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A survey on hepatic and renal trace elements status of sheep and goats in Zarrinshahr region, Isfahan, Iran: an abattoir study

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Abstract

Hepatic and renal contents of essential trace elements provide good clues to monitor the nutritional status of grazing animals. Liver and kidney samples were collected from 60 sheep and 60 goats slaughtered in Zarrinshahr abattoirs and analyzed for essential trace elements. Hepatic Fe concentrations were significantly higher in sheep than goats. There were also higher renal and hepatic Fe concentration in age group of 2-4 years compared with age groups of <2 and 4-6 years. The concentration of hepatic trace elements including Fe, Cu, Zn, Co, Ni and Cr was significantly higher than renal ones. Concentration of Cu in the liver and kidney of the sheep from Zarrinshahr were lower than normal values and findings from other countries. Fe, Cr and Ni in the organs, however, were high and Zn and Co concentrations were in normal ranges. It is concluded that high concentrations of Fe, Cr and Ni in the liver and kidney of sheep and goats from Zarrinshaher area may be related to industrial operations and should be considered with regards to animal and human health, while potentially copper deficiency may be regarded as a potential burden to flock efficiency.

Keywords: Sheep, goat, trace elements, industrial pollution, liver, kidney

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Introduction

Trace elements such as copper (Cu), zinc (Zn), iron (Fe), cobalt (Co), chromium (Cr) and nickel (Ni) have been shown to be essential for life and health in plants, animals and humans. These trace elements are part of enzymes, hormones and cells in the body (Thompson *et al*., 1991). Among those essential elements Cr and Ni have named "newer essential trace elements" and are involved in different metabolic process. However, the function of essential trace elements is paradoxical, so that deficiency or excessive intake of these elements leads to undesirable pathological conditions (Thompson *et al*., 1991; Radostits *et al*., 2007).

Essential trace elements accumulate predominantly in liver and kidney, while the metal content of muscle is generally low. Excessively higher concentrations of these metals in tissues of animals suggest an exposure either from the polluted air, water or feeds or all these sources (Jarup 2003; Wilkinson *et al*., 2003). Low concentrations or deficiency of a trace element can either be primary or secondary. A primary deficiency is that caused by an inadequate intake due to a low dietary concentration of that element and a secondary deficiency is when an adequate concentration of certain trace elements are present but other dietary components could inhibit utilization of the first element or exacerbate its excretion (Thompson *et al*., 1991).

Because in many middle eastern countries including Iran, ruminant liver and kidneys are traditionally consumed as a valuable food source, these metals can readily transferred through food chains and can pose a potential health risk (Adei and Forson-Adaboh 2008; Swaileh *et al*., 2009). On the other hand, essential trace element deficiencies among animals will potentially introduce an imbalance and element deficiencies in human diet. In addition, the intake and concentrations of essential and non-essential trace elements in internal organs of farm animals often can be mediated by the absorption and metabolism of the other elements. For example, Fe deficiency increase Ni absorption and Ni deficiency decrease tissue Zn and Cu concentrations (Spears 1984). Finally, hepatic and renal concentrations of trace elements might reflect the nutritional status of these grazing animals.

The common sources of environmental pollution are natural and anthropogenic process including various industrial activities. Several animal species particularly farm animals which reared freely on pasture have been suggested as suitable bio-indicators of the pollutant metal concentrations in the terrestrial environment (Lopez Alonso *et al*., 2002; Wilkinson *et al*., 2003). The knowledge of essential trace elements in farm animals in a pollutant environment is essentially required for assessing the effect of pollutants on animals and possible effects on human health. Moreover, hepatic and renal contents of essential trace elements collected at abbatoirs might be a good source of information in far and mountaineous areas, were collecting blood samples for monitoring trace mineral status of sheep and goat flocks is a big challenge, if not impossible.

Zarrin Shahr, Isfahan, Iran, is one of the largest industrial complexes in Iran that is surrounded by a high mountaineous climate, and a large number of sheep and goat flocks are regularly graze the region, where monitoring trace element nutritional status of the flocks is much difficult. The aims of the present study were to determine the Co, Cu, Zn, Fe, Cr and Ni concentrations in liver and kidney of sheep and goats raised in Zarrinshahr area, as well as evaluating the pattern of deficiencies or excesses in order to provide the proper formula for formulation of a trace mineral supplement that fit the requirements of the sheep and goat flocks of the region.

Materials and methods

During October – September 2012, liver

and kidney samples of sheep and goats (120 samples from each genus equally, different ages and both sexes) were collected from randomly selected healthy animals from the slaughter lines of two abattoirs in Zarin-Shahr, Isfahan, Iran. About 50g Samples were collected from the same part of each organ, caudal lob of the liver and the cranial half part of left kidney. All samples were packed individually in plastic bags, labeled and immediately transported to the laboratory and stored at -18˚C till analysis. Age of the animals was estimated using dentiture.

For liver and kidney analyses, 5g sub-samples were excised from semi-thawed tissues and processed for determination of Cu, Co, Fe, Zn, Cr and Ni using inductively coupled plasma atomic emission spectrometry (ICP – OES, Perkin Elmer Optima 4300, Germany). In ICP-OES analyses, the method detection limits of the measured elements were defined as the concentration of an element which will give a signal three times higher than the standard deviation of seven to ten replicate measurements of five reagent blanks belonging to five different sets of the experiments. The wave lengths and detection limits (DL) were given in

Statistical analysis

Data are expressed as median and interquartile range (IR). A $p < 0.05$ was considered to be significant. Normal distribution of data was tested by determining the Shapiro-Wilk W and the associated P value as well as by examining the normal probability plots. Log transformed was also tested to seek the probability of obtaining a normal distribution. Variables were tested to find significant differences related to genus, sex and age groups for all variables. When there were no significant differences, the data were combined

Table 1. All samples were analyzed in triplicate.

Elements	DL (ppb)	Wave length (nm)
Cu	0.24	327.393
Co	0.35	228.616
Fe	0.50	238.204
Zn	0.52	206.200
Cr	0.28	267.716
Ni	0.22	231.604

Table 1. The DL (detection limit) and wave length for ICP-OES elements.

Median test was used to detect significant differences among different age groups for trace element content of liver and kidney, with Mann Whitney U as post hoc test if the results were significant. Significant differences between renal versus hepatic contents of trace elements were tested using Wilcoxon signed rank test. Mann Whitney U test was used to detect significant differences in different sexes or genera. Bivariate correlation coefficient between renal and hepatic trace elements was tested using Spearman's rho, and coefficent of variation (r). Data were analyzed using a statistical software (SPSS 20, SPSS Inc, Chicago, IL, USA).

Table 1. Renal and hepatic concentrations (PPM) of Cu, Co, Zn, Cr, Ni and Fe as described by percentiles and interquartile range (IR).

Values in PPM	percentiles	Cu *	Co*	Zn*	Cr*	Ni	Fe*
Renal	25	4.54	0.11	4.32	0.2	0.38	47.6
	median	7.11	0.12	6.94	0.24	0.48	70.75
	75	8.54	0.14	10.28	0.3	0.62	88.55
	IR	11.64	0.09	17.71	0.86	0.93	159
Hepatic	25	18.13	0.25	27.8	1.11	2.45	120.75
	median	20.7	0.29	30.6	1.2	2.9	211
	75	26.45	0.32	34.6	1.36	3.29	269
	IR	46.64	0.33	30	1.55	3.6	477.6

* significant differences between hepatic and renal concentrations (Described separately in figure legends)

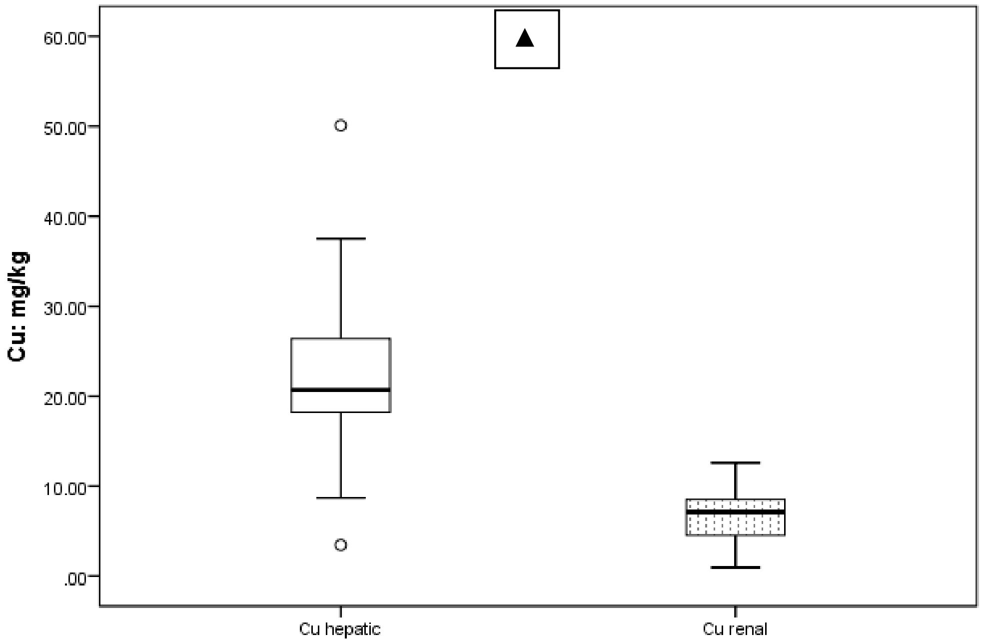

Figure 1. Hepatic versus renal copper (Cu) of sheep and goats. Circles represent outliers.
▲: significant differences between hepatic and renal Cu (p <0.0001, r=0.426, R^2=0.182).

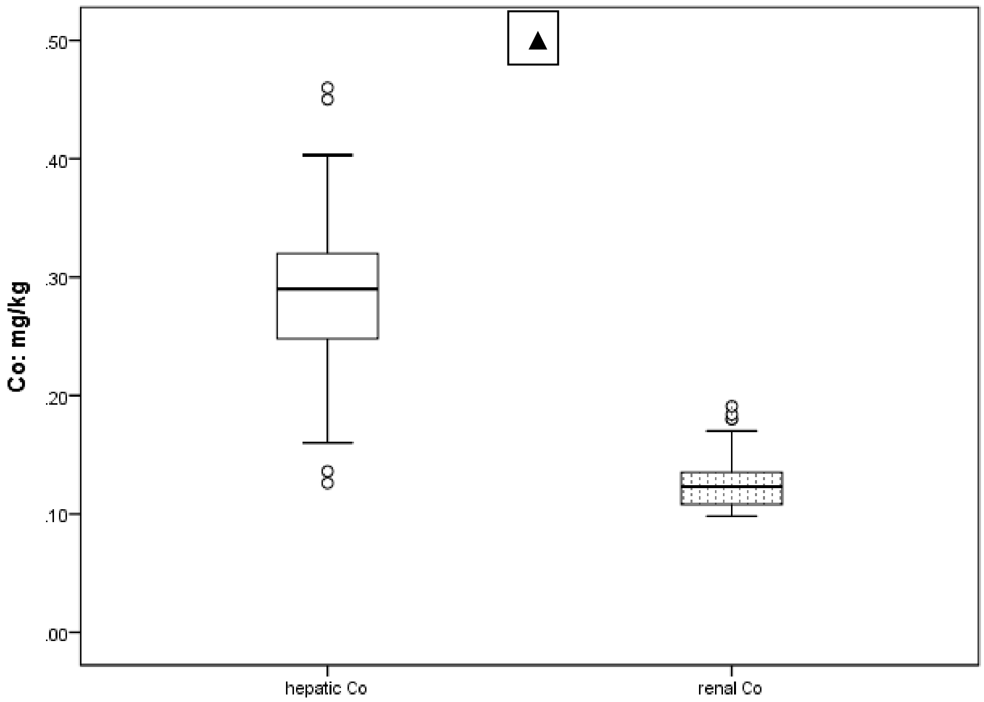

Figure 2. Hepatic versus renal cobalt (Co) of sheep and goats. Circles represent outliers.
▲: Significant difference between hepatic and renal Co (p <0.001, r=0.253, R^2=0.064).

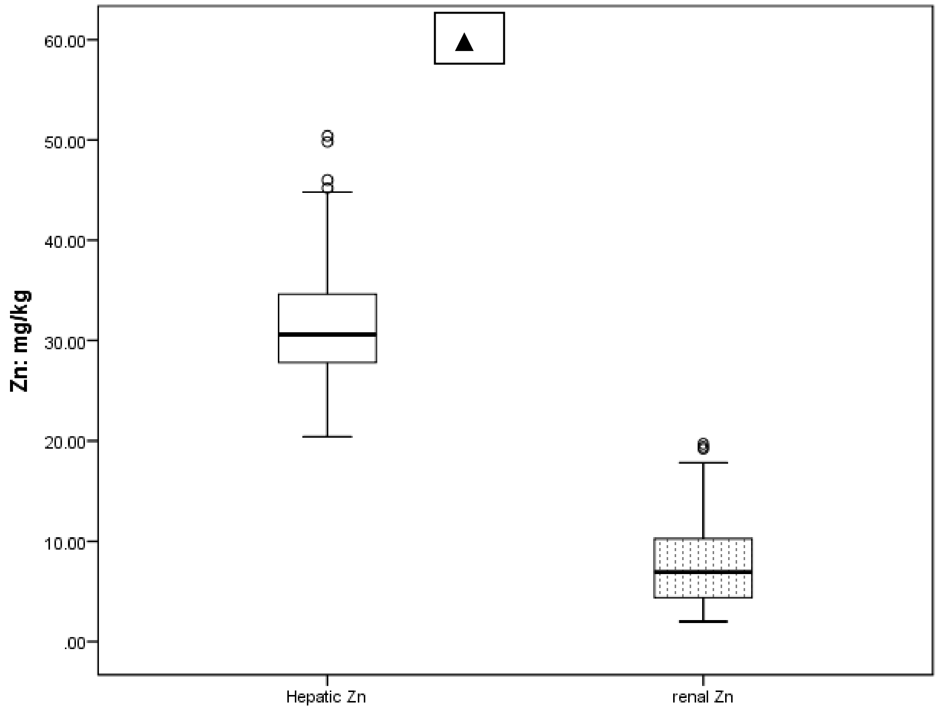

Figure 3. hepatic versus renal zinc (Zn) of sheep and goats. Circles represent outliers.

▲: significant difference between hepatic and renal Zn (p =0.0001, r=0.464, R^2=0.215).

Figure 4. hepatic versus renal chromium (Cr) of sheep and goats. Circles and asterisks represent outliers and extreme values, respectively. (p <0.05, r=-0.226, R^2=0.051)

a: significant difference between hepatic Cr of different age groups with same letters at p <0.05.

▲: significant differences between hepatic and renal Cr in all age groups (p =0.0001)

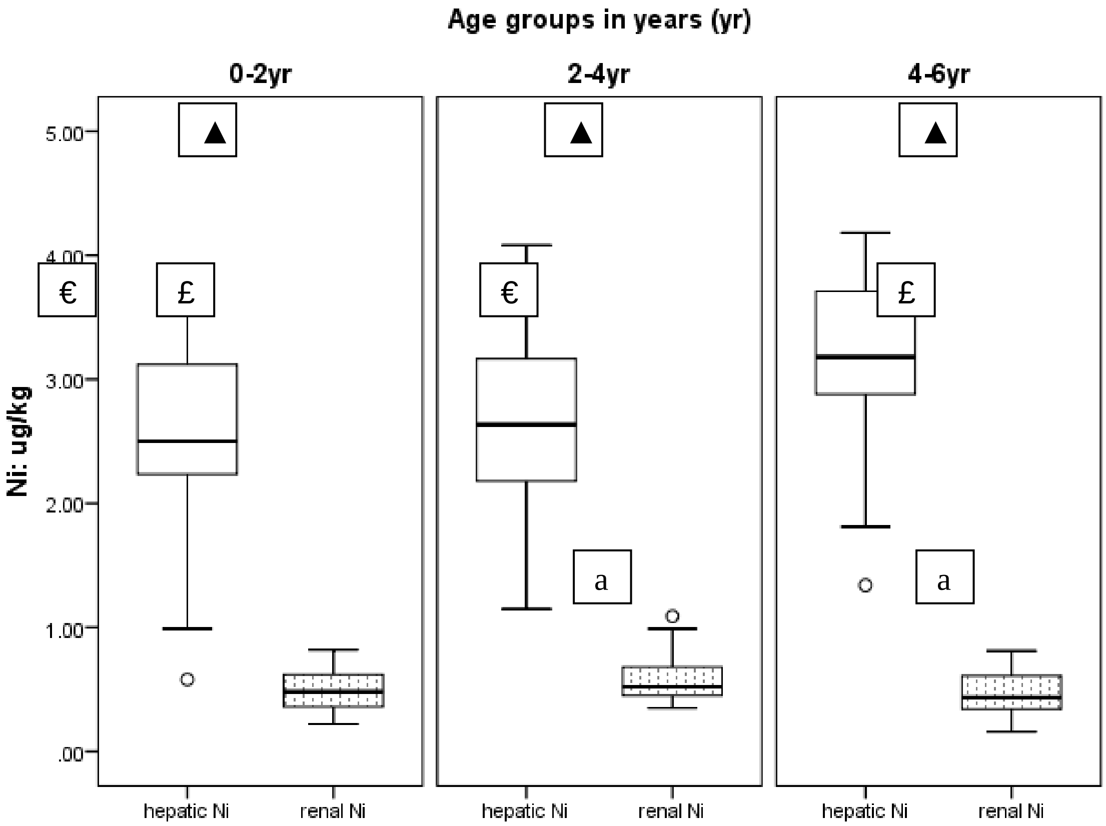

Figure 5. hepatic versus renal Nickel (Ni) of sheep and goats. Circles represent outliers.
a: significant difference between renal Cr of different age groups with same letters at p <0.05.
£,€: significant differences between different age groups of hepatic Ni with same letters (p <0.01).
▲: significant differences between hepatic and renal Ni in all age groups (p =0.0001)

Results

Hepatic Fe concentrations were significantly higher in sheep than goats (p=0.029) and it was higher in sheep. There were also higher renal and hepatic Fe concentration in age group of 2-4 year compared with age groups of <2 and 4-6 years. The concentration of hepatic trace elements including Fe, Cu, Zn, Co, Ni and Cr was significantly higher than renal ones. There was a significant correlation coefficient between hepatic iron and copper status (spearman's rho, p =0.0001, r=0.336, r^2=0.11). Collectively, no significant differences were detected between sexes (p>0.05). The descriptive results for trace elements are presented as follows:

Discussion

Trace elements such as Fe, Cu, Zn, Co, Ni and Cr are essential for normal growth, disease resistance, reproduction and production in farm animals. However, an important concept is that an essential element could also toxic and their concentrations determine the toxic or beneficial effect. Fe content of sheep liver and kidney from Zarinshahr was high or almost several times higher than in liver and kidney from Iceland (Reykdal and Thorlacius 2001), USA (Coleman *et al*., 1992) and Poland (Falandysz *et al*., 1994). Fe concentration in liver in the present study was increased with age. The concentration of Fe in the liver reflects the amount of Fe stored in the body and studies have demonstrated that aging in animals (rats) and humans is associated with increases in iron accumulation (Arvapalli *et al*., 2010).

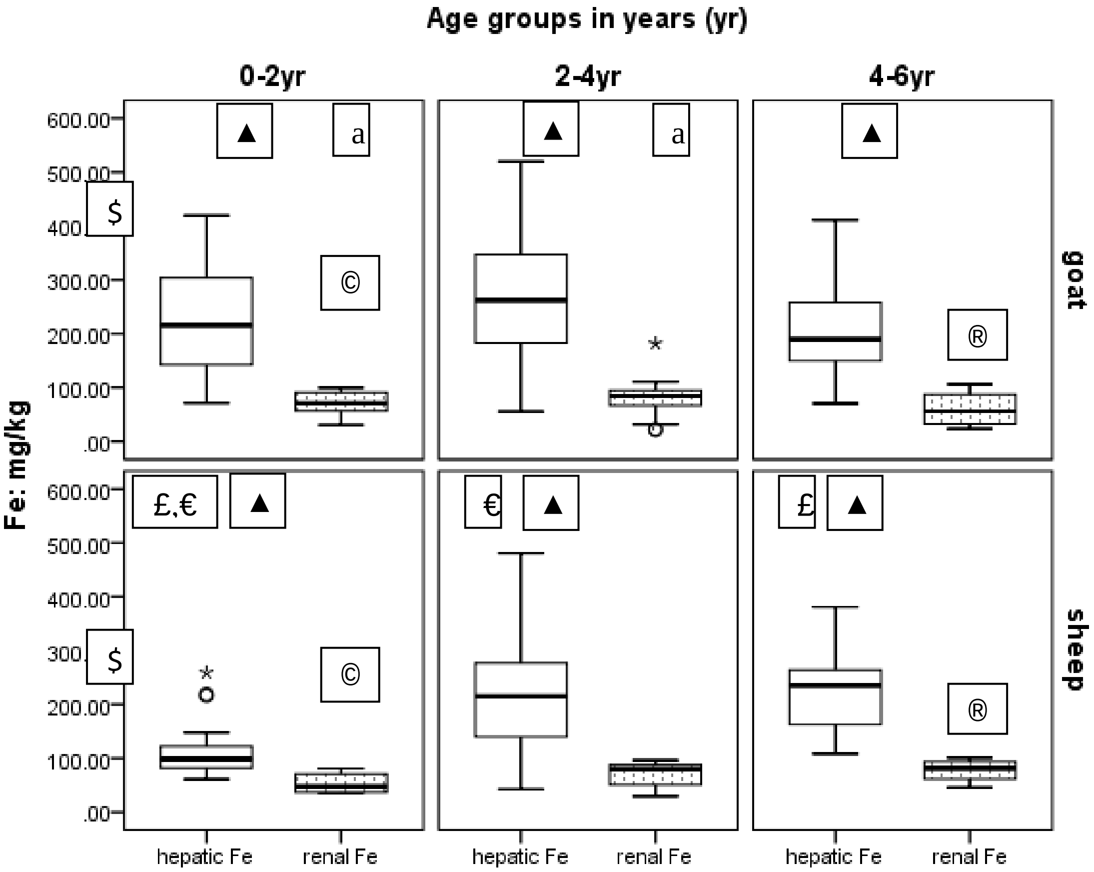

Figure 6. hepatic versus renal iron (Fe) of sheep and goats (p <0.0001 r=0.622 R^2=0.386). Circle and asterisks represent an outlier and extreme values, respectively .

a: significant difference between renal Fe of different age groups of goats with same letters at p <0.05.

€,£ : significant differences between hepatic Fe of different age groups of sheep with same symbols at p <0.01. and p <0.05, respectively.

©,®: significant difference between renal Fe of sheep and goats at specific age groups with same symbols at p <0.01 and P<0.05, respectively.

$: significant difference between hepatic Fe of sheep and goats at specific age group with same symbol at p <0.01.

▲: significant differences between hepatic and renal Fe in all age groups of sheep and goats (p =0.0001).

The concentrations of Cu in liver in this study are low in compare with other studies, but the concentrations of Cu in kidney are comparable with those reports (Salisbury *et al*., 1991; Coleman *et al*., 1992; Falandysz *et al*., 1994; Reykdal and Thorlacius 2001). On the other hand, Cu in sheep liver was very variable, the highest value being 5 times higher than the lowest value. The lower Cu values (less than 20 mg/kg) indicate possible Cu deficiency of the sheep (Radostits *et al*., 2007). Based on the results of the current study, about half of the samples had a hepatic copper less than the abovementioned threshold (20 mg/kg). So, it is an important finding that may need supplementation. It has been stated that the sheep livers with low content of Cu were rich in Fe and this might indicate a Fe-Cu interaction (Menzies *et al*., 2003). However, it is not consistent with our case while there is a highly significant correlation coefficient between hepatic cu and Fe, the effect size is negligible (p =0.0001, r^2=0.11) (Table 8). So, it seems that the region needs Cu supplementation, as well as considering the molybdenum content of the soil and the amount of sulfur that flocks ingest (Thompson *et al*., 1991).

Zn is another essential trace element which is necessary for a wide variety of physiological function. Values for Zn in sheep liver and kidney from Zarinshahr were lower than values reported in the literature (Salisbury *et al*., 1991; Coleman *et al*., 1992; Reykdal and Thorlacius 2001; Adei *et al*., 2008). Zn content of liver and kidney samples in this study did not exceed the safe permissible limit of 50 $mgkg^{-1}$ (FAO/WHO 1992).

The mean concentration of liver and kidney Co concentrations in sheep of this study were near the values in normal sheep. Co is widely distributed in the animal organ in relatively high concentration in liver and kidney. It is required for the synthesis of the vitamin B12 (Radostits *et al*., 2007). The concentrations of Cr and Ni in samples analyzed in this study are higher compared with those reported in sheep and cattle from the Netherlands (Jorhem *et al*., 1989) and Lithuania (Jukna *et al*., 2006). Cr and Ni are essential elements that are necessary for metabolic process and under natural conditions have a low potential for deficiency, however, can cause toxicosis (Thompson *et al*., 1991). There is limited available information regarding the Ni and Cr content of animal organs and their permissible values. While there is no known route of entry of these minerals into the sheep and goats rations, tracing the concentrations of Cr and Ni in the liver of sheep and goats slaughtered in the region on an annual basis could be used to gauge the potential environmental pollution by regional industries.

There were highly significant differences between hepatic and renal contents of all the above mentioned trace elements, implicating superiority of liver samples in contrast to kidney samples. Moreover, significant correlation coefficients between hepatic and renal minerals measured in this study merely shows the existence of a linear relationship between the related components, and the coefficients of variations (r^2) majorly point out to this note that the effect size is not very high (Tables 1-6). Thus, one can ignore kidney samples in similar studies, and focus on liver samples.

It is concluded that Fe, Cr and Ni concentrations in liver and kidney from sheep and goats in Zarrinshahr contained essential were higher than those reported previously in the literature, while Cu concentrations were low and less than normal range implicating the need to reassess the nutritional status of sheep and goat flocks of the region, by both the governmental and private veterinarians of the region. Grazing of sheep and goats in the vicinity of metallurgical plants have might resulted to some of these findings and hygienic control of the offal in this area should be intensified with regard to human consumption.

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بررسی وضعیت ریزمغذی های کبد و کلیه گوسفند و بزهای ناحیه زرین شهر اصفهان– ایران: یک مطالعه کشتارگاهی

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چکیده

مقدار ریز مغذی های کبد و کلیه نشانگر خوبی برای وضعیت تغذیه دام های علفخوار می باشد. در این مطالعه نمونه های کبد وکلیه ۶۰ راس گوسفند و ۶۰ راس بز از کشتارگاه زرین شهر اصفهان جمع آوری و میزان ریز مغذی ها در آنها اندازه گیری شد. غلظت آهن کبد بین گوسفندان و بزها تفاوت معنی داری نشان داد. مقدار آهن کلیه ها نیز در سنین ۴-۲ سال از سایر گروه های سنی، زیر ۲ سال و ۶-۴ سال، بالاتر بود. غلظت ریز مغذی های آهن، مس، روی،کبالت و نیکل کبد به طور معنی داری از کلیه بالاتر بودند. غلظت مس کبد و کلیه بز و گوسفندان ناحیه زرین شهر در مقایسه با سایر کشورها کمتر یافت شد. همچنین غلظت آهن، نیکل و کروم این بافت ها بیشتر از یافته های گزارشات کشورهای دیگر بود. غلظت روی و کبالت کبد و کلیه دام ها در محدوده طبیعی بودند. با توجه به نتایج، غلظت بالای آهن، کروم و نیکل کبد و کلیه گوسفند و بزهای ناحیه زرین شهر اصفهان می تواند مربوط به کارخانه جات صنعتی مستقر در ناحیه باشد و از نظر سلامتی دام ها و انسان باید مورد توجه باشد. غلظت پایین مس نیز باید بالقوه به عنوان کمبود در سطح گله ها مور توجه قرار گیرد.

واژگان کلیدی: گوسفند، ریزمغذی ها، آلودگی صنعتی، کبد، کلیه