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THE ASSESSMENT OF HEPATIC COPPER CONTENT IN LAMB, YEARLING, SHEEP AND CATTLE SLAUGHTERED IN ERBIL CITY, KURDISTAN REGION-/ IRAQ

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ABSTRACT

Liver samples from 163 slaughtered animals were studied to provide data regarding normal hepatic Copper (Cu) concentration, and Iron (Fe). Results show the mean and median liver Cu concentration in majority samples were normal. Lambs had higher ($P<0.05$) liver Cu concentration (499) compared to yearling (353), cattle (321), and sheep 200 mg /kg respectively. Cases of being at risk with Cu-toxicity were shown in 2 lambs (1797 and 2449), 1 yearling (1546), and 1 cattle) 1525 mg/kg respectively. sheep did not exhibit a high liver Cu concentration, while a six yearling, an eight sheep, and fourteen cattle revealed marginal hepatic Cu concentration. Moreover, five sheep and six cattle cases showed low hepatic Cu concentration. In addition to that the lambs and yearling cases did not reached hepatic Cu deficiency. Hepatic Fe concentration in sheep was higher ($P<0.05$) compared to lamb, yearling, and cattle, with the mean concentration of 2047, 1962, 1954, 1925 mg Fe/kg respectively. All sheep liver samples, with 33 yearling sample, and 46 lambs liver samples contained liver Fe concentrations exceeded toxic level (2450 mg/kg). While, one liver sample in cattle contained (3214 mg/kg), exceeding toxic level. There were 14 lambs and 14 yearlings, with 12 and 13 liver samples contained greater than 2000 mg of Fe/kg. In conclusion, hepatic Cu content in the majority of examined sample were normal. Though, high and low levels of Cu were also observed. The low hepatic Cu level might be related to the high hepatic Fe concentration.

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INTRODUCTION

There are twenty-two minerals shown to be essential in animal's diet to keep normal health and growth (Suttle, 2010). Those minerals are classified to macro and micro elements, the macro elements require by g/kg in the diet like Calcium (Ca) Potassium (K) Sodium (Na) Magnesium (Mg) Sulfur (S), and the micro elements require by mg/kg like Iron (Fe) Iodine (I) Zinc (Zn) Copper (Cu) Cobalt (Co) Molybdenum (Judson and McFarline,1998). Despite that the role of trace minerals in animal and human health is well established, they are the great forgotten nutrients in animal diets. Their physiological role is often underestimated and their presence in the feed in adequate quantities. However, they are necessary to maintain body function, growth and reproduction and to stimulate immune response and therefore determine the health status. Copper also has long been recognised as an essential micro element for animals' normal health and growth. Copper due to its involvement in approximately 300 enzymes and proteins. Which their functions ranging from anti-oxidant to hormone releasing proteins (Suttle, 2010). However, as light deficiency of this trace minerals can cause a considerable reduction in performance and production. Copper deficiency occurs when sheep and cattle graze pastures low in Cu or when ruminants offered a diet deficient in Cu, but more often it is associated with diet that contained high level of other minerals such as Fe (Sefdeen, 2017), molybdenum and Sulphur (Hussein, 2017).

Despite Cu being an essential trace element required for numerous vital functions in the body, it can also be extremely toxic to ruminants and cause death (Suttle, 2010). There are marked variations between domestic animals regarding their tolerance to increased dietary Cu intakes (Howell and Gooneratne, 1987). Sheep are considered to be most sensitive to Cu toxicity compared with other species (NRC, 2005). This has been attributed to their inability to increase biliary Cu excretion in response to increased Cu intake (Bremner, 1998), and limited ability to accumulate Cu bound to metallothionein (MT) in their livers (Howell and Gooneratne, 1987). Whereas, the situation reversed in the cattle. Young animals are more sensitive than adults because they have a higher efficiency of absorption (NRC, 2005, as after birth or before developing the functional rumen Cu absorption in lamb is as high as (70-85%) (Suttle, 1975). While, the absorption in the adults is low (1-10%) of dietary Cu (Suttle, 2010). Liver is the most organ in animal body that stored dietary minerals and the best biomarker for diagnosing mineral disorders in animals (Goff, 2018). To sum up, copper enable the animal to make use of the nutrients provided in the ration and when lacking may inhibit normal growth and function. Similarly, in excess they can be toxic and can lead to poor performance or even death in extreme circumstances. In the fact of view, there is poor documented data about ruminant liver Cu status in Erbil city, with the attention given to industries to give better means for supply feed contains sufficient minerals. Therefore, this study will investigate liver Cu concentration in sheep and cattle slaughtered in Erbil.

MATERIAL AND METHODS

A total of 163 sheep and bovine livers samples were taken from slaughtered animals central slaughterhouse of Erbil city, Liver samples were collected on different periods during years 2018 and 2019. Samples of approximately 50g of liver tissue were obtained using stainless steel knives. Visible fat, connective tissue and major blood vessels were excised. These samples were packed in polyethylene bags, properly identified and stored in a freezer at -20°C. Then, the frozen liver samples were oven at 60°C up to 72 hours. The dried samples were crushed using a ceramic mortar. About 2g of fine powder was used to analyse for Cu and Fe content using X-ray fluorescence spectrometer (Genius 9000 XRF, USA) following the procedures of Chelebi *et al.* (2015). All results were expressed in mg/kg dried weight DW. Reported reference values, given in wet weight (WW), then were converted to DW for comparison purposes by multiplying reported WW values by 3.5, which is the mid-point of the standard conversion rates used (range 3.0 to 4.0). Moreover, reference ranges reported in SI units (µmol/kg) were converted to mg/kg by using the conversion factors provided by Puls (1994) and Suttle (2010). The number of animals tested per animal species was summarised in Table 1. One-way analysis of variance (ANOVA) was carried out to test for significant differences in liver

Cu and Fe concentrations between species of the individuals using Genstat version 19.1 (VSN Int. Ltd., Oxford, UK). Fisher's unprotected least significant difference test was used between the means at a significant level of $p<0.05$.

Table (1): Number of liver samples analysed for each group categorised per animal species

Animals	Sample number
Lambs	47
Yearling	34
Sheep (over 2 years)	32
Cattle (local breeds)	50

RESULTS AND DISCUSSION

One of the primary goals of the present survey was to determine the liver concentration of Cu, as Cu is the most widely reported mineral deficiency in ruminant animals, but also accounts for the greatest number of cases of Cu toxicity (Suttle, 2010). Liver is the organ that generally serve as an indicator and represents the status of several trace elements concentration in ruminant animals (Langlands *et al.*, 1984). Hence, at present investigation the best method to assess the Cu status in sheep and cattle requires liver tissue (Herdt, 2000). Results from present study (mean and median) (Table 3) in comparison with the diagnostic range of liver Cu concentration of ovine and bovine (Table 2), showing the adequate liver Cu concentrations in lambs, yearling, sheep, and cattle set by Puls (1994), Kincaid (1999), and Suttle (2010). The values were ranging from 176 to 499 mg/kg DM. To our knowledge, there was no data on liver mineral concentration in ruminate, in Erbil city, for using it for comparison. Though, the mean liver Cu concentration in sheep and cow of the current study were lower compared with the liver Cu concentration of Karadi sheep (403 mg/kg DM), and cow (420 mg/kg DM) of from Sulaimaniah City-Iraq (Aljaff *et al.*, 2014). Moreover, liver Cu concentration in lambs and yearling of the current study were higher, but lower in sheep in comparison with the liver Cu concentration of lambs (300), yearling (259), and sheep (264) mg/kg DM of Karadi sheep from Sulaimaniah City-Iraq (Husain and Abd Alkareem, 2016).

Table (2): Diagnostic range for liver cooper and iron concentrations (mg/kg dried weight basis) in ovine and bovine[1]

	Cu		Fe	
	Ovine	Bovine	Ovine	Bovine
Deficient	<14	<20	53-88	<140
Marginal	70	33-125	245	350
Adequate	88-350	125-300	105-1050	1050
High	350-1750	600-1250	1500	2450
Toxic	875-3500	>1250	>1500	>2450

Pulse (1994); Kincaid (1999); Suttle (2010)

Table (3): Liver Copper (Cu) and Iron (Fe) concentration (mg/kg dried weight basis) of slaughtered local lambs, yearlings, sheep, and cattle slaughtered in Erbil city

Cu	lamb	yearling	sheep	cattle
Mean±SD	499±425	353±324	200±148	322±301
Median	350	262	178	204
Minimum	86	37	17	9
Maximum	2449	1565	495	1526
Fe	lamb	yearling	sheep	cattle
Mean±SD	1962±238	1954±227	2047±311	1925±255
Median	1940	1958	1958	1903
Minimum	1455	1044	1740	1610
Maximum	2760	2442	3490	3214

Copper in lambs' livers showed a different trend compared to yearling, sheep, and cattle. Lambs liver Cu concentrations were significantly ($P<0.05$) higher compared to yearling, sheep and cattle, with the mean value of 499, 353, 200, 321 mg of Cu/kg respectively (Fig. 1). The lower liver Cu content in cattle compared to lambs could be related to the Cu metabolism difference between these two species. Small ruminants such as lambs and sheep are more affinity to Cu toxicity compared to other species such cattle. Sheep are known to their ability in Cu storage in liver and inability to excrete excess Cu by the bile (Gooneratne et al., 1989). Whereas, the cattle are less efficient in body storage of Cu with high ability in Cu excretion, concerning Cu-deficiency.

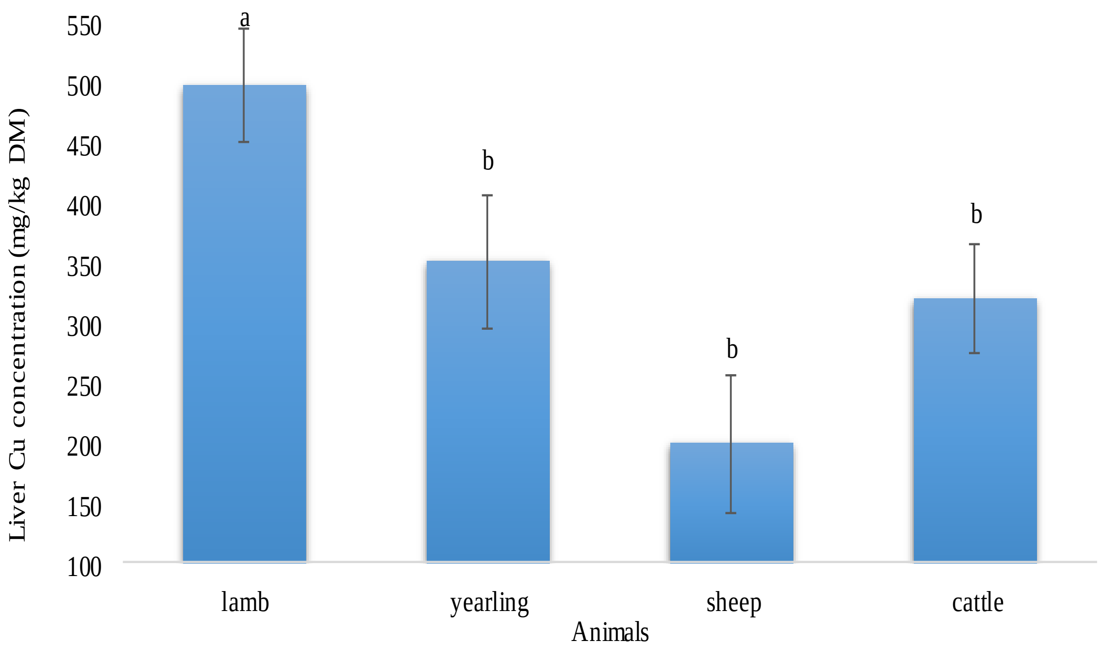

Figure (1): Liver Copper (Cu) concentration (mg/kg dried weight basis) of slaughtered local lambs, yearlings, sheep, and cattle in Erbil city abattoir. Error bars indicate SED.
[a,b] Means with different superscripts are significantly different ($P<0.05$)

There were high liver Cu concentrations (>1000) in 3 lambs, 2 yearlings, with 2 in cattle's. In lambs, 2 of them were 1797 and 2449 mg/kg, in yearling 1 was 1546 mg/kg, and in cattle, 1 was 1525 mg/kg. Hence, 2 lambs, 1 yearling and 1 cattle were at risk of being Cu-toxicity with concentrations exceeding 1500 mg kg for sheep and cattle. Although, the high hepatic Cu concentration (>1000 or 3500 mg/kg) of the individual animals in the current study is lower than level that animals died from Cu toxicity that often exceed 2000-4397 mg Cu/kg (NRC, 2005; SAC Consulting Veterinary Service, 2016). Though, there are no available data on Cu toxicity in our region. The high concentration of Cu in the liver of ruminants is related to the dietary Cu intake over a prolonged period of time (Scott, 2002), which greater than 15 mg/kg DM set by (NRC, 2005), and bioavailable Cu in the diet in ruminants (Kumaratilake, 2014). As well as, grazing pastures fertilised with Cu salts or poultry manure, or environmental contamined orchards drenched with Cu-containing chemicals, such as fungicides (Roubies *et al.*, 2008). Current finding suggested that the high liver Cu content in ruminants may be related to the feed stuff that offered to animals. As in the winter time ruminants are used to stay in the barns and feed concentrated diet, resulting in increased liver Cu concentration. The most likely explanation, well justified by published experimental findings (Crosby *et al.*, 2004), reporting that lambs reared on straw bedded floor had higher liver Cu concentration compared to those reared on expanded metal floor. Besides, the dietary Cu level in concentrate diets is usually higher than pasture Cu content (Li *et al.*, 2005). Moreover, commercial rations intended for other animal species has been suggested one of the most common causes of toxicity in sheep is the accidental feeding of foodstuffs intended for other livestock. Mineral mixes, or inappropriate formulated rations is a good example (Roubies *et al*., 2008). There is a wrong practice by the farmers and feed industries that formulate diet without calculate mineral requirements based on animal species or stage of growth and offered to all species of ruminant animals that has different requirements and metabolic process (Suttle, 2010). Therefore, a caution should be exercise and recommendation must be offered to farmers and feed industries to differentiate among ruminant animal species requirements in term of dietary Cu content and produce a diet that suitable with animal growth and species in order to avoid adverse consequence on animal health and production.

The low liver Cu concentration in the present study was also observed. As seen in Table 3, minimum liver Cu concentration from lambs (86 mg/kg) was just adequate, while yearling (37 mg/kg) and sheep (17 mg/kg) minimum liver Cu concentration were lower than marginal Cu threshold of deficiency (70 mg/kg) and tended to Cu deficiency (<14). While in cattle liver Cu were (9 mg/kg) and reached the deficiency (<20 mg Cu/kg DM). In lambs, only one liver sample was contained 86 mg/kg Cu. In yearlings, 6 samples were contained liver Cu concentrations (<70 mg/kg). While, sheep and cattle had 8 and 14 cases with liver Cu concentration contained (<70 and <125 mg/kg) respectively, with 5 of sheep and 6 samples of cattle contained deficient level of liver Cu (<20 mg/kg DM). In the recent study conducted in Basra city, south of Iraq by Al-Saad (Alsaad, 2020) reported that lambs with 124 mg/kg DM of Cu showed clinical signs of Cu deficiency including walking difficulty, frequently falling, tremors, and poor growth. Based on this threshold, there were higher number of yearlings (10) and sheep (11) had liver Cu concentration lower than 124 mg/kg DM and at risk of Cu deficiency. Similarly, low liver Cu concentration (72 mg/kg DM) in sheep has been reported in

Sudan (Ibrahim et al., 2013). Whereas, in Turkey, the normal liver Cu concentration (412 mg/kg DM) in sheep and cattle (215 mg/kg DM) has been documented (Yayayürük and Yayayürük, 2017).

The low liver Cu concentration in this study was in accordance with high liver Fe content. The mean and median liver Fe concentration in all species of animals were above high between 1903-2047 mg Fe /kg. Liver Fe concentration in sheep was higher ($P<0.05$) compared to liver Fe concentrations in lamb, yearling, and cattle, with the mean concentration of 2047, 1962, 1954, 1925 mg Fe/kg respectively (Fig 2). All sheep liver samples, with 33 yearling sample, and 46 lambs liver samples contained liver Fe concentrations exceeded toxic level 1500 mg/kg. In addition, one liver sample in cattle had liver Fe concentration (3214 mg/kg), exceeding toxic level (2450 mg Fe/kg). There were 14 liver sample of lambs and yearling, with 12 and 13 liver samples contained liver Fe concentration greater than 2000 mg/kg. Liver Fe concentration in sheep and cattle in the present investigation was several times higher compared to liver Fe concentration in sheep (237 mg/kg DM) and cattle (180 mg/kg DM) from Turkey (Yayayürük and Yayayürük, 2017). Although, higher levels of liver Fe concentration in sheep (3325 mg/kg DM) and cows (2779 mg/kg DM) were reported by (Aljaff *et al.*, 2014). Ruminants grazing on pasture or consuming forage-based diets are often exposed to high level of dietary Fe (> 500 mg/kg DM) (NRC, 2005). In addition, the adverse effect of Fe on Cu status in ruminants has long been reviewed and documented (Gould and Kindall, 2011; Sefdeen, 2017). The Fe levels in all animals in this study were high and reached to toxic level and adverse effect of Fe on liver Cu concentration can be seen when liver Fe concentration exceeds 1500 in sheep, and 2450 in cattle mg/kg. To our knowledge there are no available data about Fe levels in animal tissues from Kurdistan region. The high liver Fe accumulation (1373-1493 mg/kg DM) has been reported in Brazil and it is linked with Cu deficiency in sheep (Sousa *et al.,* 2012). They suggested that the antagonism effect of Fe on Cu status could be due to this element may have been an important factor in triggering Cu deficiency in ruminant animals. Although, the exact mechanism by which Fe reduced Cu status in animals is still unclear. Generally, liver Fe concentrations in lamb and yearling were higher compared with sheep and cattle. This is consistence with lower liver Cu concentration in sheep and cattle in comparison with lamb and yearling. The results of the present study confirm Cu-Fe antagonism by which Fe may resulted in reduce Cu availability, reducing liver Cu concentration in studied animals. Therefore, the low liver Cu concentration in this study could be attributed to Cu-Fe interaction. In addition to low dietary Cu content in ration. Hence, the findings of this study suggest to reduce the dietary Fe in ration by reducing Fe supplementation in feed premixes. In addition, offering diet to ruminant animals based on animal species, stage of growth, and production.

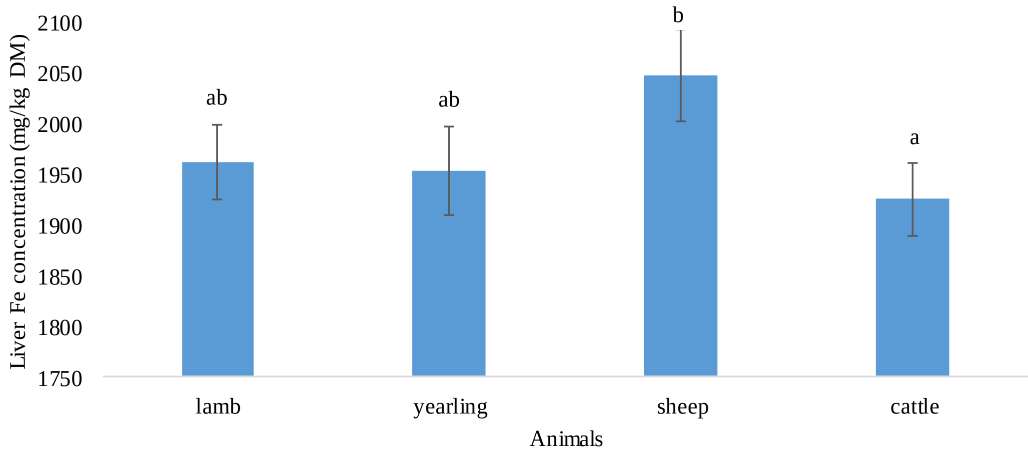

Figure (2): Liver Iron (Fe) concentration (mg/kg dried weight basis) of slaughtered local lambs, yearlings, sheep, and cattle in Erbil city abattoir. Error bars indicate SED.
[a,b] Means with different superscripts are significantly different (P<0.05).

CONCLUSION

The analysed livers samples of slaughtered ruminants in Erbil city in this study were generally had an adequate liver Cu concentration. The highest liver Cu concentration were in lambs following yearlings, but sheep and cattle had lower liver Cu concentrations. However, the maximum liver Cu concentration in a small number of lambs, yearling, and cattle were tended to toxic levels. Liver Fe concentration of majority of samples were high and sheep had the highest liver Fe concentration. The low liver Cu was in an accordance with high liver Fe concentration.

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تقييم محتوى النحاس الكبدي في الحملان ، الحوليات ، الأغنام ، والماشية في مدينة أربيل، آقليم كردستان – العراق

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الخلاصة

تم دراسة 163عينه كبد من الحيوانات المذبوحة لتوفير بيانات عن القيم الطبيعية لتركيز النحاس(Cu) في الكبد و مع المعادن المضادة لها مثل الحديد(Fe) . أظهرت نتائج أن متوسط تركيز Cu في الكبد في معظم العينات كان الطبيعي. وكان لدى الحملان تركيز أعلى (P<0.05)من Cu في الكبد (499) مقارنة مع الحوليات (353) والأبقار (321) والأغنام (200) ملغم/كغم على التوالي. كانت حالات التعرض لخطر التسمم بالنحاس فى الحملان (1797 و 2449) ، والحوليات (1546) وماشية واحدة 1525 (ملغم/كغم) على التوالي. كانت هناك الحوليات (6)، والأغنام (8)، والماشية (14) معهم التركيز Cu الطفيف في كبد. كما لوحظ 5 (الأغنام) و 6 (الماشية) حالات من تراكيز Cu الطفيف في كبد. الحملان و الحوليات لم تصل لحالات نقص النحاس في الكبد. كان تركيز Fe في كبد الأغنام أعلى (P<0.05) مقارنةً بالحملان والحوليات والابقار، بمتوسط تركيز 2047 ، 1962 ، 1954 ، 1925 ملغم/كغم على التوالي. جميع عينات كبد الأغنام، مع 33 عينة من كبد الحوليات، و 46 عينة من كبد الحملان احتوت على تراكيز عاليه من Fe تجاوزت المستوى السمي. بينما احتوت عينة كبد واحدة من الأبقار على (3214 ملغم/كغم) متجاوزة المستوى السام (2450 ملغم Fe / كغم). كانت هناك 14 حالة من الحملان والحوليات ، مع 12 و 13 عينة من الكبد تحتوي على أكثر من 2000 ملغم/كغم. الأستنتاج، التركيز Cu في الكبد لغالبية العينات التي تم فحصها كان طبيعيًا. على الرغم من ذلك، لوحظ أيضًا مستويات عالية ومنخفضة. قد يكون انخفاض تركيز النحاس في الكبد مرتبطًا بارتفاع تركيز Fe.

الكلمات المفتاحية: الكبد، النحاس ،الحديد، الآغنام، الآبقار.

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