Deposition of copper in cattle and buffaloes edible tissues slaughtered in Assiut city, Egypt

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Abstract

A total of 168 samples of livers, kidneys and muscles (part of the diaphragm) were screened. The samples were subjected to preparation and for measurement the level of copper (Cu) by using Atomic Absorption/Flaming Emission Spectrophotometer. Buffaloes organs showed variations in their copper content. As for liver, the mean copper concentrations were 11.52 ± 5.41 with a range varied from 2.34 to 21.88 µg/g wet weight. Whereas in buffalo kidneys copper level varied from 2.34 to 15.13 with a mean value of 5.85 ± 4.34 µg/g wet weight. Moreover, the concentrations of copper in buffalo muscles varied from1.56 to 15.50 µg/g wet weight with a mean value of 4.35 ± 3.96. On the other hand, copper in cattle liver varied in its concentrations where the mean value was 11.59 ± 5.79, with a range varied from 3.13 to 25.00 µg/g wet weight. Copper in cattle kidneys varied from 2.50 to 16.3 with a mean value of 4.18 ± 3.12 µg/g wet weight. Besides, the concentrations of copper in cattle muscles were 3.23 ± 2.48, 1.88 and 14.34 µg/g wet weight, respectively as a mean, minimum and maximum. In conclusion, 1% - 26% of the examined samples of both cattle and buffalo were higher than the results obtained by Egyptian Organization Standardization and Quality Control (2008), in addition, livers samples have high concentrations of copper than kidneys and muscles.

Keywords

Copper Toxicity In Cattle, Copper Toxicity In Buffaloes, Egypt

1. Introduction

Copper is an essential micronutrient required in the growth of both plants and animals. In humans, it helps in the production of blood haemoglobin. Copper is indeed essential, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper normally occurs in drinking water from Cu pipes, as well as from additives designed to control algal growth. While Cu's interaction with the environment is complex, research shows that most Cu introduced into the environment is, or rapidly becomes, stable and results in a form which does not pose a risk to the environment. In fact, unlike some man-made materials, Cu is not magnified in the body or bioaccumulated in the food chain[1]. Copper supplemented feeds are prepared for animals. The metal tends to be accumulated in the liver and kidney. However, there have been no reported cases of toxicity to human to this source[2]. The richest sources of copper in human dietaries are crustaceans and shellfish especially oysters and the organs of meats, particularly lamb or beef liver. The extent of copper absorptions influenced by the amount and chemical form of the copper ingested by the dietary level of several other metal ions and organic substances and by the age of the animal. For example, mature sheep normally utilize less than 10% of the copper they ingest, while young lambs prior to weaning utilize four to seven times this proportion of dietary copper [3]. Chronic copper poisoning may occur in animals under natural grazing conditions, as a consequence of excessive consumption of copper-containing salt licks or mixtures, from the unwise use of copper containing drenches, from contamination of feeds with copper compounds and from agricultural or industrial sources. Abnormally high liver copper levels are characteristic of a number of diseases in man. These include Mediterranean anemia, hemochromatosis, cirrhosis and yellow atrophy of the liver, severe chronic diseases accompanied by anemia and Wilson's disease[4]. Therefore, the current study investigated copper concentrations in the muscles (part of the diaphragm), livers and kidneys of cattle and buffalo which are slaughtered in Assiut city by using of Atomic Absorption/Flaming Emission Spectrophotometer (Shimadzu model AA 630-02) and assessed the human risk of this metal.

2. Material and Methods

2.1. Collection of Samples

A total of 168 samples of liver (part of caudate lobe), kidney and muscles (part of diaphragm) were collected from 23 male cattle and 33 male buffaloes 2-3 years old (livers, kidneys and muscles specimens from each species) slaughtered in Assiut abattoirs. Each sample was about 50 grams weight and was individually placed in polyethylene bags and labeled with the date, kind, age and sex of each animal. The collected samples were immediately taken to the laboratory in an ice box where they were kept deeply frozen at -20°C until preparation, digestion and analysis.

2.2. Preparation of Equipments

All glass utensils were thoroughly cleaned with water and then dipped in glass jars containing mixture of hydrochloric acid 25% and nitric acid 10% in ratio of 1:1 and leftover night. After that all utensils and instruments were thoroughly washed by distilled water and dried.

2.3. Preparation of N/10 Hydrochloric acid

According to A.O.A.C.[5] 8.9 ml conc. Hydrochloric acid were added to one liter of distilled water. The prepared stock solution of N/10 Hcl was preserved for dilution of the digested samples.

2.4. Laboratory Technique

2.4.1 Digestion Procedure

The applied technique recommended by Fahmy[6] was followed. In a clean dry Kjeldhal flask of 250-300 ml capacity, one gram of wet sample, 5 ml of 50% sulphuric acid and 5 ml of concentrated nitric acid were added. The flasks were heated gently over a low flame of a minor-burner until clear fumes of nitric and sulphuric acids appeared, where the flame was turned off and the flasks were allowed to cool. On reheating the dark brown liquid formed in the flask is gradually disappeared. Complete digestion is indicated by colorlessness of the liquid. Stronger heating was continued for sometimes to drive off most of the nitric acid in the flask.

To hasten digestion, it was found better to use only 3 ml of the concentrated acid initially and then the other 2 ml were added after cooling.

2.4.2 Filtration

10 ml Hcl N/10 were dissolved in 90 ml distilled water in volumetric glass cylinder to obtain 100 ml. About 50 ml of the prepared solution were added to the digested particles previously heated and cooled in a glass flask. The mixture was agitated well for thorough mixing.

The obtained mixture was filtrated through a glass funnel containing filter paper, where the filtrate was collected in a glass cylinder. The remainder 100 ml of the prepared solution were added also to the digested flask to dissolve any other digest particles which may be still adhered on the flask wall, and the mixture was thoroughly agitated, then filtrated.

The obtained filtrate for every sample was put in two special vials each of 50 ml capacity, stoppered and preserved at room temperature. Every laboratory technique was done in duplicate for each sample.

2.4.3 Estimation of Copper

The previously digested and filtrated samples were prepared for measurement the level of copper in each sample in Biochemistry Department, Faculty of Medicine, Assiut University, by using the Atomic Absorption/Flaming Emission Spectrophotometer (Shimadzu model AA 630-02), using an air acetylene flame and hallow cathode lamp.

$$C = \frac{R}{S} \quad \text{X conc. of standard X dilution}$$

C = concentration of heavy metal µg/g wet weight.

R = reading of element conc. on digital scale of Atomic Absorption Spectrometry (AAS).

S = Reading of standard.

Copper: The wave length was adjusted to 324.7 n.m. and the used lamp current was 10 mA.

2.5. Statistical Analysis

Data was represented as (Mean ± SD).

T-test was used to compare between any two groups with normal data.

Mann-Whitney Rank Sum test was used to compare between any two groups with skewed data.

3. Results

Table 1. Comparisons (Mean ± SD, range) between concentrations of copper (µg/g wet weight) in the examined buffaloes and cattle.

Kind of animal	Buffalo			Cattle		
	Liver*	Kidney*	Muscle*	Liver**	Kidney**	Muscle**
Copper	11.52±5.41 2.34-21.88	5.85±4.34 2.34-15.13	4.35±3.96 1.56-15.50	11.59±5.79 3.13-25.00	4.18±3.12 2.50-16.13	3.23±2.48 1.88-14.34

* No. of samples= 33; ** No. of samples= 23

Table 2. Differences of copper concentrations (µg/g wet weight) in livers, kidneys and muscles of buffaloes and cattle.

Metal	Site			Significance		
	Liver	Kidney	Muscle	Liver vs Kidney	Liver vs Muscle	Kidney vs Muscle
Cattle	11.59±5.79 3.13-25.00	4.18±3.12 2.50-16.13	3.23±2.48 1.88-14.34	***	***	***
Buffalo	11.52±5.41 2.34-21.88	5.85±4.34 2.34-15.13	4.35±3.96 1.56-15.50	***	***	***

* Significant ($p < 0.05$); ** Highly significant ($p < 0.01$); *** Very highly significant ($p < 0.001$).

Table 3. Percentages of samples that contain copper higher than that recommended by the Egyptian Standards*:

Metal	Permissible limit	Buffalo			Cattle		
		Liver	Kidney	Muscle	Liver	Kidney	Muscle
Copper	15 mg/kg	25%	3%	3%	26%	1%	0%

* E.O.S.Q.C. (2008).

4. Discussion

4.1 Comparisons (Mean ± SD, range) between concentrations of copper (µg/g wet weight) in the examined buffalo and cattle:

Buffaloes organs showed variations in their copper content (**Table 1**). As for liver, the mean copper concentrations were 11.52 ± 5.41 with a range varied from 2.34 to 21.88 µg/g wet weight. Whereas in buffalo kidneys copper level varied from 2.34 as a minimum to 15.13 as a maximum with a mean value of 5.85 ± 4.34 µg/g wet weight. Moreover, the concentrations of copper in buffalo muscles were 1.56, 15.50 and 4.35 ± 3.96 µg/g wet weight as a minimum, maximum and mean respectively.

On the other hand, copper in cattle liver varied in its concentrations where the mean value was 11.59 ± 5.79, with a minimum of 3.13 and a maximum of 25.00 µg/g wet weight. Copper in cattle kidneys varied from 2.50 to 16.3 with a mean value of 4.18 ± 3.12 µg/g wet weight. In addition, the concentrations of copper in cattle muscles were 3.23 ± 2.48, 1.88 and 14.34 µg/g wet weight, respectively as a mean, minimum and maximum.

Nearly similar results were obtained by many researchers [7-12], while lower results stated by Iwegbue [13].

4.2 Differences of copper concentrations (µg/g wet weight) in livers, kidneys and muscles of buffaloes and cattle.

As for variations in concentrations of copper (µg/g wet weight) in livers, kidneys and muscles of buffaloes recorded in (**Table 2**), showed that there is a very highly significant differences in copper concentrations in livers versus kidneys, livers versus muscles, as well as kidneys versus muscles.

While for variations in concentrations of copper (µg/g wet weight) in livers, kidneys and muscles of cattle recorded in (**Table 2**) pointed out that there is a very high significant differences in copper concentrations in livers versus kidneys, livers versus muscles, as well as a very high significant difference in kidneys versus muscles.

4.3 Percentages of samples that contain copper higher than that recommended by the Egyptian Standards:

According to E.O.S.Q.C. [14], copper levels higher than the Egyptian Standards (15µg/g) recorded in (**Table 3**) were detected in 25% in buffalo livers, 3% buffalo kidneys and 3% buffalo muscles. While in cattle higher levels could be detected in cattle liver (26%) and cattle kidney (1%). Higher mean copper levels in liver can occur by contamination of pasture and by industrial emissions.

5. Conclusion and Recommendations

The information given by the achieved results proved that all the examined livers, kidneys and muscles of both buffalo and cattle were found contaminated with copper. Some tissues showed higher levels of copper than that recommended by the Egyptian Standards.

So, for limitation of the risk of copper in carcass of slaughtered animals the following recommendations should be taken in considerations:

1. Monitoring programme must be carried out periodically to measure Cu concentration in soil and plants intended for animals to assess their load of copper to avoid their hazards on animals and humans.
2. Contamination of both cattle and buffaloes is transferred to animals via direct exposure, polluted water and crops grown on industrial effluents. Another important source of contamination is from pesticides, fungicides, and often release contaminated water into the drainage system which leads to streams and other water bodies. Therefore, it is important to keep monitoring the various pollutants in the meat since most of the butcheries sell their meat strategic places like roadsides and near bus stops.

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