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Author(s)	El-Ghareeb, Waleed Rizk; Darwish, Wageh Sobhy; Meligy, Ahmed Meligy Abdelghany
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Metal contents in the edible tissues of camel and sheep: human dietary intake and risk assessment in Saudi Arabia

Waleed Rizk El-Ghareeb[1, 2], Wageh Sobhy Darwish[1,*] and Ahmed Meligy Abdelghany Meligy[3, 4]

[1] Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt
[2] Department of Veterinary Public Health and Animal Husbandry, College of Veterinary Medicine, King Faisal University, Saudi Arabia
[3] Department of Central Lab of Chemical Analysis, College of Veterinary Medicine, King Faisal University, Saudi Arabia
[4] Physiology Department, Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt

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Abstract

Camel and sheep meats are considered major sources of animal-derived protein, vitamins and minerals in certain parts of the world such as Arabian Peninsula, India and Middle East countries. Despite these health benefits, the safety of sheep and camel meat may be compromised by the presence of heavy metals. The monitoring of metal concentrations is therefore important to ensure compliance with food safety regulations and consequent consumer protection. This study was undertaken to estimate the toxic metals (lead, cadmium and arsenic) and the essential trace elements (copper, zinc and iron) contents in the edible tissues (muscle, livers and kidneys) of both camel and sheep slaughtered at Al-Ahsa, Saudi Arabia. The dietary intake and human health risk assessment of the examined metals due to consumption of these tissues by Saudi population were estimated. The results indicate that the tested metals were detected in all the tissue samples with variable concentrations. Residual concentrations of all examined metals were within the maximum permissible limits set by European Union, Food and Agricultural Organization except for the arsenic. Human health risk assessment revealed that the detected concentrations of cadmium and lead might not possess health hazards. Nevertheless, care is imperative regarding As especially in Saudi Arabia because of the excessive meat consumption.

Key Words: Camel, Risk assessment, Saudi Arabia, Sheep, Trace elements

Introduction

Arabian camel (*Camelus dromedarius*) is an important animal worldwide, especially in the Arabian Peninsula and Middle East countries. Camel plays an important role in the culture and lifestyle of people living in these areas for more than 2000 years. Camels are known for their survivability on the extremely hard environmental conditions[19]. Camels are reared in these regions

*Corresponding author: Wageh Sobhy Darwish (Ph.D), Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt
E-mail: wagehdarwish@yahoo.ca; wagehdarwish@zu.edu.eg
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for its meat, milk, wool and leather production[21]. Camel is considered as a major source of meat supply in the Arabian Gulf area, some parts of Africa, India and Australia and additionally, the camel meat production represents about 0.7% of the world meat production[13]. Camel meat is considered healthier than that of beef and mutton, in terms of less fat amount, less cholesterol and high protein content[19].

Sheep (*Ovies aries*) is domesticated since ancient times and share same environment with humans, thus they are considered as ideal bioindicators for human exposure to environmental pollutants, and furthermore their meat and offal represent ideal sources for animal-derived protein worldwide[8].

Metals are classified into essential and toxic elements. Essential metals such as zinc (Zn), cupper (Cu) and iron (Fe) are needed to maintain the normal body functions and blood synthesis. Toxic metals such as lead (Pb), cadmium (Cd) and arsenic (As) are found in the contaminated food of both animal and plant origins, leading to several biological implications. Thus monitoring the levels of these elements in human food is of particular importance for both food safety and human health[4].

Camel and sheep like other animals during their lifetime are exposed to different xenobiotics, such as heavy metals[9]. However, there is insufficient information about metal content in the edible tissues of these animals, particularly in the camel. In Saudi Arabia, like in many Middle East countries and Arabian Gulf area, people consume large amount of camel and sheep meat and offal. However, the information about the Saudi population dietary exposure and health risk assessment due to consumption of such meat is scarce. Therefore, the present study aimed at the estimation of the metal content in meat, liver and kidney of both camel and sheep slaughtered at Al-Ahsa slaughterhouse, Saudi Arabia. The measured toxic metals were Pb, Cd and As and the essential metals were Zn, Cu and Fe). Furthermore, the investigated metals-dietary intake and health risk assessment were estimated.

Materials and Methods

The experiments were done according to the regulations and guidelines of King Faisal University. All chemicals were purchased from Merck, Darmstadt, Germany and were of HPLC grade or the highest quality available.

Collection of Samples: A total of 120 samples of muscles, liver and kidney were collected from randomly selected Camels and sheep ($n = 20$). Samples were collected from Al-Ahsa central slaughterhouse, Saudi Arabia (25°22′18.1″N 49°26′21.1″E), directly after slaughter. This area is considered rural depends mainly on livestock production with limited industrial activities. Samples were collected during the period of July to December 2017. Age of animals was determined by visual examination of teeth during the ante-mortem inspection[25]. The average age of the slaughtered camel and sheep was estimated to be 31.65 ± 24.27 and 12.45 ± 7.94 months, respectively. All animals were apparently healthy, active, and free from any disease. Sampled tissues for metal analysis were collected in plastic falcon tubes and stored at −20°C until extraction and measurement.

Sample preparation and extraction: Extraction of trace elements was done according to the method described before[11]. In short, one gram of each tissue was digested in 10 mL of 3 : 2 mixture of nitric acid (65%) and perchloric acid (70%). This mixture was kept overnight for digestion at room temperature, then heated for 3 h in a water bath with shacking at 70°C. After complete digestion, prepared sample solutions were kept in acid-leached polyethylene bottles at room temperature until metal analyses.

Trace element measurement: Metal concentrations

(Cd, Pb, As, Zn, Cu and Fe) were measured using an atomic absorption spectrophotometer (Shimadzu AAS 6800, Shimadzu, Japan), using hollow cathode lamps, equipped with air-acetylene flame. Mean recoveries ranged from 95 to 106 %. Calculation of metal concentrations was performed based on standard curves done for all examined metals. All recorded results were on wet weight (ww) basis.

Quality assurance and control: The accuracy of the analysis was checked by measuring IAEA-142/TM from IAEA's certified reference materials (muscle homogenate) (Vienna, Austria). Recovered concentrations of the certified samples were within 5% of the certified values. All samples were analyzed in triplicates.

Estimated daily intake (EDI): To calculate the estimated daily intake (EDI) of the examined metals; the following equation described by the Human Health Evaluation Manual (US Environmental Protection Agency)[27] was used: $EDI = C_m \times F_{IR} / BW$

Where EDI is based on μg/kg/day; C_m is the concentration of the metal in the sample (mg/kg wet weight); F_{IR} is the meat ingestion rate in Saudi Arabia, which was estimated at 146 g/day[2]; BW is the body weight of Saudi adults and children, which was estimated at 70 kg for adults and 30 kg for children[4].

Health risk Assessment: The non-cancer risk imposed on Saudi population (adults and children) by consumption of metal-contaminated edible tissues was calculated based on the guidelines recommended by the US Environmental Protection Agency[27]. The hazard ratio (HR) was calculated by the following equation: Hazard Ratio (HR) = EDI/RfD $\times 10^{-3}$

Where EDI, is the estimated daily intake and RfD is the recommended reference doses in mg/kg/day (0.001 for Cd, 0.004 for Pb, 0.0003 for As, 0.3 for Zn, 0.04 for Cu and 0.7 for Fe)[27]. The hazard ratios (HRs) can be summed to generate a hazard index (HI) to estimate the risk of mixed metals. HI was generated by using the following equation:

$HI = \sum HRi$

Where *i* represent each metal

A HR and/or HI of >1 indicates that there is potential risk to human health, whereas a result of ≤ 1 indicates no risk of adverse health effects.

Statistical analysis: Statistical significances and correlation analysis were evaluated using Tukey-Kramer HSD difference test (JMP) (SAS Institute, Cary, NC, USA). A $P < 0.05$ was considered to be significant. Values were expressed as means $\pm$ standard deviation.

Results

The results recorded in Table 1 showed that the liver and kidney tissues of camel had significantly the highest mean concentrations of Cd (μg/kg ww) (2.18 ± 0.71 and 1.83 ± 0.63, respectively) followed by the sheep samples, liver (1.79 ± 0.66); kidney (1.18 ± 0.37) and muscle (0.44 ± 0.17); while the muscle of camel had the lowest Cd residues (0.29 ± 0.27). The liver of sheep had significantly the highest Pb concentrations (10.81 ± 4.54 μg/kg ww), while the muscle of camel had the lowest Pb residues (2.26 ± 0.67 μg/kg ww).

It is clear from the recorded results that liver had significantly the highest As residues, followed by kidney and finally muscle in both sheep and camel. Copper content ranged from 0.06 to 5.02 mg/kg ww in the edible tissues of camel and from 0.11 to 3.35 mg/kg ww in the sheep samples. The mean concentrations of Cu in the liver of camel and sheep were 1.66 ± 0.92, 1.74 ± 0.73 mg/kg ww, respectively.

Zinc was recorded in the examined tissues in the following order liver $>$ kidney $>$ muscle in both camel and sheep. Similar to Zn, liver of both camel and sheep had the highest Fe concentrations followed by kidney and finally

Table 1. Heavy metal contents in the camel and sheep meat and offal

	Cd		Pb		As		Cu		Zn		Fe	
	Range (Median)	Mean ±SD	Range (Median)	Mean ±SD	Range (Median)	Mean ±SD	Range (Median)	Mean ±SD	Range (Median)	Mean ±SD	Range (Median)	Mean ±SD
Camel kidney	1.08–2.92 1.82	1.83 ±0.63^{a}	5.05–11.88 8.22	7.91 ±2.39^{b}	11.14–55.56 22.24	24.14 ±12.85^{b}	0.06–0.92 0.18	0.26 ±0.25^{b}	1.33–3.55 2.82	2.54 ±0.66^{bc}	8.92–17.64 12.24	13.22 ±2.52^{b}
Camel liver	1.12–3.95 1.95	2.18 ±0.71^{a}	4.66–15.23 9.11	9.26 ±3.32^{ab}	14.76–99.95 36.89	38.41 ±25.15^{a}	1.04–5.02 1.23	1.66 ±0.92^{a}	3.07–6.77 3.58	3.96 ±1.02^{a}	10.91–20.98 18.47	18.23 ±2.22^{a}
Camel muscle	0.11–0.89 0.15	0.29 ±0.27^{c}	1.12–3.25 2.19	2.26 ±0.67^{c}	7.14–26.14 12.65	12.89 ±4.29^{c}	0.11–0.19 0.15	0.15 ±0.02^{c}	0.22–1.99 1.13	1.16 ±0.52^{d}	2.14–8.89 4.12	4.15 ±1.62^{c}
Sheep kidney	0.17–1.78 1.17	1.18 ±0.37^{b}	4.44–15.33 7.78	8.32 ±2.97^{ab}	10.74–76.78 23.91	30.34 ±20.86^{a}	0.11–0.98 0.19	0.32 ±0.29^{b}	1.11–11.78 3.09	3.03 ±2.26^{ab}	10.08–23.62 16.69	16.66 ±4.15^{a}
Sheep liver	0.85–3.58 1.73	1.79 ±0.66^{a}	5.54–19.28 9.69	10.81 ±4.54^{a}	10.57–84.41 18.54	30.29 ±22.57^{a}	1.05–3.35 1.48	1.74 ±0.73^{a}	2.22–8.58 3.43	4.21 ±1.67^{a}	10.57–20.62 17.55	17.09 ±2.84^{a}
Sheep muscle	0.09–0.84 0.41	0.44 ±0.17^{c}	1.56–4.13 2.71	2.71 ±0.68^{c}	6.97–18.33 8.48	10.05 ±3.77^{c}	0.11–0.33 0.17	0.18 ±0.05^{c}	1.01–3.99 1.16	1.54 ±0.79^{cd}	2.02–10.09 6.67	6.16 ±2.55^{c}

Residual concentrations of cadmium (Cd), lead (Pb), arsenic (As) and copper (Cu) are in μg/kg ww; while that of zinc (Zn and iron (Fe) are in mg/kg ww in the examined edible tissues of camel and sheep (n = 20 each). Measurements were done in triplicate for each sample. Means in the same column carrying different superscript letter are significantly different at $P < 0.05$.

muscle (Table 1).

Scatter-plots made in Fig. 1 showed correlation analysis between the measured metals in the camel liver. Significant positive correlations in case of Cd-Pb ($r = 0.684$, $P = 0.0005$); Cd-As ($r = 0.753$, $P = 0.0005$); Pb-As ($r = 0.701$, $P = 0.0003$) and Cu-Zn ($r = 0.284$, $P = 0.04$) were observed (Fig. 1). Significant positive correlations were recorded also in sheep as in case of Zn-Pb ($r = 0.589$, $P = 0.004$); Zn-As ($r = 0.724$, $P = 0.003$) and Fe-As ($r = 0.296$, $P = 0.002$) (Fig. 2). Age had positive correlations with the examined metals in both camel and sheep except for Cu and Fe in both camel and sheep and Zn in camel (Fig. 1 and 2).

The ranges of EDI (μg/kg/day) values were 0.001–0.007 (Cd); 0.005–0.036 (Pb); 0.021–0.134 (As); 0.375–5.811 (Cu); 2.419–14.033 (Zn) and 8.656–56.976 (Fe) among Saudi children and adults (Table 2). Non-carcinogenic hazard ratios and hazard indices due to consumption of camel and sheep edible tissues were shown in Table 3. All HR and HI values were far below 1.

Discussion

Camel and sheep are considered valuable sources for animal derived protein, vitamins and essential trace elements worldwide, especially for people living in the Middle Eastern and Arabian countries. These animals might expose to heavy metals and trace elements either due to environmental pollution or in the form of mineral mixtures introduced as food supplements. However, there is a clear lack of information about metal load in the edible tissues in such animals, in particular, the camel. Thus, estimating the residual levels of these metals, especially the toxic ones, and assessment of the possible human health risks are of high priority.

Estimation of metal concentrations and their correlations in camel and sheep edible tissues

Cadmium was detected in all examined

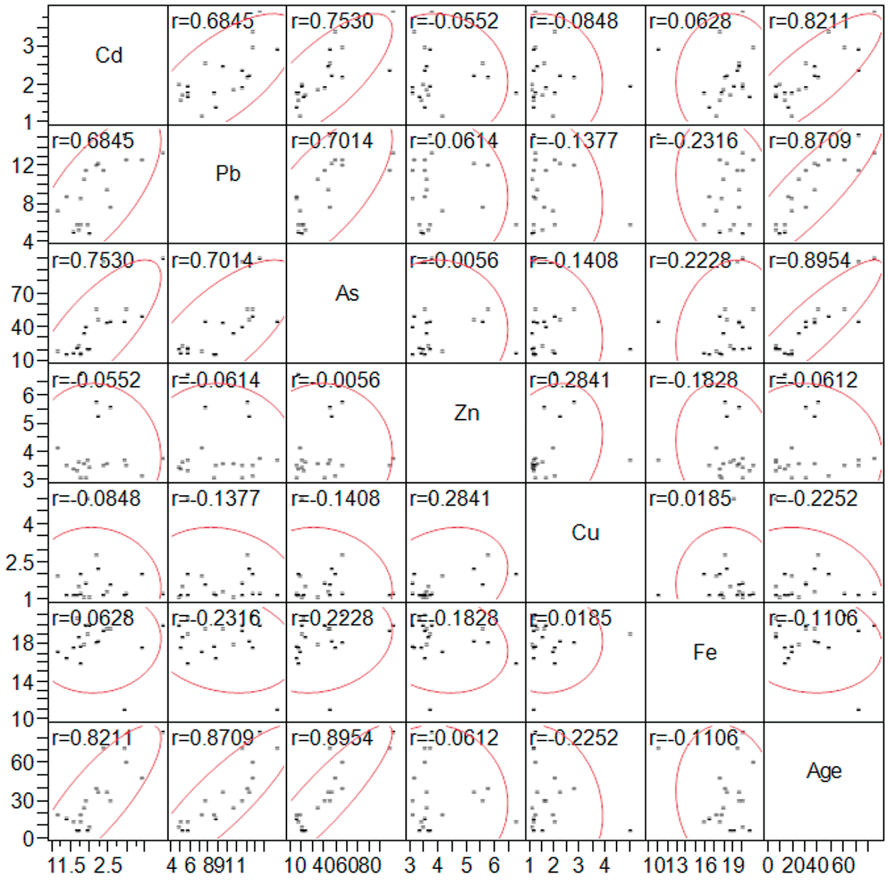

Fig. 1. Correlations between different metals and age in the liver of camel. Scatterplots between different metal concentrations in the liver and age of camel (n = 20), r refers Pearson correlation. This analysis was done using JMP software (SAS Institute, Cary, NC, USA).

samples from both camel and sheep. The recorded concentrations of Cd in sheep in this study were lower than that recorded in sheep and cattle offal collected from Egypt, Ghana and Serbia[4,8,26]. Lower concentrations were recorded in mutton, pork and beef collected from Taiwan[6]. The recorded concentrations of Cd in this study were within the maximum permissible limits (MPLs) of Cd in the muscle and offal (0.5 to 1.0 mg/kg ww)[10]. However, Cd concentrations were higher than EU limits in hunted game animals in Slovakia[15].

Lead was detected in all examined samples in this study. Levels of Pb in the edible tissues of camel and sheep from our study were lower than the levels reported by Bortey-Sam *et al.*[2] in sheep and goat from Ghana. Additionally, Darwish *et al.*[8] recorded higher Pb concentrations in the liver and kidney of sheep and cattle slaughtered in Egypt. However, Chen *et al.*[6] recorded lower Pb levels in the mutton collected from Taiwan. None of the tested samples in this study exceeded MPLs of Pb (0.1 and 0.5 mg/kg ww for muscle and offal, respectively) set by European Commission[10]. Unlikely, Pareja-Carrera *et al.*[23] reported that 87.5% of the collected samples from livestock living in the ancient mining area of Sierra Madrona and Alcudia Valley, Spain exceeded these MPLs.

Arsenic was detected in all muscle and offal samples tested in this study. There was no significant differences between the same tissues in both of camel and sheep ($P < 0.05$). EC[10] set 0.05 mg/kg ww was standard value for As concentrations in meat and offal. It is worth noting that 20% and 10% of liver and kidney

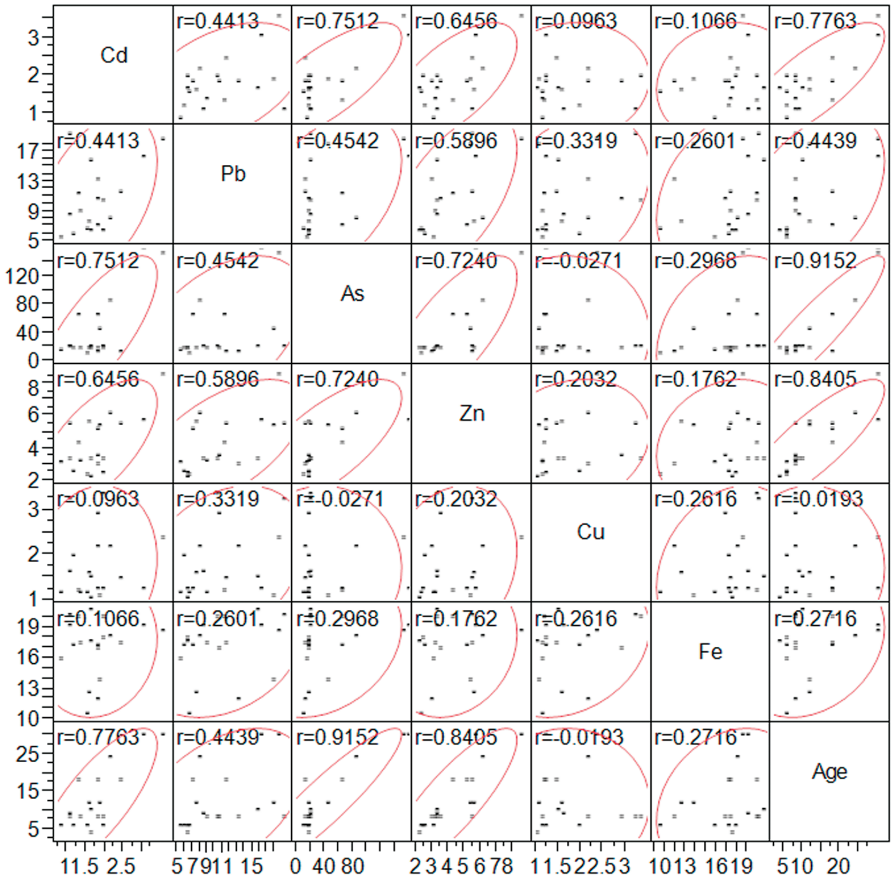

Fig. 2. Correlations between different metals and age in the liver of sheep. Scatterplots between different metal concentrations in the liver and age of sheep (n = 20), r refers Pearson correlation. This analysis was done using JMP software (SAS Institute, Cary, NC, USA).

samples of camel exceeded this level. However, in sheep 25% and 20% of liver and kidney samples exceeded that value. None of the examined muscle samples exceeded MPL of As. Levels of As in our study were nearly similar to those reported by Bortey-Sam *et al.*[4] in sheep and goat offal collected from Ghana. Unlikely, Chen *et al.*[6] detected lower concentrations of As in meat samples collected from Taiwan. One possible reason for the high load of As in the examined tissue samples is probably due to the use of arsenic based feed additives during the intensive livestock production as in many parts of the world[16].

Copper was detected in all examined samples with variable concentrations. Livers of sheep and camel had significantly ($P < 0.05$) the highest concentrations of Cu residues. Copper concentrations in our study were within the recommended MPLs (40 mg/kg ww)[10]. It is important to mention that the recorded levels of Cu in this study were comparable to that recorded in sheep offal in Ghana[4], game animals and beef in Germany[24]. However, higher Cu concentrations were recorded in livers and kidneys of sheep and goat raised in Egypt and game animals hunted in Slovakia[1,15].

Zinc is an essential element, which is necessary for various body functions. Zn was recorded in all examined samples with variable levels. Liver had the highest content of Zn compared to other tissues. There was no significant differences between camel and sheep samples. The recorded levels in this study did not exceed MPLs of Zn (10 mg/kg ww)[10]. Higher concentrations of Zn were recorded in edible

Table 2. Dietary trace elements intake (μg/kg/day) due to ingestion of camel and sheep meat and offal

	Cd		Pb		As		Cu		Zn		Fe	
	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult
Camel kidney	0.006	0.004	0.026	0.016	0.081	0.051	0.867	0.542	8.467	5.298	44.066	27.573
Camel liver	0.007	0.005	0.031	0.019	0.128	0.081	5.533	3.462	13.211	8.259	60.766	38.022
Camel muscle	0.001	0.001	0.008	0.005	0.042	0.027	0.467	0.292	3.866	2.419	13.833	8.656
Sheep kidney	0.003	0.002	0.028	0.017	0.101	0.063	1.066	0.667	10.111	6.319	55.533	34.748
Sheep liver	0.006	0.004	0.036	0.023	0.134	0.084	5.811	3.629	14.033	8.781	56.967	35.645
Sheep muscle	0.001	0.001	0.009	0.006	0.033	0.021	0.611	0.375	5.133	3.212	20.533	12.848
TDI	1.00		3.57		No		500		1000		800	

TDI: Tolerable daily intake according to FAO/WHO (2010)

Table 3. Heavy metal risk assessment due to ingestion of camel and sheep meat and offal

	HR												HI	
	Cd		Pb		As		Cu		Zn		Fe			
	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult	Child	Adult
Camel kidney	0.006	0.004	0.007	0.004	0.268	0.168	0.022	0.014	0.028	0.018	0.063	0.039	0.394	0.247
Camel liver	0.007	0.005	0.008	0.005	0.427	0.267	0.138	0.087	0.044	0.028	0.087	0.054	0.711	0.446
Camel muscle	0.001	0.001	0.002	0.001	0.143	0.089	0.012	0.007	0.013	0.008	0.019	0.012	0.19	0.118
Sheep kidney	0.004	0.002	0.007	0.004	0.337	0.211	0.027	0.017	0.034	0.021	0.079	0.049	0.488	0.304
Sheep liver	0.006	0.004	0.009	0.006	0.448	0.281	0.145	0.091	0.047	0.029	0.081	0.051	0.736	0.462
Sheep muscle	0.001	0.001	0.002	0.001	0.112	0.069	0.015	0.009	0.017	0.011	0.029	0.018	0.176	0.109

tissues of sheep from Egypt and Ghana[1,4]. Additionally, beef, veal and game animals from Germany and Slovakia had also higher Zn levels than that reported in this study[15,24].

Iron is similar to Zn as it is considered as an essential element needed at certain levels for some essential physiological functions and blood synthesis. Iron was detected in all examined samples. None of the samples exceeded the MPL of Fe (30 mg/kg ww)[10]. The Fe load in the examined tissues in this study was comparable to that reported in sheep kidney and muscle raised in Turkey[5]. However, the recorded levels of Fe were lower than the recorded Fe levels in the edible tissues of sheep and goat in Ghana[4].

The reasons behind the differences in the metal-accumulation patterns among this study and studies from other parts of the world are mainly due to the differences in the environmental pollution scenarios and differences in the rearing systems. Furthermore, inter-species differences in metal accumulate might be attributed to the inter-species differences in their xenobiotic metabolizing enzymes and metal-detoxification abilities[20]. The high level of toxic metals and trace elements in the liver and kidney of camel and sheep in this study compared with the muscle may be attributed to their significant role in xenobiotics metabolism and detoxification[17].

Metal-metal interactions are considered as an approved bio-adaptation mechanism for the toxic metals as some trace elements tend to increase in the tissue by the increase of the toxic metals[7]. In this study, such correlations were also observed between the measured elements. We have chosen liver to calculate metal-metal relationships as it showed the highest accumulation level of all examined metals, beside its major role as organ of xenobiotics metabolism and detoxification. Scatter-plots between the measured metals in the camel liver showed significant positive correlations in case of Cd-Pb, Cd-As, Pb-As and Cu-Zn. Similarly, significant positive correlations were recorded in sheep

especially between essential trace elements and toxic ones as clear in case of Zn-Pb; Zn-As and Fe-As. Nearly similar findings were reported before between Cd-Pb[8] and As-Hg[22,31]. Attempting to explain correlation data for biological samples is not simple because of differences in the source of metals and differences in metal metabolism. However, a possible explanation might be the induction of metallothionein, a protein that is linked to metal-detoxification[20].

Age is considered as a major factor for exposure not only to heavy metals but also to other environmental pollutants. In this study, strong and significant positive correlations were reported between the age of animals and the load of metals in the liver. For instances, in camel, age showed significant ($P < 0.0001$) positive correlations with Cd, Pb and As with r values of 0.821, 0.870 and 0.895, respectively. Similar phenomenon was observed in the case of sheep, as age had significant positive correlations with Cd ($r = 0.776$, $P = 0.016$); Pb ($r = 0.443$, $P = 0.006$); As ($r = 0.915$, $P = 0.001$) and Zn ($r = 0.840$, $P < 0.0001$). Age is reported to have positive correlation with toxic metals accumulated in cattle, sheep and boars in studies conducted in Egypt, Serbia and Slovakia[8,15,25]. However, slightly negative correlations were recorded in the current study between age and essential metals such as in Cu and Fe in the camel and sheep. Interestingly, Zn showed a positive correlation with age in sheep, which was negative in camel. This may indicate inter-species differences in xenobiotics metabolism. Thus, future studies should be conducted in order to declare the complete relationships between age and such essential metals among domesticated and wild animals.

Dietary intake and Health risk assessment

There is no report indicating the health risk assessment associated with metal exposure of Saudi population due to consumption of camel and sheep offal. Thus, health risk assessment among Saudi adults and children was evaluated in this study via estimation of dietary intake (estimated daily intake (EDI)), hazard index (HI) and hazard ratio (HR) of the examined trace elements. Comparing the recorded EDI values in this study with FAO/WHO[14] tolerable daily intake of metals revealed that dietary intake of all metals was acceptable except for As. FAO/WHO expert committee on food additives[14] recorded an increase in the incidence of lung cancer due to As exposure, so the tolerable daily intake of As was no longer appropriate. It is worth noting that despite the dietary intake of Saudi population of As is too low; these levels should be reduced to minimum. Metal dietary intake in Saudi population was comparable to the levels reported in New Zealand, Egypt and China[8,18,28].

Risk analysis in this study was conducted via estimation of non-carcinogenic hazard ratios (HRs) and hazard indices (HIs) as shown in Table 3. All HR and HI values were far below 1, which indicates no potential exposure to risk due to ingestion of camel and sheep meat and offal. However, great care should be taken as repeated ingestion of even small concentrations of these metals might interfere with some biological functions. HR and HI values reported in this study were lower than that reported in Ghana and Egypt[4,8].

The examined metals in this study had significant contribution to human health. For instances, chronic exposure to Cd may cause renal dysfunction and pulmonary effects[32]. Lead poisoning was recorded to be the cause of children deaths in China, Nigeria and Zambia[3,29,30]. Arsenic was linked to multi-organ cancers, skin lesions, developmental and reproductive disorders[12]. Zn, Cu and Fe are essential trace elements that is needed for the normal body functions including skin integrity, wound repair, fertility, blood synthesis and metal detoxification[14].

Conclusions

Metals with confirmed health risks were detected in all examined samples. This may reflect potential health hazards among consumers especially, in children due to the high consumption of meat in the study area. Future studies are still needed to declare the exact relationships between metals and the age of the animal, in addition to the inter-species differences in xenobiotics metabolism.

Conflicts of interest

None

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