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Heavy metal concentrations in bovine tissues (muscle, liver and kidney) and their relationship with heavy metal contents in consumed feed

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ABSTRACT

Toxic (lead, cadmium and mercury) and essential trace (copper and zinc) metals were measured in muscle, liver and kidney samples of bovine and their relationships with heavy metal concentrations in consumed feed were studied. A total of 216 tissue samples from 72 cows and 216 feed samples from 18 farms were collected during four seasons and analyzed for heavy metals by inductively coupled plasma-optical emission spectrometry after wet digestion. The arithmetic mean concentrations (mg/Kg wet weight) of lead (Pb), cadmium (Cd) and mercury were respectively, 0.221, 0.028 and 0.003 in muscle, 0.273, 0.047 and 0.002 in liver and 0.244, 0.114 and 0.003 in kidney. All measured concentrations (with the exception of Pb in muscle) were below the European Union maximum residual limits (MRL). The Cd contents of the kidney were significantly higher than which observed in other tissues. Although, copper (Cu) and zinc (Zn) levels in all of samples were below MRL, but results showed that many cattles may be suffering from Cu and/or Zn deficiency. Significant and positive correlations between Pb ($p < 0.05$, $r = 0.163$) and Cd ($p < 0.01$, $r = 0.303$) concentrations in feed and studied organs were observed. As a considerable amount of metals above MRL were noticed in our study, continuous monitoring of these metals is recommended to avoid hazardous transfer to human through the food of animal origin.

1. Introduction

Fat and protein contents and sensory characteristics have been considered as mainly determinants for meat quality in traditional meat market while healthiness of meat should be contemplated as a critical concept in meat and edible offal quality (Lopez-Alonso et al., 2016). Heavy metals are known as chemical contaminants for meat and edible offal. Metals such as lead (Pb), cadmium (Cd) and mercury (Hg) are toxic even at trace levels and play no useful role in human physiology. Trace essential metals like copper (Cu) and zinc (Zn) are required at low concentration in living organisms to ensure normal growth, development and maintenance, and however their deficiency can cause serious health problems or even death in livestock, although they pose a risk for human health in exposure to values higher than optimal levels (Nriagu et al., 2009; Ugwu, 2010). Accumulation of metals in livestock can be passed on to human through the consumption of their products. Cadmium has been classified as certainly carcinogenic to human (International Agency for Research on Cancer, 1993).

Therefore, it is necessary to monitor heavy metal residues in meat and animal feed in an accurate manner. Various spectrometric techniques such as flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, inductively coupled plasma-optical emission spectrometry and inductively coupled plasma-mass spectrometry have been used for evaluating heavy metals in bovine edible tissues (Sedki et al., 2003; Demirezen and Uruc, 2006; Lopez-Alonso et al., 2016; Ubong et al., 2016). Some clear advantages, including simultaneous measurement capability of multiple metals and very low detection limits have embossed inductively coupled plasma as choice technique for monitoring the levels of toxic metals in food (Nardi et al., 2009).

Meat, liver and kidney are important sources of essential micro-elements (notably Fe, Cu, Zn and Se) in the human diet but may also carry toxic metals as residues. Establishment of regulations for heavy metals in these organs has become important in many countries. For instance maximum residue limits (MRL) of Pb and Cd (mg/g wet weight) have been prescribed as 0.1 and 0.05 in meat, 0.5 and 0.5 in liver and 0.5 and 1 in kidney of bovine by Commission of the European communities (Commission of the European communities, 2006). The Codex Alimentarius Commission has established a limit for Pb in meat and edible offal of bovine that are the same as those prescribed by

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Commission of the European Communities (Codex Alimentarious Commission, 1996). In the case of Hg, the European Union has no legal limits in meat products. No regulations has yet been established for this issue in Iran. Fars province with 0.4 million heads cattle ranks in the first of cow population in the south of Iran, and play an important role in the supply of protein with animal origin in the country (Agriculture Statistics of Iran, 2016). In spite of the considerable production of red meat in this region, there are no data on contamination of heavy metals in meat and edible organs. This study was to determine occurrence and levels of toxic (Cd, Pb and Hg) and trace (Cu and Zn) elements in meat, liver, kidney and feedstuffs consumed by cattle in farms of the Fars province (south of Iran). Comparison of results with different legal regulation, between polluted and unpolluted regions and different seasons was also done.

2. Material and methods

2.1. Study area and sampling

A total of 216 muscle, liver and kidney samples from 72 cows were collected from slaughterhouses during four seasons of the year, in different areas of Fars province (south of Iran). This province contains 29 counties and locates between latitude 27°3′ to 31°40′ N and longitude 50°36′ to 55°35′ E in an area about 133000 km^2 with mean annual rainfall of about 230 mm. The counties of Fars province sorted on the basis of environmental pollution status and Shiraz, Marvdasht and Kazerun counties from the top of the list and Nayriz, Zarrin Dasht and Bavanat from the bottom of the list were selected as polluted and unpolluted areas, respectively.

Approximately 100 g of muscle, liver and kidney were taken from each animal at slaughter and were packed in plastic bags and transported to the laboratory in ice boxes. The samples were taken from the diaphragm, caudate lobule of liver and anterior half of the right kidney (Miranda et al., 2001). In addition, 216 different animal feedstuff samples were collected from three farms in each of the mentioned counties (in total 18 farms and a maximum 3 samples in each farm), during four seasons of the year. Selected farms for feed sampling were the origin of meat samples. Mixed ration was used for sampling and it composed from feedstuffs that provided either through the region's farms or from animal feed factories. Approximately 500 g of different animal feedstuffs were taken according to the procedure described by the EC (Commission of the European communities, 2009). The samples were sealed in plastic bags and transported to the laboratory. Each sample was ground in a laboratory mill (IKA-Werke GmbH, Model MF10, Staufen, Germany), passed through a 1-mm sieve and thoroughly mixed before packaging. All of samples were kept frozen at −4 °C until analysis.

2.2. Samples preparation and analyses

Meat and offal samples were defrosted to room temperature and cleaned from gross fat and tendons at the time of analysis. Then each of sample was homogenized by a home-mixer and prepared by wet digestion method under laminar flow hood conditions. In the next step, one g from homogenized sample was taken into a 15 ml glass tube and digested by acid attack technique using 0.7 ml of nitric acid (HNO_3) and 0.3 ml perchloric acid ($HClO_4$). In this technique, the easily oxidized components are decomposed by HNO_3 and the rest of the components, which are more difficult to oxidize, decomposed by $HClO_4$ (Aras and Ataman, 2006). A HNO_3-$HClO_4$ mixture is recommended for foods containing proteins and carbohydrates and no fat (Pomeranz and Meloan, 1994).

All inorganic acids were of analytical grade, Merck brand (Darmstadt, Germany). The contents of tubes were heated for 16 h in water bath at 80 ºC and then filtered through a Whatman no. 41 filter paper in a Falcon volumetric tube and adjusted to 2 ml with nitric acid. The Pb, Cd, Hg, Cu and Zn concentrations in the prepared samples were quantified using inductively coupled plasma atomic emission spectrometer (Optima 7300 DV, Perkin–Elmer, Norwalk, CT, USA).

Operating conditions of this instrument was as follow; frequency 40 MHz, radio frequency power 1200 W, Plasma, auxiliary and nebulizer gas flow rates 15, 0.5 and 0.8 L/min respectively, sample pump rate 2 ml/min, auto integration time 1–5 s, read delay time 60 s, replicates times 3 and Nebulizer type concentric.

2.3. Quality control and assurance

A standard reference material 1577b, lyophilized bovine liver, was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and used to verify the accuracy of the method. The certified value for Hg was not available in the reference material, then for this element 10 spiked samples at a concentration that gave absorbance values 2–10 times greater than the normal levels in muscle was prepared. Limit of detection (LOD) and limit of quantification (LOQ) were determined based on the standard deviation of the blanks and were calculated as three and ten times the standard deviation of the reagent blanks, respectively.

2.4. Analysis of data

Statistical Package for the Social Sciences for Windows (SPSS Inc., Version 16, Chicago, IL) was used for performing all statistical analyses. Normality and equality of variances of the data were analyzed using Kolmogorov-Smirnov and Levene tests, respectively. As the collected data were not normally distributed, data were transformed into log. T-test and one-way analysis of variance (ANOVA) followed by Duncan's multiple range test were used to seek for any significant effect of the main factors on parameters studied. The level of significance was set at $\alpha < 0.05$.

3. Results and discussion

Accuracy assessment showed a good agreement between the analyzed and the certified values and the measured values were within 10% of the certified values (97.0%, 93.6%, 95.1% and 100.3% for Pb, Cd, Cu and Zn, respectively). Mean recovery for Hg was 93%. Limits of detection were 0.003, 0.002, 0.0008, 0.005 and 0.002 mg/L for Pb, Cd, Hg, Cu and Zn, respectively and the values of below these limits were considered as zero.

Geometric and arithmetic means, standard error, maximum, minimum and MRL values and the percent of sample above MRL of Pb, Cd, Hg, Cu and Zn found in meat, liver, kidney and animal feed are presented in Table 1. The average concentration of Pb and Hg did not differ significantly in various samples. With regard to the Pb, the arithmetic averages found in the liver (0.273 mg/kg) and kidney (0.244 mg/kg) were below the MRL (0.500 mg/kg), however, 15.3% and 13.9% samples of liver and kidney, respectively had Pb levels above the MRL. Averages Pb concentrations in the liver and kidney in the current study are comparable to 0.231 and 0.226 mg/kg respectively found in Brazil (Alkmim Filho et al., 2014) but they are lower than 0.415 mg/kg in liver and 0.534 mg/kg in kidney that reported in other parts of Iran (Sobhanardakani et al., 2012). The mean concentrations of Pb in muscle samples in this work were above the accepted limits and 75% samples exceeded the MRL.

The concentrations of Pb in liver and kidney are relatively high in the present work when compared respectively with 0.016 and 0.026 mg/Kg in Korea or 0.08 and 0.04 mg/Kg in southern Nigeria (Kim et al., 2016; Maxwell Azubuike Iwegbue, 2008). These differences are probably attributable to variations in environmental contamination, feed sources and age of the animals (Waegeneers et al., 2009; Alkmim Filho et al., 2014; Kim et al., 2016). Any contact of animal with sources of Pb, such as paints, fluids, and batteries can be ways for entering of