary treatments was showed on livers weight or the total DM percentage between the lambs in all dietary groups at week 12 (Table 8). Clinical sign of Cu deficiency can be observed when the liver Cu concentration reduced to less than 20 mg/kg DM, which is

determined as a threshold value for Cu deficiency as estimated by NRC (26), or to less than 10 mg kg<sup>-1</sup> DM (41). However, with the current study, dietary Fe supplements with medium or high S supplements did not cause symptoms of hypocuprosis or general ill health in lambs throughout the study being in agreement with the findings reported by other studies in sheep (30, 43) and cattle (20, 6, 28).

**Table 6. Effect of dietary iron supplementation in presence of sulphur on liver minerals concentration of growing lambs (mg kg<sup>-1</sup> DM)**

ID	Dietary treatments			s.e.d.	P value
	L:L	H:M	H:H		
Cu	310.00±13.72	263.00±23.46	161.00±22.31	70.000	0.13
Fe	547.00±28.31	774.00±37.31	657.00±37.25	81.300	0.045
Mn	15.50±1.31	15.00±1.37	14.70±1.30	4.440	0.98
Mo	2.19±0.23	1.61±1.5	2.30±1.70	0.347	0.14
Zn	138.70±13.37	146.70±11.31	159.90±10.02	12.350	0.26

L:L= Low Fe: Low S; H:M= High Fe: Medium S; H:H= High Fe: High S.

**Table 7. Effect of dietary iron supplementation in presence of sulphur on total liver minerals storage of growing lambs (mg liver<sup>-1</sup>)**

ID	Treatments			s.e.d	P value
	L:L	H:M	H:H		
DM%	27.4±2.79	28.5±3.18	27.7±2.45	0.96	0.52
Cu	39.2±1.33	32.9±1.21	18.7±1.31	12.17	0.26
Fe	59.8±21.47	101.6±24.05	102.1±26.11	10.09	0.001
Mn	6.1±2.77	4.6±2.32	6.9±2.36	0.91	0.07
Mo	0.6±0.23	0.6±0.17	0.7±0.21	0.06	0.11
Zn	22.6±18.25	22.5±22.14	26.5±21.30	1.97	0.11

In an experiment by Grace *et al.* (15, 16) where the lambs fed different supplemental levels of S alone (3.9 to 7.9 g/d, for 105 days) displayed no symptoms of Cu deficiency throughout the study; this is in line with the results observed currently. Pogge *et al.* (29) also found no effect of S level (2.4 and 6.8 g kg<sup>-1</sup> DM, as sodium sulphate) on DMI and DM or OM digestibility of steers. In contrast, it has found a 20% reduction in DMI, 9.03% in DLWG, and 16.25% in FCR in steers given 2.5 g S kg<sup>-1</sup> DM compared with those given 1.5 or 2.0 g S kg<sup>-1</sup> DM (45). In another study, Spears *et al.* (37) found no effect of supplemental S (0, 1, or 3 g kg<sup>-1</sup> DM) on the DMI of steers during the growth stage; however, steers given higher level (3 g S kg<sup>-1</sup> DM) showed lower DLWG than steers given 0 or 1 g S kg<sup>-1</sup> DM. These results were disagreed with those observed currently. This might have been due to the difference in species or to unknown dietary effects. The performance measurements were not influenced by the

dietary treatments, being no effects of supplemental Fe on DM and nutrient digestibility was observed by Sefdeen (33), so the energy and protein utilisation will remain the same and no effects will be expected on lamb's performance. These results are in accordance with the results found by other studies in sheep (30, 16, 43), and cattle (9, 7, 6, 28, 13). In the current experiment, dietary Fe with low or high S level had no effect on the plasma Cu concentrations of lambs throughout the experimental period and plasma Cu level was decreased at all treatments, but the level of reduction was higher in the groups fed H:M and H:H diets. However, in week 12, decreasing trend was observed in lambs given H:M and H:H diets compared with those fed L:L diet (10.78 and 10.38 vs. 12.11 µmol/l, respectively). This may due to the short experimental period and also to the liver Cu concentration of the lambs (19). Plasma Cu concentration of 3 - 9 µmol l<sup>-1</sup> was determined to identify marginal Cu

deficiency in sheep, goats and cattle (39). Animal liver accumulates Cu, nevertheless when intake of Cu is inadequate the liver releases Cu into the blood to meet the physiologic demands of animals (19). Although there was non-significant increases in the plasma Fe concentration between the dietary treatments throughout the experimental period, but the higher plasma concentration in the groups of lambs given supplemental Fe during the trial period indicates the highly availability of the form of Fe used (21). This results are in agreement with those observed by Prabowo *et al.* (30) where supplemental Fe had no significant effects on plasma Fe concentration of lambs following 84 days of the trial. Similar findings were also found on the effect of dietary treatments on the plasma Mn concentration of lambs. This results were in line with those found by Sefdeen (33) in Swaledale and Texel cross lambs fed diet supplemented with dietary Fe. Similarly, Standish *et al.* (38) observed no effect of dietary Fe on plasma Mn concentration of cows. Plasma Mo increased in the groups of lambs given supplemental Fe being similar with those observed by Sefdeen (33) in growing lambs Humphries *et al.* (20) in cattle. High dietary Fe may reduce liver and plasma Cu by different mechanisms, which could be different from Mo effect and have not yet been specified. The increases in the biliary Mo concentrations observed in the current experiment in groups fed diet supplemented with 800 mg Fe and 3 g S/kg DM was parallel with the increases in the plasma Mo concentration of lamb groups given supplemental Fe. Increasing in the blood and biliary Mo concentration may influence reducing liver Cu storage of lambs given Fe supplements or a combination of Fe and S diet. In the current experiment, plasma Zn was significantly lower in the group of lambs fed H:H diet from week 2 to week 12. However, there was no significant differences in the plasma Zn concentration between the lamb groups fed L:L and M:H diets. The significant decreases observed in the plasma Zn concentration of the lambs fed supplemental dietary Fe could be due to the competition between Fe and Zn absorption through the apical membrane of the enterocytes as both of

these minerals uses the same transporter protein (3).

#### **Liver mineral concentrations**

The initial liver mineral concentration of the experimental lambs that slaughtered at day one of the experiment was normal and within normal reported ranges (26, 41). Dietary treatments have affected liver Cu concentration of lambs in all three groups. However, the effect on liver Cu concentrations of lambs in group fed L:L diet was minimal which may insure that the Cu content of the diet was adequate (41). At the end of the study, the lambs fed L:L, H:M, and H:H diets have lost -0.46, -1.01, -2.23  $\mu\text{g Cu d}^{-1}$ , respectively. The observations of decreasing the liver Cu concentrations of the groups fed experimental diet confirmed the antagonistic effects of supplemental Fe and S on liver Cu concentrations. The non-significance trend of the results might be due to the period of experiment or number of replicates per treatment. The results observed currently were in agreement with those reported by Rosa *et al.* (32) in adult sheep given diet supplemented with 1000 mg Fe/kg DM as  $\text{FeCl}_3$ , on the liver Cu storage of mature sheep. Several studies found a significant decrease in the hepatic Cu concentration of sheep and cattle fed Fe supplemented diets (500 – 800 mg Fe  $\text{kg}^{-1}$  DM) (28, 30, 43, 33). The antagonistic effects of Fe in ruminants has been suggested to rise from the interaction with sulphide produced in the functional rumen (40). This interaction may result in trapping sulphide and enhancement of its ability to limit Cu absorption in the small intestine (40). The significant increases in the liver Fe concentration of the lambs fed diets supplemented Fe and S (H:M and H:H diets) insures the highly availability of the form of Fe supplemented compared to those fed control diet which is in accordance to the results reported by (4, 33). Dietary treatments had no effect on liver Mn Mo and Zn concentration which is in agreement with those observed by Sefdeen (33) in growing lambs and by Williams (43) in lambs.

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