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Determination of minerals in liver and blood of sheep fed with high poultry manure doses for prolonged periods

Determinación de minerales en hígado y en sangre de ovejas alimentadas con altas dosis de pollinaza por periodos prolongados

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ABSTRACT

The objective was to measure the levels of minerals in the liver and blood of sheep, fed with high levels of poultry manure. Two experimental groups were considered: a treated group (ovine bellies that consumed poultry manure for at least three years) and a control group (lambs in fattening that did not consume poultry manure). Six animals (n=6) from each group were randomly sacrificed, blood samples were collected prior to slaughter, and hepatic tissue samples were subsequently taken. The minerals analyzed were copper (Cu), iron (Fe) and zinc (Zn). In the treated group, concentrations of Cu, Fe and Zn were 176.23, 9.58, and 72.63 mg/L, respectively. While in the treated group they were 85.35, 13.41 and 112.0 mg/L, respectively. The Cu determinations of the treated group were higher in both liver and blood ($p < 0.05$). However, in spite of the time of exposure to poultry manure consumption, the animals showed no signs of copper intoxication, which could be conditioned by the low concentration of the mineral in the poultry excreta.

Keywords: ovines, health, copper, iron, zinc, poultry manure.

RESUMEN

El objetivo fue medir los niveles de minerales en hígado y en sangre de ovinos alimentados con altos niveles de pollinaza. Se consideraron dos grupos experimentales, un grupo tratado (vientres ovinos que consumieron pollinaza por al menos tres años) y un grupo testigo (corderos en engorda que no consumieron pollinaza). Se sacrificaron al azar seis animales de cada grupo (n=6), previo al sacrificio se colectaron las muestras sanguíneas, y posteriormente se tomaron muestras de tejido hepático. Los minerales analizados fueron cobre (Cu), hierro (Fe) y zinc (Zn). En el grupo tratado, las concentraciones en hígado de Cu, Fe y Zn fueron de 176.23, 9.58, y 72.63 mg/L, respectivamente; mientras que en el grupo testigo fueron de 85.35, 13.41 y 112.0 mg/L, respectivamente. Las determinaciones de Cu del grupo tratado

fueron superiores tanto en hígado como sangre ($p<0.05$). Sin embargo, a pesar del tiempo de exposición al consumo de pollinaza, los animales no presentaron signos de intoxicación por cobre, lo que pudo estar condicionado por la baja concentración del mineral en las excretas avícolas.
Palabras clave: ovinos, minerales salud, cobre, hierro, zinc, pollinaza.

INTRODUCTION

In Mexico, sheep production is limited (Martínez *et al*., 2011), among other things, due to food issues, since the costs are very high, and sheep production in Mexico is still from backyard, as a secondary activity; which leads to the use of industrial waste such as poultry manure in animal feeding. Mexico is consolidated as the fifth largest producer of chicken and egg in the world (SAGARPA, 2016), so the production of poultry manure is very high, which makes it a potential residue for sheep feeding.

The use of poultry manure as a dietary supplement is due to its ease of acquisition and its moderate cost. Among the advantages is that it represents an important contribution of protein and minerals for animals, especially available phosphorus. Among the disadvantages is its high copper content, which may lead to intoxication (Castellanos, 2007). This happens, despite the recommendations of not using the poultry manure for prolonged periods or high levels (Ríos *et al*., 2005), when the producer abuses the poultry manure use, including high levels in the diet, thinking of feeding better and cheaper to their cattle, which can be counterproductive (Castellanos, 2007).

Copper (Cu) is an essential trace element for most biological processes in plants and animals. It is useful for normal iron metabolism, elastin and collagen synthesis, melanin production, and central nervous system integrity (Kimberling, 1998). It is also a cofactor of many cuproenzymes, but it is extremely toxic in excess (Horn and Tumer, 1999; Mercer, 2001).

All living organisms have developed highly specialized homeostatic mechanisms to recruit, remove and remove copper, and neutralize its toxic effect (Dameron and Harrison, 1998; Harris, 2000; Mercer, 2001). Several species of animals show variation in their tolerance to increases in copper levels in the diet (Howell and Gooneratne, 1987).

Undoubtedly, sheeps are the most susceptible to chronic copper toxicity; however, in terms of resistance to copper toxicity, there are differences among breeds, as some authors comment that copper toxicity occurs more frequently in the Dorper breed than in Merino one (Bath, 1979; Harrison *et al*., 1987).

On the other hand, there is the question of food safety, some authors (Ríos *et al*., 2005) comment on the importance of animal health, especially when evaluating the chicken bed and chicken dung as a supplement in animal feed, because the products that are generated from these animals will be consumed by the man. Therefore, the objective of the present study was to determine the concentrations of hepatic and blood copper and to evaluate the health of the animals in a farm that supplemented their sheep with high content of poultry manure in the diet.

MATERIAL AND METHODS

The work was carried out in the farm "El Refugio" which is located in Lo de García, Tepic, Nayarit. The region has a warm subhumid climate with rainfall in summer with an average annual temperature of 21.3 ºC, with an average rainfall of 1152.3 mm, with an altitude of 915 meters above sea level (m.a.s.l) (INEGI, 2006) with 200 ovine bellies (Treated group, supplemented with poultry manure), and 30 lambs in fattening (control group, fed without poultry manure).

The poultry manure was acquired in a poultry company of the region; to this poultry manure was realized analysis of the minerals of Cu, Fe and Zn. The excreta management was carried out following the indications of Official Mexican Standard (NOM-044-ZOO-1995).

Experimental groups

Treated group: the breed of these females was Pelibuey/Katahdin; the ages ranged from 10 months to 5 years. The body condition was between 2 and 3.5 following the classification of De Lucas (2007). The management of the bellies (during the last three years) was in a semi-frozen feeding system, with grazing and ad libitum access to the supplement. This supplement contained 60% poultry manure, 10% maize grain, 28% molasses and 2% commercial mixture of minerals for sheep. Each kilogram of this mineral mixture contained: Calcium (Ca) 130g, Phosphorus (P) 50g, Sodium (Na) 109g, Chlorine (Cl) 200g, Iron (Fe) 4.3g, Magnesium (Mg) 10g, Manganese (Mn) 3.3g, trace minerals and wheat bran residue, cane molasses and vegetable flavoring).

Control group: the average age of these animals was six months, Pelibuey / Katahdin breed. In these animals, the operating system was fully stabbed. In this way, the diet was continuous and consumed a commercial diet based on maize grain, corn stubble, grain sorghum, soybean, molasses, canola and 2% of the commercial mixture of minerals for sheep already described.

Sacrifice of animals

Six animals (randomly chosen) from each group were sacrificed. Although the treated group tried to ensure that the animals were more than four years old, in order to ensure that they had consumed poultry manure for three years.

In order to carry out this work and the care of the animals, the guidelines of the Official Mexican Standard (NOM-062-ZOO-1995) were taken into account: Technical specifications for the care and use of laboratory animals, livestock farms, and farms, centers of production, reproduction and breeding. The animals were sacrificed following the recommendations of Official Mexican Standard (NOM-033-ZOO-1995): Humanitarian slaughter of domestic and wild animals.

Blood sampling was performed before the animals were slaughtered by jugular venipuncture with a 21G Vacutainer needle, the blood obtained was placed in 3 cc heparinized tubes. Once the animals were dead, liver tissue samples were taken from the left lobe. Concentrations of Cu, Fe and Zn were determined; in blood, liver and poultry, by means of atomic absorption spectrophotometry with the aid of Varian Mark Spectra AA equipment. The blood samples were analyzed with a 1:1 (vol:vol) dilution with distilled water and immediately read in the atomic absorption equipment with the respective lamp for each element. In the case of liver and poultry manure samples, a gradual digestion process was required in a digestion plate, starting at 50 ºC until reaching 250 ºC, for the total destruction of the tissue. After the digestion process was completed, a clear sample was obtained, and the sample was then diluted into 25 ml volumetric flasks with distilled water and the elements were read in the atomic absorption equipment. The acids used were nitric acid with perchloric acid, reactive grade, in a ratio of 2: 1 (vol: vol) (Alcántar and Sandoval, 1999). MediCalc® recommendations (MediCalc®, 2017) were used for the conversion to international units (from µg/dl to µmol/L).

Statistical analysis

A descriptive analysis was performed that included the mean value, the standard deviation, minimum and maximum. The hypothesis test was performed using a t-Student test for independent samples. These analyzes were performed with the statistical software SPSS Version 20.0 (IBM, 2011).

RESULTS AND DISCUSSION

Values in poultry manure

From the samples of poultry manure analyzed the iron was the one that was found in greater concentration, followed by the zinc and finally the copper (Table 1), being there statistical difference among the three. The values of the analyzed minerals follow the same trend of presentation as that described by Pacheco *et al*. (2003), that is concentrations are highest followed by zinc and finally copper.

Table 1. Mineral levels in poultry manure (ppm, fresh basis).

	Mean	Typical deviation	Minimum	Maximum
Cu mg L^{-1}	2.06[a]	2.75	0	6.6
Fe mg L^{-1}	182.68[b]	166.46	35.2	638.2
Zn mg L^{-1}	41.38[c]	9.142	27.7	56.6

[a,b,c] Different literals indicate significant differences between minerals ($p<0.05$).

The values of copper in poultry manure (3.096 ppm) are lower than those published by Pacheco *et al*. (2003), who reported an overall average of 82 ppm. Deshck *et al*. (1998) found values of copper in poultry manure in a range of 29.0-122.7 with an average of 51.4, values that are higher than those found by us.

It is known that diets in sheep must contain copper; however, controversy exists as to ideal concentrations. Taking into account feeding tables, they mention that sheep tolerate up to 25 ppm (NRC, 1985), Hartmans (1975) comments that diet for sheep with more than 15 ppm (15 mg/kg) of copper can cause Copper, and whereas Plumlee (2004) comments that the toxic dose begins with 30 ppm and as normal ranges mentions of 10-20 ppm. The level of copper administered in the present investigation was 1.85 ppm (1.85 mg/kg), which is well below those considered advisable.

Although copper concentration was low, both the high inclusion rate of poultry manure in the diet (60%) and the long exposure time (three years) should be taken into account, which could lead to accumulation of copper in liver.

As for the inclusion percentage of poultry manure, there are studies that have included levels from 50% (Pérez, 2004), 60% (Vivas, 2002) and up to 85% (Mavimbela *et al*., 2000) with a period of exposure of maximum 4 months.

As for exposure time to Cu, necessary to produce intoxication in sheep, the opinions are diverse (Cantón *et al*., 1994), as these investigators did not register mortality within 91 days of feeding with 87 ppm of Cu, while other working groups describe mortality from 67 days, and even from 30 days, exposure to copper, with lower levels of this mineral in the diet (67 ppm and 60 ppm, respectively) (Bostwick, 1982; Zervas *et al*., 1990).

Values in liver

The results of the concentrations of copper, iron and zinc, determined in liver, are described in Table 2. In this table can observe that copper and iron were higher in the treated group, although only the Copper reached significant statistical difference (p <0.05). On the other hand, zinc was higher in the control group, although without statistical significance.

The range of hepatic copper values published by Underwood and Suttle (1999) is 33.3-100 μmol/l, so the values of hepatic copper found by us in the treated and control groups can be considered as high (2819.2 and 1365.6 μmol/l respectively).

Table 2. Mean concentrations (standard deviation) of minerals in sheep liver (ppm, fresh basis).

		N	Mean	Typical deviation	Minimum	Maximum
Cu mg L^{-1}	60% (Treated)	6	176.2[a]	59.2	104.9	243.7
	0% (Control)	6	85.4[b]	13.7	65.0	99.8
Fe mg L^{-1}	60% (Treated)	6	72.6[a]	30.9	26.2	111.0
	0% (Control)	6	112.0[a]	70.7	48.8	239.2
Zn mg L^{-1}	60% (Treated)	6	9.6[a]	3.5	3.1	13.1
	0% (Control)	6	13.4[a]	2.9	8.5	16.1

[a,b] Different literals indicate significant differences among treatments (p<0.05).

Plumlee (2004) reports that in most ruminants the accumulation of more than 250 ppm of copper in the liver is considered as toxic, this value is higher than that found in the liver of the treated group (176.2 ppm) and almost triple of the control group value (85.3 ppm).

The lambs sampled by Sivertsen and Løverg (2014) in their first year of study (November, March and June) averaged a value of 110 mg/kg on a wet basis, whereas in the second year they averaged a total of 114 mg/kg. These values are higher than those described in the present investigation in 5-month-old lambs (85.35 mg/kg), this difference may be due to the consumption of pastures containing high levels of copper.

Oruc *et al*. (2009) reported values of 302 mg/kg on wet basis, these samples came from three sheep that had died and showed signs of copper intoxication, these values being almost twice that of lambs that consumed poultry manure 176.2 mg/kg).

Another factor that could contribute is the resistance of the breed, as Harrison *et al*. (1987) states that, in terms of copper metabolism, there are genetic differences among sheep breeds. In this sense Lewis *et al*. (1997) mentions that the Suffolk breed is particularly at risk, whereas Bath (1979) found that copper toxicity occurs more frequently in the Dorper breed than in the Merino one. The copper determinations of the treated group were superior both in blood and in liver, however, no animal showed intoxication symptoms.

Values in blood

The levels of copper, iron and zinc analyzed in blood serum were higher in the treated group than in the control group, although only copper showed significant statistical differences (p <0.05) (Table 3).

Table 3. Mean value (standard deviation) of sheep blood minerals (ppm, fresh basis).

		N	Mean	Typical deviation	Minimum	Maximum
Cu mg L^{-1}	60% (Treated)	6	0.35[a]	0.17	0.23	0.62
	0% (Control)	6	0.12[b]	0.03	0.06	0.14
Fe mg L^{-1}	60% (Treated)	6	36.25[a]	30.91	8.90	95.20
	0% (Control)	6	17.40[a]	9.88	6.50	31.40
Zn mg L^{-1}	60% (Treated)	6	0.98[a]	0.84	0.02	2.06
	0% (Control)	6	0.93[a]	0.36	0.40	1.37

[a, b]Different literals indicate significant differences between treatments (p<0.05).

The results of sheep that consumed pollinaza were slightly lower than those published by Mohammed *et al*. (2014) independently of the physiological state and of the season. This may be due to race (Bath, 1979; Lewis *et al.*, 1997), although further studies would be needed to support the claim that the Pelibuey breed is more resistant to copper intoxication.

CONCLUSION

The iron and zinc levels analyzed in blood serum and liver of the treated group with respect to the control group showed no statistical difference. The copper determinations of the treated group were superior both in blood and in liver, however, no animal showed intoxication symptoms.

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