Full length research article

ASSESSMENT OF LEVELS OF COPPER, CADMIUM AND LEAD IN SECRETION OF MAMMARY GLAND OF COWS GRAZED ON OPEN FIELDS.

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ABSTRACT

The levels of copper, cadmium and lead were determined in milk samples from cows grazed on open fields. The use of H_2O_2 cleared the residual colours of the metal solutions following digestion with HNO_3 acid. The results of the Atomic Absorption Spectrophotometric analysis of the metal solutions from the milk samples showed the metal concentrations in the order Pb (0.531±0.299 mg/dm^3)>Cd (0.257±0.127 mg/dm^3)>Cu **(**0.062±0.026 mg/dm^3). The levels of Pb and Cd in the milk samples studied exceeded the permissible maximum daily intake recommended by W.H.O. in the health criteria.

Keywords: Copper, Cadmium, Lead, Cow, mammary gland, secretion

INTRODUCTION

Mammary glands in Cows are the most physiologically active part and therefore, the output of heavy metals in their organs may be clearly reflected through milk. Heavy metals such as copper (Cu), cadmium (Cd) and lead (Pb) are considered important among contaminants, because of their toxicity, long survival time, accumulation in nature and circular course in biosphere. These metals may be introduced into animals like cows, through the food chain (Ayodele *et al.* 1999; Ayodele & Madu 2004) and subsequently into man through the mammary secretion from cows (Vidovic *et al.* 2003; Ayodele & Hassan 2001a). The presence of cadmium and lead in biological fluids such as milk and blood have not been found to possess any evidence that suggest that they may be involved in normal metabolic processes in man. Lead inhibits ferrochelatase, the enzyme responsible for the incorporation of iron in the porphyrin ring resulting in an accumulation of protoporphrin IX in the erythrocyte (Ayodele & Bayero 2002). The inhibition of α-amino levulinic acid dehydratase acivity, decrease in red cell survival, decrease in the rate of globin synthesis and an inhibition of the haeme synthesis are also attributable to lead. Cadmium has been implicated in osteoporosis with impaired general health, lung and kidney damage (Friberg 1948; Vidovic *et al.* 2003). Long term chronic exposure to cadmium has been associated with anemia, anosmia, osteomalacia and cardiovascular diseases especially hypertension (Ayodele *et al.* 1999).

The most typical feature of chronic cadmium exposure is renal glomerular and tubular damage (Goyer 1989). Copper, unlike cadmium and lead is a common component of some biological fluids and essential for certain biological activity, but it becomes toxic when present in high concentrations as a result of bioaccumulation (Abdulrahman & Tsafe 2004). Copper is absorbed as an amino acid complex and combines with albumin when it enters the blood stream. Albumin carries copper to the liver where it is incorporated into the ceruloplasmin. ceruloplasmin is a protein containing copper and carbohydrates. It functions as a copper carrier and has the ability to oxidize iron and hence known as ferroxidase with an oxidase ability towards a number of amines and other substances. Copper is also a component of cytochrome oxidase enzymes and supper oxide dismutase, which is especially active in red blood cells. Copper is stored in the liver and other tissues with protein called metallothionine. Excess copper in the body leads to Wilson's disease which is characterized by deficiency of ceruloplasmin. When ceruloplasmin is reduced, more copper accumulates in the brain and liver with resultant neurological changes and liver damage. Clinical interests in trace metal analyses for the diagnosis and prognosis of diseases and nutritional status have been stimulated by increasing awareness of the prevalence of poisonings from occupational, environmental and nutritional exposures to toxic metals (Ayodele & Hassan 2001b). It is also recognized that measurements of milk trace metal levels could provide a prognostic index. This paper reports the levels of Cu, Cd and Pb in cow milk aimed at indexing heavy metal accumulation from milk consumption.

MATERIALS AND METHODS

A total of 24 cow milk samples were collected from the Fulani settlement in Kudenda village around Nassarawa in Kaduna State, Nigeria. The number of samples collected was determined by the chain-fodder- milk values reported by Vidovic *et al.* (2003). From this work, percentage transfer of metals was indexed by lead (Pb) and Zinc (Zn). Transfer of Pb from fodder to milk was 4 ± 0.07% while transfer of Zn was 3 ± 0.01%. Average metal transfer from fodder to milk was found to be 3.5 ± 0.04%.

Mean transfer = 3.50%
Standard deviation (s) = ± 0.04%

$$n \geq \frac{(ts)^2}{d}$$

n = Number of samples to be taken.
t = Degree or level of confidence at specific replication.
s = Standard deviation
d = Distance from population mean

Muhammed *et al.* (1996) had earlier reported the average amount of metals in plants from the sampling location to be 275mg/kg, with Standard deviation 175.90

Therefore the estimated transfer of metals from plant to milk (S_o)

$$= \frac{3.50 \times 275}{100} = 9.63$$

Corrected standard deviation on average amount of metal in plants from the sampling location was obtained from

$$\frac{0.04 \times 175.90}{100} = 0.07$$

$S_o \times S = 0.07$
$d \pm \leq 0.08$

At 95% confidence limit and 4 replications, t = 2.776 ≈ 2.78

$$\geq n = \frac{(2.78 \times 0.07)^2}{0.08} = 5.9$$

$=> n \geq 6$

Therefore minimum number of samples required for the study was 6, and thus 6 healthy cows were selected for the study and marked C1, C2, C3, C4, C5, and C6. Separate milk samples were collected from the cows in the morning hours, into sterile plastic bottles on every sampling day. Four replicate samples were taken at 4 days interval to obtain the total milk samples studied. The samples were immediately taken to the laboratory and stored in the refrigerator prior to preparation and analysis.

To prepare the sample for analysis, 10cm^3 of each milk sample was measured into porcelain crucible and heated in a furnace at 500^{o}C for 4 hrs. The white ash was allowed to cool and 20cm^3 of conc. HNO_3 was added in a 250cm^3 conical flask. The mixture was heated at 160^{o}C to evaporate most of the liquid, cooled and 10cm^3 of H_2O_2 was added. This mixture was heated at the same temperature to dryness. The residue was dissolved in 20cm^3 of water and the solution filtered into 50cm^3 volumetric flask and made up to mark with deionised water. The filtrate was stored in plastic sample bottle for Atomic Absorption Spectrophotometric analysis using Buck Scientific 210 VGP. Triplicate digestions of samples were carried out.

RESULTS

The daily mean concentration of Cu is given in Table 1 which ranged from 0.041-0.099 mg/dm^3. It varies on the separate days of sampling, from not detected in some samples to 0.25mg/dm^3. Mean total concentration of Cu was found to be 0.062mg/dm^3 with a standard deviation of 0.026. However the mean concentration of Cu over the sampling days for each cow was 0.046-0.166 mg/dm^3 with standard deviations of 0.0369 and 0.119 respectively.

To determine the relationships between the six cows studied, bivariate statistics was used to determine any correlation between the milk samples from the cows. With respect to Cu concentrations, milk samples from the cow labeled C1 showed strong Correlations with cows C2 and C3 with Pearson coefficients of 1.000 in each case at the 0.01 level. Animal C3 also showed very strong correlations with C1, C2, and C6 with correlation coefficients ranging from 0.606 – 1.000. Both C2 and C3 showed negative correlations with C4 (-1.000) and C2 also showed negative relationships with C6.

The levels of Pb in cows sampled on different days ranged from a daily mean of 0.263-0.846mg/dm^3 (Table 2) but the mean total Pb concentration was 0.531 ± 0.299mg/dm3 for all the cows studied. Mean Cd concentrations varied from 0.123-0.428mg/dm^3 per day, but the mean total Cd for the cows studied was 0.257± 0.127mg/dm^3 (Table 3).

DISCUSSION

The analysis of the results of Cu concentration showed high standard deviations which may be attributed to variation in the daily sources of food that could accumulate Cu in the cows (Vidovic *et ai.* 2003). Another reason may be the individual inherent capacity of the cows to excrete Cu probably through their urine or dung. The correlations observed between the cows with respect to Cu concentration may be an indication of group grazing of the cows. The similarity in the concentrations of Cd and Pb in the milk samples may also be indicative of the suspected group grazing behaviour of the cows on different fields within the study area.

The results showed that the milk samples contain more Pb than other metals. The lead content exceeds the permissible maximum daily intake of 0.05mg/kg body weight (WHO 1984). The likely source of Pb that must have made its way through the food chain, include traffic emissions (Lawal & Mohammed 2002; Lawal & Audu 2004). From the results of the analysis, only cow C1 showed a significant correlation with C6 at the 0.05 levels with regards to Pb concentration. The nature of the correlations between the cows in terms of Pb concentration in the milk samples may be pointing at genetic differences between the cows and dependence of metal levels on the phase of lactation, in addition to confirming possible uniform distribution of Pb on the grazing fields (Ayodele & Hassan 2001b).

The concentration of Cd in the milk samples was also found to be higher than the recommended dietary intake of 0.02-0.06mg/day (WHO 1984). The correlation patterns were similar to those obtained for Cu. Some of the animals may be showing ability to excrete Cd out of their system as demonstrated by the irregularity in the detection of the metal in the samples. This was supported by the lack of correlation in the levels of Cd in some of the animals.

The mean total concentrations of metals in the milk samples are in the order Pb > Cd > Cu. Since the concentrations of Pb and Cd exceeded the permissible maximum daily intake, consumption of milk for a protracted period from such cows could lead to the accumulation of the elements in the tissues or organs of the consumer. Therefore consumers of natural milk from cattle raised in the study area should be screened periodically for heavy metals which may be accomplished by analyzing the urine, hair and fingernails.

TABLE 1. THE LEVELS OF COPPER (Cu) IN COWS SAMPLED ON DIFFERENT DAYS

Days	Cows						Mean day^{-1} mg/dm3
	C1	C2	C3	C4	C5	C6	
10-8-06	ND	0.160	0.018	0.160	ND	0.027	0.061
14-8-06	ND	ND	ND	ND	0.198	0.045	0.041
18-8-06	0.082	0.010	0.030	ND	0.081	0.082	0.048
22-8-06	0.250	0.220	0.091	0.03	ND	ND	0.099
Mean total Cu							**0.062±0.026**

TABLE 2. THE LEVELS OF LEAD (Pb) IN COWS SAMPLED ON DIFFERENT DAYS

Days	Cows						Mean day^{-1} mg/dm3
	C1	C2	C3	C4	C5	C6	
10-8-06	0.080	0.482	0.289	0.45	0.316	0.105	0.287
14-8-06	0.158	0.158	0.145	0.812	0.025	0.278	0.263
18-8-06	0.665	0.702	1.033	0.779	1.012	0.882	0.846
22-8-06	0.728	0.496	0.539	0.818	0.995	0.787	0.727
Mean total Pb							**0.531±0.299**

TABLE 3. THE LEVELS OF CADMIUM (Cd) IN COWS SAMPLED ON DIFFERENT DAYS

Days	Cows						Mean day^{-1} mg/dm3
	C1	C2	C3	C4	C5	C6	
10-8-06	0.16	ND	ND	ND	ND	0.58	0.123
14-8-06	0.09	0.45	ND	0.57	0.41	0.02	0.257
18-8-06	0.50	0.10	0.47	0.14	0.51	0.85	0.428
22-8-06	ND	ND	ND	0.76	0.44	0.12	0.22
Mean total Cd							**0.257±0.127**

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