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COPPER AND MERCURY LEVELS IN LOCAL JORDANIAN AND IMPORTED SHEEP MEAT AND ORGANS

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Summary

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A total of two hundred and forty random samples were evenly collected from mutton, liver and kidney of 40 local (Group A) and 40 imported (group B) sheep – 40 of each tissue from each sheep group. The samples were freshly collected from sheep 12–18 months old and slaughtered in abattoirs in Jordan in 2002. Moreover, another 40 fresh mutton samples of imported sheep (group C) were collected from different markets. The samples were analyzed by atomic absorption spectroscopy (AAS) to estimate their contents of Cu and Hg. Results indicated that imported mutton group B had significantly higher ($P<0.05$) mean values of Cu and Hg pollution than the local and imported group C muttons. The mean concentration of Cu and Hg in the liver of imported sheep (group B) were significantly higher ($p<0.05$) than that recorded in the liver and kidney of local sheep. The percentages of local, imported group B and imported group C mutton that exceeded the permissible limit of Cu (20 mg/kg) were 5%, 27.5% and 0.00% respectively. However, mutton samples that contained Hg above permissible limit (0.5 mg/kg) were recorded only in imported sheep from group B at a rate of 7.5%. The reproducibility and accuracy of the results obtained have been studied, judging from quality control (QC) samples and reproducible results. The between run precision and accuracy of the calibration standards were < 5% to the relative standard deviation. The effect of Cu and Hg on animal and human health is discussed.

Key words: copper, mercury, meat, heavy metal pollution

INTRODUCTION

Heavy metals have recently come to the forefront dangerous substances and are considered as serious chemical health hazards for men and animals (Lars, 2003; Massadeh & Snook, 2002). The presence of Cu and Hg at significant concentrations in the meat and edible offal's is considered a potential health hazard to human and animals (Abou-Arab, 2001).

Copper is an essential trace element for animals, it is required for normal biological activity of many enzymes, haemoglobin formation and hair keratin (Underwood, 1977; Prohaska & Gybina, 2004) The distribution of the total body Cu among the tissues varies with the species, age and Cu status. The total body Cu in sheep is distributed as follows: Liver

72–79%, muscles 8–12%, skin and wool 9%, and skeleton 2% (Walter, 1986).

Data on the Cu concentration in meat vary greatly between countries. The Cu concentration vs wet tissue weight was 59.7 ppm in the liver of Finnish cattle, 8.8 ppm in the liver of Irish cattle and 22.1 ppm in the liver of Polish cattle (Doyle & Spaulding 1978). The Cu concentration in meat, liver, and kidney of sheep slaughtered in northern part of Poland was 0.52–7.30, 3.80–88.0 and 2.80–15.0 mg/kg wet weight, respectively (Falandysz, 1991). In New Zealand, the mean levels of Cu in examined muscles, liver and kidney, of slaughtered sheep were 2.9 ppm, 7.1 ppm and 4.4 ppm, respectively (Solly *et al.*, 1981). Furthermore, the permissible limit for Cu is 20 ppm as recommended by Food Stuff Cosmetics and Disinfectant Act Regulation (Anonymous, 1972).

Mercury has long been known as toxic environmental pollutant presenting an occupational hazard. The increased use of Hg in industrial processes, as a fungicide and increased reports of Hg poisoning in humans have prompted this concern (Centers for Disease Control and Prevention, 2005). It enters the body through ingestion and inhalation (Kazantzis, 2002, Prohaska & Gybina, 2004). In general, Hg compounds are highly toxic, because they are fat soluble and easily absorbed and accumulated in erythrocytes and the central nervous system (Vinas *et al.*. 2002; Castoldi *et al.*, 2003). However, the metabolism of Hg and the degree of its toxicity are affected by the chemical form, route of entry, length of exposure, and dietary content of interacting elements, especially selenium (Underwood, 1977; Reykdal & Thorlacius 2001; Ikemoto *et al.*, 2004; Prohaska & Gybina, 2004). Animal may be exposed to air borne Hg or it may enter the body orally through contaminated water or feed (Cang *et al.*, 2004). Mercury residues are found in the kidney, liver and skeletal muscle of sheep. Elevated kidney and liver Hg levels have been reported in sheep grazing near a chlor-alkali plant (Lodenius & Tulisalo, 1984; Walter, 1986). Mercury residues in poisoned sheep were higher in both kidney and liver than in skeletal muscle (Luckow, 1982). Previous studies indicated that the mean values of Hg in liver and kidney of slaughtered sheep varied greatly (Falandysz, 1991; Reykdal & Thorlacius 2001; Seady, 2001) On the other hand, the concentration of Hg in sheep meat was found to be low in several studies (Ward, 1987; Seady, 2001) It was reported that, the means of Hg in muscle, liver and kidney of sheep fed on 4 mg of inorganic mercury/day for 28 days were 1.580 mg/kg, 0.064 mg/kg and 9.054 mg/kg, respectively, while, the means of Hg in the control sheep group were 0.091 mg/kg, 0.026 mg/kg, and 0.128 mg/kg respectively (Kacmar *et al.*, 1992). The toxic dose of Hg for sheep was 4 g, and it is considered as a cumulative poisonous element because of its slow excretion from the intestine and kidney. According to the FAO/WHO Codex Alimentarius Commission (1992) and the Egyptian Organization for Standardization and Quality Control (1993), the recommended mercury concentration of 0.5 mg/kg in meat is considered safe.

The objectives of this study were: to estimate the mean concentration of Cu and Hg in local and imported mutton and to estimate the prevalence rates of mutton, liver and kidney with exceeding permissible limits of Cu and Hg in local and imported sheep.

MATERIALS AND METHODS

Reagents

All reagents used were of the highest purity available. Digestion mixture consisted of 60 mL of HNO_3 and 40 mL of $HClO_4$.

Instrumentation

A Perkin Elmer model (Spectra- AA10, USA) flame atomic absorption spectrometer (AAS) with computer system was employed throughout the experiment for determination of Cu and Hg. The concentrations of Cu in the solutions were determined by AAS which was adjusted at wavelengths of 324.8 nm and 253.7 nm for Cu and Hg, respectively. Absorbance and concentration were recorded on the digital scale of the AAS. The obtained results of Cu and Hg contents in the examined samples were calculated as ppm (mg/kg) on wet weight.

Collection of samples

A total of 240 random meat, liver and kidney samples were collected from 40 local (group A) and 40 imported (group B) sheep carcasses (40 of each tissue from each sheep group) slaughtered in different slaughterhouses in Jordan in 2002. The local and imported (group B) sheep selected were of the same age group 12–18 months. Another 40 random mutton samples of imported sheep (group C) was collected from different markets to estimate their Cu and Hg concentrations. The imported sheep were not raised in Jordan and were slaughtered within one month of importation date. Samples were collected in polyethylene bags. All bags were put in ice box and transferred immediately to the Meat Hygiene Laboratory in the Faculty of Veterinary Medicine, Jordan University of Science and Technology, and the collected samples were analyzed to estimate the level of Cu and Hg residues by AAS.

Washing procedure

Washing of the glassware and plastic film is an important process to avoid any sort of contamination especially when trace elements or heavy metals are analyzed. The test tubes, polyethylene tubes and glassware were soaked in water and soap for 2 hours then rinsed several times with tap water. After that glassware has been rinsed once with distilled water, once with mixture (consisting of 520 mL deionized water, 200 mL concentrated HCl and 80 mL H_2O_2), and once with washing acid (10% HNO_3). Finally, they were washed with deionized water then air-dried in incubator away from contamination or dust (El-Mowafi, 1995).

Digestion procedure

One gram of each sample was processed by a sharp scalpel in a screw capped tube. Five milliliters of the digestion mixture were added to the tissue sample (Clark, 1989). The tubes were tightly closed and the contents were vigorously shaken and allowed to stand overnight. Then, the tubes were heated for 3 hours in water bath adjusted at 70^{o}C to ensure complete digestion of the samples. The digestion tubes were vigorously shaken at 30 min intervals during the heating period. Finally the tubes were cooled at room temperature and then diluted with 5 mL deionized water, and filtered through filter paper (Wattman No.42). The filtrate was collected in polyethylene tubes. These tubes were capped with polyethylene film and kept at room temperature until analyzed for Cu and Hg contents (Seady, 2001). Blanks and standards were prepared in the same manner as for wet digestion and by using the same chemicals.

Preparation of blank and standard solution

Blank tubes were used to determine the heavy metal contamination that may be present in the chemicals used for wet digestion. Standard solutions using pure certified metal standard were prepared for Cu and Hg. Serial standard solutions for Cu and Hg were prepared at ideal adequate strength (Hassan, 1993).

Statistical analysis

Data concerning Cu and Hg concentrations were analyzed by ANOVA, t-test and Chi-squared test. The values were expressed as mean concentrations (mg/kg) ± SD. P values < 0.05 were considered to be significant.

RESULTS

Copper and mercury mean concentrations (mg/kg) in mutton from the three groups of meat is presented in Table 1. The mean Cu concentration in imported mutton group B was significantly higher (P<0.05) than those of the local and imported mutton groups A and C respectively (by ANOVA test).

Generally, the Cu concentration was 4.14 ± 3.38 mg/kg and ranged from 2.40 to 23.10 mg/kg for local mutton, 20.58 ± 4.71 mg/ kg within 17.40 to 40.30 mg/kg for imported mutton group B and 0.31 ± 0.18 mg/kg (within 0.10 to 0.80 mg/kg) for group C (Table 1). The mean concentration of Hg in mutton was 0.11 ± 0.07 mg/kg ranging from 0.04 to 0.18 mg/kg for local sheep and 2.97 ± 2.23 mg/kg ranging from 1.10 to 6.10 mg/kg for group B, whereas the Hg content of imported mutton group C was below detection threshold. Moreover, the mean concentration of Hg was significantly higher (P<0.05) in mutton from group B than that in either groups A or C mutton.

The Cu concentrations in liver samples of local and imported sheep group B were relatively high. For local sheep liver, they were 36.88 ± 22.5 mg/kg (within the range from 5.50 to 100 mg/kg) and for sheep liver from group B 56.39 ± 25.98 mg/kg (18.90–106 mg/kg). The mean concentrations of Cu in liver of imported sheep group B were significantly higher (P<0.05) than those in the liver of local sheep (Table 2). On the other hand, the concentrations of Hg in liver samples of local sheep were 0.47 ± 0.16 mg/kg and ranged from 0.31 to 0.63 mg/kg, whereas Hg concentrations in liver samples of imported sheep group B were higher, ranging from 0.38 to 2.80 mg/kg and the mean was more than twice of that observed in group A as shown in Table 2.

Table 1. Comparison between Cu and Hg concentrations (mg/kg) in local (group A) and imported sheep mutton (groups B and C) (mean ± SD)

Sheep origin	Sample size	Heavy metal	
		Cu	Hg
Local (Jordan, group A)	40	4.14 ± 3.38	0.11 ± 0.07
Imported (group B)*	40	20.58 ± 4.71 #	2.97 ± 2.23 #
Imported (group C)*	40	0.31 ± 0.18	ND

* sheep imported live and slaughtered within one month; ND = not detected, # significantly different vs groups A and B (P<0.05) by ANOVA.

Table 2. Comparison between Cu and Hg concentrations (mg/kg) in organs of local (group A) and imported sheep group B (mean ± SD)

Sheep origin	n	Organ			
		Liver		Kidney	
		Cu	Hg	Cu	Hg
Local	40	36.88±22.51	0.47 ± 0.16	8.66±4.65	0.83±0.37
Imported (group B)	40	56.39±25.98#	1.35 ±1.02 #	21.43±10.21	3.49±1.83 #

significantly different (P<0.05) by t-test vs. local sheep.

Table 3. Acceptability of 40 mutton samples according to Cu and Hg permissible limit and the sheep country origin

Groups	n	Copper concentration exceeding permissible limit		Mercury concentration exceeding permissible limit	
		n	%	n	%
Group A	40	2	5.0	0	0
Group B	40	11	27.5#	3	3.75
Group C	40	0	0	0	0

significant (P<0.05) by Chi-squared test for higher rate in group B; Cu: permissible limit 20 mg/kg ; Hg: permissible limit 0.5 mg/kg.

In sheep kidney, the mean concentration of Cu in the imported sheep group B was still extremely higher 3 times than that observed in the local sheep as shown in Table 2. The concentration of copper in kidney samples was 8.66 ± 4.65 mg/kg (ranging from 3.90 to 24.80 mg/kg) for the local sheep and 21.43 ± 10.21 mg/kg (ranging from 16.00 to 82.70 mg/kg) for imported sheep group B. The concentration of Hg in sheep kidney from imported sheep group B was 4 times higher than that found in the local sheep. It amounted to 0.83 ± 0.37 mg/kg (ranging from 0.32 to 1.35 mg/kg) for local sheep and 3.49 ±1.83 mg/kg (ranging from 0.48 to 6.10 mg/kg) for group B as shown in Table 2.

Three samples out of 40 of the sheep mutton group B exceeded the safe standard limit of Hg. In contrast, all examined sheep mutton from groups A and C were found to be within the standard limit (0.5 mg/kg) of the Egyptian standard (Egyptian Organization for Standardization and Quality Control, 1993), as shown in Table 3. According to the Egyptian standard permissible limit for Cu (20 mg/kg) and Hg (0.5 mg/kg), the prevalence rates of sheep liver with Cu or Hg concentration exceeding the safe permissible limits are shown in Table 4. The prevalence rate of copper that exceeded the permissible limit in the liver of imported sheep group B (92.5%) was significantly higher (P<0.05) than the rate found in the liver of local sheep (72.5%) as shown in Table 4. However, the prevalence rates of Hg in the liver that exceeded the permissible limit were very low in both local and imported sheep group B. They were 2.5 % and 5%

Table 4. Prevalence rates of heavy metals with exceeded permissible limit in internal organs of local and imported sheep

Sheep origin	n	Organ							
		Liver				Kidney			
		Cu		Hg		Cu		Hg	
		n	%	n	%	n	%	n	%
Local (group A)	40	29	72.5	1	2.5	4	10.0	3	7.5
Imported (group B)	40	37	92.5 #	2	5.0 ns	14	35.0 #	7	17.5 ns

significant ($P<0.05$) by Chi-squared test vs. group A; *ns* = not significant. Cu: permissible limit 20 mg/kg ; Hg: permissible limit 0.5 mg/kg.

respectively, and the difference between them was not significant ($P>0.05$) (Table 4). The prevalence rates of Cu that exceeded the permissible limit in the kidneys of imported sheep group B was 35% and it was significantly higher ($P<0.05$) than the rate found in the kidney of local sheep (10%) as shown in Table 4. The prevalence rates of Hg concentration that exceeded the permissible limit in kidneys were low in both local and imported sheep (groups A and B): 7.5% and 17.5% respectively, with no significant difference ($P>0.05$).

DISCUSSION

Regardless the country origin of sheep, the liver samples contained the highest mean level of Cu followed by kidneys and meat samples (Table 4). On the other hand, 92.5%, 35% and 27.2% of liver, kidneys and meat respectively of imported sheep group B exceeded the safe permissible limit (20 mg/kg). At the same time, 72.5%, 10% and 5% respectively of examined samples of local sheep exceeded the permissible limit. From the above mentioned results, it is obvious that the majority of liver samples had high levels of Cu exceeding the guideline standard limit, while the lowest concentrations of this metal were detected in the meat samples of local and imported sheep. The current results are in agreement with those reported by Seady (2001) that the mean values of Cu concentrations in meat, liver and kidney samples of sheep were 6.92 mg/kg, 29.20 mg/kg and 12.02 mg/kg, respectively. Higher mean values of Cu in the liver 190 mg/kg, were recorded by Ward (1987) while in this study the mean liver Cu was 36.88 mg/kg in local sheep and 56.39 mg/kg in imported one (group B). However, other studies recorded lower mean values of Cu in meat and organs of sheep than those found in the present one (Solly *et al.*, 1981; Falandysz, 1991; El-Mowafi, 1995; Reykdal & Thorlacius, 2001). Copper occurs in foods in many chemical forms and combinations, which affect its availability to the animal. It is known to be essential at low concentration for both human and animals but it is toxic at high levels. The lethal dose of Cu is 100 ppm but food with copper concentration of 5–7 ppm becomes repulsive for human consumption. Thus, there is no danger for human to get Cu poisoning. (Vos *et al.*, 1991). On the other hand, chronic exposure to Cu fumes leads to the syndrome called "metal fume fever" which is characterized by nasal congestion, fever up to 39 ^{o}C, chills, malaise and shortness of breath.

The highest concentration of Hg, in sheep tissues was found in the kidneys followed by liver then meat. This finding is in agreement with other studies where mercury reported to be concentrated mainly in kidneys (Falandysz, 1991; Reykdal & Thorlacius, 2001; Cang *et al.*, 2004). Furthermore, the mean Hg residues were recorded at significantly higher level (P<0.05) in meat and in offal of imported group B sheep than that found in the local sheep (Table 4). Results showed that imported group C meat samples tested were free from toxic Hg (Table 1). The variation observed in the levels of Hg residues in local sheep (group A) and imported group B may be attributed to the differences in pasture, amount and type of sludge treatment and type of feed. Much lower concentration of Hg in sheep meat and offal were detected in most other studies. For example, Falandysz (1991), recorded lower levels of Hg residues in liver (0.002 mg/kg) and kidneys (0.120 mg/kg) of Polish slaughtered sheep. Also, Vos *et al.* (1991) detected much lower level of Hg in sheep meat, liver and kidneys (0.001, 0.002 and 0.007 mg/kg, respectively). However, all mutton samples from the local and imported group C sheep were within the permissible limit for Hg (0.5 mg/kg) and only 7.5% of the imported sheep group B exceeded the permissible limit. The prevalence rates of liver and kidneys that exceeds mercury permissible limit were low in local and imported sheep group B and were not significantly different (Table 4).

Copper concentration in mutton indicated that 5% and 27.5% of the local and the imported group B sheep respectively, exceeded the safe permissible limit of Cu, stipulated by the Egyptian Organization for Standardization and Quality Control, (1993), which recommended 20 mg/kg as guide line for Cu in mutton and offal (Table 3). However, none of the examined imported mutton group C exceeded this limit as shown in Table 3. There is a significant association (P<0.05) by Chi-squared test between the rate of copper concentration exceeding permissible limit in mutton, and country origin of sheep.

Mercury in the mutton of imported sheep group B is considered a moderate public health problem where 3.75% of the samples have exceeded the permissible limit, while mutton from local or imported sheep group C might be considered safe.

In general, copper is concentrated more in liver than other tissues, while mercury is concentrated more in the kidneys. Mutton of imported group C sheep has the lowest heavy metals contamination when compared with the mutton of local and imported sheep (groups A and B). The mean Hg concentrations in the mutton samples marketed in Jordan were very low in the local (group A) and imported sheep group C but significantly higher in the group B. Mercury in mutton marketed in Jordan is not a public health problem since only 3.75% of the samples from imported sheep group B had exceeded permissible limit while none of the local (group A) and imported group C sheep samples had exceeded permissible limit.

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