HEPATIC COPPER, ZINC AND COBALT IN BEEF CATTLE HEALTHY AND AFFECTED BY FASCIOLASIS IN COLIMA, MEXICO

COBRE, ZINC Y COBALTO HEPÁTICO EN BOVINOS SANOS Y AFECTADOS POR FASCIOLASIS EN COLIMA, MEXICO

Barragán A.[1], Macedo R.[1*], Barragán F.J.[2], Rodríguez A.[2], García L.J.[3], Prado O.[1]

[1]Facultad de Medicina Veterinaria y Zootecnia. Universidad de Colima. México. [*]macedo@ucol.mx.

[2]Facultad de Ciencias Químicas. Universidad de Colima. México.

[3]Centro Universitario de Investigación y Desarrollo Agropecuario. Universidad de Colima. México.

Keywords: *Fasciola hepática*; Minerals; Beef production; Food safety.

Palabras clave: *Fasciola hepática*; Minerales; Producción de bovinos de carne; Seguridad alimentaria.

ABSTRACT

A study was conducted with the objective to determine and compare the hepatic copper, zinc and cobalt concentrations in beef cattle healthy and affected by fasciolasis in Colima, México. A total of 42 samples of liver, 21 confiscated by the presence of *Fasciola hepatica* and 21 collected in randomly selected butchers, were analyzed by atomic absorption spectrometry to determine copper, zinc and cobalt concentration. There was no difference ($p>0.05$) in the concentration of the three minerals between healthy and infected livers. The overall average hepatic concentration of copper, zinc and cobalt was 54.03, 11.99 and 0.28 mg/kg WW respectively. However average copper concentration fulfilled the limits established by the Mexican Official Norm for human consumption, the high variability observed caused that a high proportion of the samples exceed the maximum level set by the norm.

RESUMEN

Se realizó un estudio con el objetivo de determinar y comparar la concentración de cobre, zinc y cobalto hepático en ganado bovino sano y afectado por fasciolasis in Colima, México. Un total de 42 muestras de hígado, 21 confiscadas por la presencia de *Fasciola hepatica* y 21 recolectados en carnicerías seleccionadas al azar fueron analizados por medio de espectrometría de absorción atómica para determinar la concentración de cobre, zinc y cobalto. No existió diferencia ($p>0.05$) en la concentración de los tres minerales entre los hígados sanos e infectados, observándose una concentración general promedio de cobre, zinc y cobalto de 54,03, 11,99 y 0,28 mg/kg BH respectivamente. No obstante que la concentración promedio de cobre se ubicó dentro de los límites establecidos por la Norma Oficial Mexicana para su consumo humano, la alta variabilidad observada ocasionó que una gran proporción de las muestras excedieran el nivel máximo establecido por la norma.

INTRODUCTION

Beef is an essential part of the human diet since it constitutes an important source of protein and minerals. However, sometimes it can contain both, high and low concentrations of certain trace elements that which can negatively affect health (NRC, 2005; Pérez *et al*., 2010). Previous studies carried out in Mexico have found potentially toxic concentrations of copper in livers of cattle intended for human consumption (Alcocer *et al*., 2007; Huerta, 2010). Other studies have documented a moderate zinc deficiency in certain population groups such as pregnant women and preschool children in rural and marginal urban areas (Hunt *et al*., 1985; Rosado, 1998; Rosado *et al*., 1995; Valenzuela *et al*., 2008).

On the other hand, fasciolasis is an economically important worldwide parasitic disease caused by trematodes of genus *Fasciola*, which affects the health of a wide variety of domestic and wild animals and

Received: 08.05.2016; Accepted: 11.10.2016; On-line: 15.12.2016

even humans (Quiroz, 2005). It also has great significance for public health and its epidemiological and zoonotic importance in Mexico is clearly documented (Carrada-Bravo, 2006; Carrada-Bravo, 2007).
Taking in to consideration that previous studies have found a relationship between the presence of *Fasciola hepatica* and the concentration of heavy metals in the liver of some species (Symonds *et al*., 1983; Vengust *et al*., 2003; El-Khadrawy *et al*., 2008) and that currently, the consumer of agricultural products demands higher standards of quality and safety, the objective of this study was to determine and compare the hepatic copper, zinc and cobalt in cattle healthy and affected by fasciolasis in Colima, México.

MATERIALS AND METHODS

Twenty-one 250 g samples of bovine liver confiscated by the presence of *Fasciola hepatica* in the Municipal Meat Processing of Colima and 21 similar samples of healthy liver intended for human consumption randomly collected in butchers of the metropolitan area of Colima, México were taken for heavy metals determination.
Copper, zinc and cobalt concentrations were determined at the Multidisciplinary Laboratory of the Faculty of Chemistry of the University of Colima using atomic absorption spectrometry employing a Varian Fast-Sequential, model 220 FS spectrophotometer, following specifications of the Mexican Official Norm NOM-117-SSA1-1994.
Samples were ground, dried in an oven at 90°C for 12 hours until constant weight and stored in a desiccator. Subsequently, 5 g of dry sample were placed in porcelain capsules with 1 ml of peroxide and were calcined for 6 hours at 450°C. At the end of calcination, the crucible walls were washed with 2 ml of 2N hydrochloric acid and the sample was placed on a heating plate at 120°C to remove the excess of acid, holding for the required time and repeating this procedure until the samples were free of carbon. The ashes were dissolved in 5 ml of 2N hydrochloric acid and transferred to a 50 ml propylene tube. The crucible was washed with two 5 ml aliquots of 2N hydrochloric acid and transferred to the same tube and mixed volumetrically to obtain a volume of 50 ml. A reagent blank and a fortified sample were run by each series of digestion, calibration standards were prepared at concentrations of 1, 2, 3 and 4 mg/L for each metal and finally readings were performed on the atomic absorption spectrometer.
Data were analyzed using Student's T test for independent samples and significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

The concentration of copper, zinc and cobalt was statistically similar ($P> 0.05$) between healthy and infected livers. It is important to note the great variability in the content of these minerals in both groups studied (table I).

Table I. Copper, zinc and cobalt concentration (mg/kg wet weight), in healthy and infected beef cattle livers in Colima, México (*Concentración de cobre, zinc y cobalto (mg/kg de peso seco) en hígados de bovinos para carne sanos e infectados en Colima, México*).

Trait	Copper	Zinc	Cobalt
Healthy liver	52.76 ± 31.42^a	11.40 ± 2.70^a	0.24 ± 0.09^a
Infected liver	55.30 ± 28.36^a	12.58 ± 3.09^a	0.32 ± 0.29^a
Average	54.03	11.99	0.28
p-value	0.78	0.20	0.23

According to McGavin & Zachary (2007), fasciolasis causes a colangiocistohepatitis resulting in necrosis of hepatocytes, which in turn causes that copper and zinc are not metabolized which result in their accumulation in liver tissue and blood subsequently, condition not observed in this study. Contrary, Vengust *et al*. (2003) found that in fallow deer (*Dama dama*) the concentration of copper in the liver was lower in animals affected by fasciolasis compared to healthy animals, whereas the concentration of this mineral in the blood was higher in infected animals. Other study carried out in female buffalo, mentioned that serum copper concentration was higher in healthy animals, while zinc concentration was similar (El-Khadrawy *et al*.,

2008). An earlier study indicated that the excretion of copper and zinc in bovine bile is not associated with the presence of the parasite (Symonds et al., 1983).

From the viewpoint of livestock management, taking in to consideration that copper concentration in bovine liver is considered deficient below 10 mg/kg and toxic when exceeding 250 mg/kg (Bagley *et al*.,1997), all livers analyzed had a normal content of this mineral. In contrast, the hepatic concentration of zinc indicates a deficiency of the mineral, since according to McDowell *et al*. (1997) deficiency-toxicity levels are set at 40-150 ppm respectively. Given that cobalt deficit level is below 0.05 ppm, the hepatic concentration of this mineral in the studied livers were normal (McDowell *et al*., 1997). Symptoms of cobalt toxicity are rare and difficult to observe since cattle can tolerate up to 100 times the level of its needs (Alvarez, 2001) and toxic levels are about 3000 times greater than the requirements in most of domestic species (Miller et al., 1991).

The great variability in the concentration of the three minerals can be explained by the differences in the feeding system used for fattening cattle. In the case of copper, hepatic concentration is higher in cattle fed supplements containing poultry litter compared with those fed pastures (Alcocer *et al*., 2007). These authors found an average hepatic copper concentration of 187 ppm. The same results were found in other countries like Spain, where it was observed that hepatic copper and zinc concentrations of cattle slaughtered in Galicia exceed acceptable values for human consumption (Lopez *et al*., 2000).

Although the average copper content in liver samples collected from butchers was below the limit set by Mexican Official Norm NOM-004-ZOO-1994 for human consumption which is 60 ppm, 43% of the samples showed higher values. With regard to international standards, the average copper concentration of both, healthy and infected livers showed values outside the maximum limit, set at 40 mg/kg (AAFCO, 1996), and only 43% of those livers intended for human consumption met that standard. This demonstrates the existence of a real risk of poisoning in humans by bioaccumulation of this element.

Some studies carried out in rural areas of Mexico suggest the existence of a moderate zinc deficiency in certain population groups as pregnant women and preschool children (Hunt *et al*., 1985; Rosado *et al*., 1995). Madrigal *et al*. (1986) mention the low contribution of food of animal origin (rich in highly bioavailable zinc) to the diet of the rural and the urban marginal population, among the causes of this deficiency. Furthermore, these populations consume a diet rich in corn, beans and vegetables, which are high in phytic acid and dietary fiber, which inhibit the absorption of zinc (Sandström & Lönnerdal, 1989). This situation may become worse considering the low content of this element in food of animal origin, as happens with the liver samples analyzed in this study.

CONCLUSIONS

Hepatic concentration of copper, zinc and cobalt was similar in healthy and infected livers. Average concentration of copper fulfilled the limits established by the Mexican Official Norm for human consumption, but the high variability observed caused that a high proportion of the samples exceed the maximum level set by the norm.

BIBLIOGRAPHY

AAFCO. 1996. *Association of American Feed Control Officials*. Atlanta, Georgia.

Alcocer V.V.M., Castellanos R.A.F., Herrera C.F., Chel G.L.A. & Betancur A.D.A. 2007. Detección de metales pesados y dicloro difenil tricloro etano (DDT) en músculos y órganos de bovinos en Yucatán. *Técnica Pecuaria en México* 45, 237-47.

Álvarez C.J.L. 2001. Bioquímica nutricional y metabólica del bovino en el trópico. 1ª ed. Editorial Universidad de Antioquía. Medellín, Colombia. 202 pp.

Bagley C.V., Stenquist N.J. & Worwood D.R. 1997. Copper deficiency in Utah. All Archived Publications. Paper 1210. Utah State University. URL http://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=2209&context=extension_histall

Carrada–Bravo T. 2006. Fasciola hepatica: investigación clínico-epidemiológica. *Revista Gastroenterología Mexicana* 71:169-73.

Carrada–Bravo T. 2007. Fasciola hepatica: ciclo biológico y potencial biótico. *Revista Mexicana de Patología Clínica* 54, 21-7.

El-Khadrawy H.H., Faragalla M., El Moghazy M.M., El Aziz A. & Ahmed, W.M. 2008. Field investigation on the correlation between ovarian activity and fascioliosis in buffalo-cows. *American-Eurasian Journal of Agricultural & Environmental Sciences* 3, 539-46.

Huerta B.M. 2010. Cobre en tejidos animales. *Nacameh* 4(Supl. 1.), 1-13.

Hunt I.F., Murphy N.J., Cleaver A.E., Faraji B., Swendseid M.E., Browdy B.L., Coulson A.H., Clark V.A., Settlage R.H. & Smith, J.C. 1985. Zinc supplementation during pregnancy in low-income teenagers of Mexican descent: Effects on selected blood constituents and on progress and outcome of pregnancy. *American Journal of Clinical Nutrition* 42, 815-28.

López A.M., Benedito J.L., Miranda M., Castillo C., Hernández J. & Shore, R.F. 2000. Toxic and trace elements in liver, kidney and meat from cattle slaughtered in Galicia (NW Spain). *Food Additives & Contaminants* 17, 447-57.

Madrigal H., Chávez A., Moreno-Terrazas O., García T. & Gutiérrez, G. 1986. Consumo de alimentos y estado nutricional de la población del medio rural mexicano. *Revista de Investigación Clínica* 38, 9-19.

McDowell L., Velázquez P.J. & Valle G. 1997. Minerales para rumiantes en pastoreo en regiones tropicales. Diagnóstico de deficiencias de desbalances minerales. 3ª ed. Universidad de Florida. Gainesville, Florida. 151 pp.

McGavin M.D. & Zachary J.F. 2007. Pathologic Basis of Veterinary Disease. 4th ed. Elsevier-Mosby Saunders. St. Louis, Mo. 1488 pp.

Miller E.R., Lei X. & Ullrey D.E. 1991. Micronutrientes in agriculture. In: *Trace Elements in Animal Nutrition*. 2nd ed. Soil Science Society of America, Inc., pp. 593-662.

NOM-004-ZOO-1994. Modificación a la Norma Oficial Mexicana NOM-004-ZOO-1994. Control de residuos tóxicos en carne, grasa, hígado y riñón de bovinos, equinos, porcinos y ovinos, por lo que ahora se denominará grasa, hígado, músculo y riñón en aves, bovinos, caprinos, cérvidos, equinos, ovinos y porcinos. Residuos tóxicos. Límites máximos permisibles y procedimientos de muestreo. URL http://www.senasica.gob.mx/?doc=522

NOM-117-SSA1-1994. Bienes y Servicios. Método de prueba para la determinación de cadmio, arsénico, plomo, estaño, cobre, fierro, zinc y mercurio en alimentos, agua potable y agua purificada por espectrometría de absorción atómica. URL http://www.salud.gob.mx/unidades/cdi/nom/117ssa14.html

NRC. 2005. *Mineral Tolerance of Animals*. 2nd ed. Committee on Minerals and Toxic Substances in Diets and Water for Animals. National Academy Press. Washington, D.C. 495 pp.

Pérez C.A., Pérez G.M.L. & Fernández C.A. 2010. Presencia de arsénico en tejidos de origen bovino en el sudeste de la provincia de Córdoba, Argentina. *Investigación Veterinaria* 12, 59-67.

Quiroz R.H. 2005. Parasitología y enfermedades parasitarias de animales domésticos. Editorial Limusa. México, D.F. 827 p.

Rosado J.L. 1998. Deficiencia de zinc y sus implicaciones funcionales. Salud Pública de México, 40(2), 181-88.

Rosado J.L., Bourges H. & Saint-Martín B. 1995. Deficiencia de vitaminas y minerales en México. Una revisión crítica del estado de la información: I. Deficiencia de minerales. *Salud Pública de México* 37, 130-39.

Sandström B. & Lönnerdal, B. 1989. Promoters and antagonists of zinc absorption. In: *Zinc in Human Biology*. (ed. by Mills C.F). Springer Verlang, pp. 57-78.

Symonds H.W., Mather D.L., Mallinson C.B. & Hughes D.L. 1983. Bile flow, bile salt secretion and the excretion of iron, copper, zinc and manganese in the bile of calves infected with *Fasciola hepatica*. Research in Veterinary Science 35, 69-74.

Valenzuela V.C., Letelier C.M.A., Olivares G.M., Arredondo O.M. & Pizarro A.F. 2008. Determinación de hierro, zinc y cobre en carne de bovino. *Revista Chilena de Nutrición* 35, 139-46.

Vengust G., Klinkon M., Bidovec A. & Vengust A. 2003. *Fasciola hepatica*: effects on blood constituents and liver minerals in fallow deer (*Dama dama*). *Veterinary Parasitolgy* 112, 51-61.