



Determination of concentration of some heavy metals in the blood of Holstein-Friesian cattle on a farm in Nasarawa State, Nigeria

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The study was carried out to determine the presence and concentration of some heavy metals in the blood of Holstein-Friesian cattle in a private farm in Nasarawa State Nigeria. Blood samples were obtained from 22 Holstein-Friesian cattle consisting of 21 cows and 1 bull, of ages between 3.5 and >3.5 years. The samples were analyzed using Atomic Absorption Spectrophotometer (AAS). The mean lead (Pb), Iron (Fe) and Cadmium (Cd) concentrations were 1.160, 47.681 and 0.051 mg/kg, respectively. There was no significant ($P>0.05$) effect of age and sex on the concentration of Pb, Fe and Cd. The study found the presence of Pb, Fe and Cd in blood samples collected from Friesian cattle. The FAO/WHO permissible limit of Cd and Pb in blood is 0.5mg/kg. Pb was detected above the maximum limit. The high concentration of these metals recorded could be as a result of prolong use of inorganic agricultural products and likely irrigation water which contaminate the feed. High levels of these metals in Holstein-Friesian can cause decreased milk production, placenta damage, mineral deficiency and respiratory failure among others. Inorganic agriculture should be replaced with organic methods; industrial activities close to pasture yards should be avoided. Sources of irrigation water should be free from heavy metals contamination.

Keywords: Blood, Cadmium, Holstein-Friesian, Iron, Lead, Nigeria

Introduction

Food safety concerns has necessitate the need for an in-depth research and reports of prevalence of toxic heavy metals in food animals due to the utilization of these animals and their products for human consumption. It has been shown by Casas & Sordo (2006) that Pb, Cd and Ar are considered to be one of the major environmental pollutants, it continued to pose health hazards to animals and humans in Nigeria and other part of the world and has been incriminated as a cause of accidental poisoning in domestic animals more than any other substance.

Human activities such as mining, use of fertilizer, pesticides and herbicides for agricultural activities,

indiscriminate dumping of waste on land and water bodies, contribute immensely to the occurrence of toxic metals in the ecosystem (Jayasekara *et al.*, 1992). The inability to degrade these metals makes them to stay in our environment permanently (Baykov *et al.*, 1996). Iron (Fe) is an essential trace element required by all forms of life, it is required in life of man and animals for the synthesis of haem proteins and in many enzyme systems (FAO, 2011). Meat and grain constitute the significant part of dietary source of Fe. Coup & Campbell (1964) have shown that irrigation water from borehole is a source of Fe and will be precipitated and deposited on pasture upon exposure to air. Cattle can get

exposed to Pb through ingestion and inhalation. Other anthropogenic sources of heavy metal contamination include agricultural activities, such as pesticide and herbicide application, contaminated irrigation water, municipal waste used for fertilization of crops (Alloway & Jackson, 1991), mineral fertilizer containing traces of heavy metals and direct waste disposal on farmland (Merian *et al.*, 2004). Minnesota department of health (MDH, 1999) have shown that Cd, Pb and Ar are found in phosphorus fertilizers and these metals poses the most important health concern. Ketut *et al.* (2017) conducted their study in Bali where they detected high levels of Pb in the plasma of cattle that had ingested contaminated grass and polluted water raised from unsanitized and polluted farm. Fe may be present in drinking water and irrigation water. The FAO (2011) have reported that Fe is used as coagulants and in the corrosion of steel and cast-iron pipes.

Agenin (2002) in his study conducted in the Savannah region of Nigeria have reported an increase levels of some heavy metals in the soil as a result of farmyard activities and chemical fertilizer application. Cattle reared under intensive management system can get expose to sources of heavy metals via ingestion of contaminated pastures and crops produced by application of inorganic agricultural products as shown in the study of Tomza-Marciniak *et al.* (2010) conducted in Poland. Cattle can have elevated levels of Pb in their blood without showing any outward signs of clinical disease. However, prolong consumption of meat and milk containing heavy metals will have deleterious effects to man. High levels of Pb in humans can be toxic, it has negative effect in the synthesis of haemoglobin, kidneys, gastrointestinal tract, joints and reproductive system and acute or chronic damage to nervous system (Pandey & Madhuri, 2014).

Somasundaram *et al.* (2005), in their study conducted in India established that giving animals feeds from areas with elevated content of trace elements results in their bioaccumulation in edible tissues, eggs and milk. Michael *et al.* (2018) in there study conducted in Southern Nigeria have shown that contaminated food is one of the main sources of exposure to heavy metals in man and an increased dietary heavy metal intake may contribute to the development of various disorders. Pb has been found to decreased sperm count in male and spontaneous abortions in female (Hertz-Picciotto, 2000), Hwua & Yang (1998) showed that Cd can induce the expression of several gene types and can result in the development of cancer in man. The effects of high levels of Fe in animal include depression, coma, convulsion, respiratory failure

and cardiac arrest. In human, excess Fe intake may result in siderosis (deposition of Fe in tissue) in liver, pancreas, adrenals, thyroid, pituitary and heart (FAO, 2011). Heavy metals have negative effect to cattle production, for example, Cd has been reported by Smith *et al.* (1991) to cause reductions in both intestinal zinc absorption and hepatic zinc reserves in cattle as a result of competition for the cation-binding sites. Ahsan *et al.* (2017) also reported that Cd can be absorbed through the intestinal tract, passes through the placenta of pregnant animal and damage placenta membrane and DNA. Coup & Campbell (1964) conducted an experiment in New Zealand to see the effect of excess Fe intake in intensively managed dairy cows, they show that high Fe intake caused scouring, loss of bodyweight and lowered production of milk and butterfat. It has been reported by Pandey & Madhuri (2014) that excess Cd levels in blood can cause osteomalacia and osteoporosis.

Patra *et al.* (2008) reported significant correlation between blood Pb and milk Pb concentrations in lactating cows. This shows that heavy metals in the blood can be transferred to the milk of lactating cows which will subsequently be consumed by man. The relative importance of heavy metals associated with toxicity in cattle and human and the continuous application of inorganic materials, fertilizers, herbicides on crops and pastures, the use of boiler discharge as sources of irrigation and the intense industrial activities in the study area have necessitated the need for this study. The aim of the study is to assess the levels of heavy metals in dairy cattle as well as the effect of age and sex on the concentration of these metals in Holstein-Friesian cattle.

Materials and Methods

Study area and site

The study was conducted in an integrated dairy farm in Keffi Local Government Area of Nasarawa State. The town falls within the guinea savannah agro-ecological zone and is located 8.85 latitudes and 7.87 longitudes. The town has a population of 92,664 at the 2006 population census (NPC, 2006) making it the second biggest town in the state. Keffi town is about 50km from Nigeria's Federal Capital, Abuja. The dairy farm is situated in Gauta Village, which is 4km from the city town. The farm has both local and exotic dairy cattle which are used to produce dairy products for human consumption. The farm has vast hectares of land used to grow and produced crops, hay, silage and pasture all year round using irrigation system, inorganic fertilizers (N.P.K) and chemical herbicides and pesticides. The source of irrigation water is borehole, dam and flowing stream water in which the local community

also utilizes for washing, bathing and other domestic activities. Discharged water from boilers also serves as source of irrigation water. There was on-going massive construction of a flour mill factory side by side the dairy farm. Tomza-Marciniak *et al.* (2011) have reported high levels of heavy metals in the blood of cows raised from inorganic farm compared with cows from organic farm. Thus, the industrial and inorganic agricultural activities taking place on the farm can expose these cattle to high levels of heavy metals. Additionally, the Scientific Committee on Animal Nutrition (SCAN, 2003) have shown that contamination of animal feeds by toxic metals cannot be eliminated completely because of the levels of their distribution in the environment

Sample population

The sampling population consists of 121 Holstein-Friesian cattle managed intensively. The animals were kept for dairy purpose and milking is usually done twice a day using an automated milking machine. They were fed on fresh pasture, hay, silage and concentrate.

Sample size

A total of 22 Holstein-Friesian cattle consisting of 1 bull and 21 cows were randomly selected from the population.

Blood collection and digestion

The Venipuncture method of blood collection was used as described by Charles *et al.* (2017). In brief, about 5ml blood samples were collected from jugular vein of Holstein Friesian cattle and introduced into a plastic Ethylenediaminetetraacetic acid (EDTA) bottles in order to prevent coagulation of the blood samples. Conventional Wet Acid Method of Digestion as described by Miranda *et al.* (2005) was used. 5ml of freshly prepared mixture of concentrated HNO₃ (nitric acid) and H₂O₂ (hydrogen peroxide) in a 4:2 v/v ratio was poured into a Pyrex flask containing 2ml of the blood samples and left for 15 minutes. Watch glass was used to cover the flask prior to digestion. A total of 3 ml of HNO₃ and few drops of H₂O₂ were used to treat the digest followed by subsequent heating at 80°C on a hot plate to obtain

a clear digested solution. The excess acid mixture was evaporated to a semi-dry mass and thereafter cooled and diluted with 0.2ml nitric acid. A 100 ml volumetric flask was used to transfer the mixture and diluted with distilled water to obtain a 60 ml volume. The samples were stored in a refrigerator at 4°C awaiting Atomic Absorption Spectrophotometer (AAS) analysis.

Analysis of samples

Buck scientific VGP 210 model Atomic Absorption Spectrophotometer was used to evaluate the concentrations of heavy metals in the digested samples. A calibration of the AAS was done with standard solutions after which they were aspirated into the AAS equipment for analysis. Concentration of Pb, Cd and Fe were evaluated using descriptive statistic while Student *t*-test was used to analyze the effect of age and sex on the concentration of the metals using Statistical Package for Social Sciences (SPSS, 2010) version 20.0 for Windows.

Results

The results for the mean concentrations (mg/kg) of Pb, Fe and Cd detected from the blood of the twenty-two Holstein-Friesian cattle sampled from an intensively managed dairy farm is presented in Table 1. All the metals were detected above 0.00mg/kg. The mean concentration (mg/kg) for Pb, Fe and Cd were 1.160, 47.681 and 0.051, respectively.

Table 2 shows the mean concentration (mg/kg) of Pb, Fe and Cd from two different age groups of Holstein-Friesian cattle sampled. Pb and Fe concentrations were found to be higher in the younger (0-3.5 years) age group compared to the older (>3.5 years) group. However, there was no statistical significant difference ($P>0.05$) from this variation. The two age groups have nearly equal concentrations of blood Cd level with no significant difference ($P>0.05$). The result for mean blood concentrations (mg/kg) of Pb, Fe and Cd in Holstein-Friesian according to sex is shown in Table 3. There was no significant effect ($P>0.05$) of sex on the concentration of the metals in the samples, though Mean concentration of Pb and Cd was higher in females than male cattle.

Table 1: Mean concentration (mg/kg) of heavy metals in blood of Holstein-Friesian cattle

Metal	No. examined	Mean (mg/kg)	SD
Pb	22	1.160	1.16
Fe	22	47.681	20.58
Cd	22	0.051	0.02

No. = number; SD = standard deviation

Table 2: Mean concentration (mg/kg) of lead (Pb), iron (Fe) and cadmium (Cd) from different age group of Holstein-Friesian cattle

Metal	0-3.5 years		SD	L
	(19)	>3.5 years (3)		
	(mg/kg)	(mg/kg)		O
Pb	1.340	0.019	1.16	S
Fe	48.335	43.542	20.58	N
Cd	0.050	0.053	0.02	S

SD= standard deviation; LOS = level of significance; NS = not significantly different (P>0.05)

Table 3: Mean concentration (mg/kg) of lead (Pb), iron (Fe) and cadmium (Cd) in Holstein-Friesian cattle according to sex

Metal	Male (1)	Females (21)	SD	LOS
	(mg/kg)	(mg/kg)		
Pb	0.341	1.232	1.16	NS
Fe	55.415	47.313	20.58	NS
Cd	0.048	0.051	0.02	NS

SD= standard deviation; LOS = level of significance; NS = not significantly different (P>0.05)

Discussion

Environmental pollution has been positively correlated with the presence of heavy metals in the food chain by several studies. Organic agriculture is safer and can prevent food-chain disease transfer to human (Gabryszuk *et al.*, 2010). The obtained mean concentration (mg/kg) of Pb, Fe and Cd from this study were 1.160, 47.681 and 0.051 respectively. The values obtained for this study have exceeded the FAO/WHO maximum permissible limits for Pb in blood as 0.50mg/kg. Blood Pb levels above 0.35 µg m/L is toxic for ruminants while 1 µg mL⁻¹ in blood is fatal for animals (Swarup *et al.*, 2005). Tomza-Marciniak *et al.* (2011) reported a lower mean blood Pb concentration of 0.017mg/kg from inorganic raised cows in Poland. Also, they obtained a maximum blood Pb concentration (mg/kg) of 0.078 which is about forty times lower than the maximum value of 3.468 obtained from this study. The maximum blood Pb value from this study is in contrast to the study conducted in Bali by Ketut *et al.* (2017) where they recorded a maximum blood Pb value of 23.204mg/kg. Also, they obtained a mean Pb concentration of 7.350mg/kg in the blood of cows, a concentration that is two times higher than obtained in this study. Ogabiela *et al.* (2011) have reported in Northern Nigeria high levels of heavy metals from the blood of cattle grazed in polluted environment than those raised in non-polluted environment. Their obtained mean blood Pb concentration of 0.79mg/kg from the polluted environment is lower than the value from this study. Elevated levels of Pb in the blood interfere with the availability of other essential minerals.

Miller *et al.* (1990) have shown that the level of Pb in blood has a significant correlation with the levels and/or metabolism of essential trace metals. The results of the study conducted by Nwude *et al.* (2010) in Awka Southern Nigeria on the blood Pb of cows shows a mean concentration (mg/kg) of 2.79, a value lower than obtained from this study. Blood Pb levels in lactating cows have been shown to be transfer into milk. Patra *et al.* (2008) have reported significant correlation between blood and milk Pb concentration in lactating cows. This means high concentration of heavy metals in blood of lactating cows will result in the transfer and formation of heavy metals residues in milk. The results of the study of Ahmed *et al.* (2007) conducted in India indicated significant association between elevated blood Pb levels and the risk of anaemia. Cd is among heavy metals that have no any beneficial biological role. Pandey & Madhuri (2014) have reported that excess Cd levels in blood can cause increased blood pressure and myocardial disease in animals.

The concentration of Cd in this study is lower than the 0.5mg/kg permissible limit of FAO/WHO for tissues of cow. However, our value was high compared to concentration (mg/kg) range of Cd in blood (0.001 – 0.019) reported by Lucky & Temitayo (2017), but lower to the mean blood Cd levels of 5.67mg/kg reported by Okareh & Oladipo (2015) in their study conducted in cows from Southern Nigeria. The environment where these cattle are raised is also contaminated with heavy metals, Miranda *et al.* (2009) have reported that cattle and other ruminants are indicators of environmental contamination with heavy metals. Tomza-Marciniak

et al. (2011) detected a mean blood Cd concentration of 0.0007mg/kg from inorganically raised Friesian cows in Poland, even though the management system is similar to our area of study, the Cd value detected in our study is seventy times higher than their obtained value. The higher values of the three metals obtained in our study indicates most likely, the environment and the feed are highly contaminated with heavy metals as a result of inorganic farming activities and industrial activities. The long-time application of phosphorus fertilizers and herbicides to the pastures and crops is one of the most probable sources of Cd and Pb which originated from the contaminated soil. This can be supported by the study of Asad (2015) conducted in Jordan on different farms to see the effect of phosphorus fertilizer and herbicide applications to soil and plants. His results show that increase in the levels of Cd and Pb on plants correlates with an increase on the levels of these heavy metals on soil, he concluded that the most likely sources of Cd and Pb in the soils and plants were pesticides and phosphorus fertilizer. The 0.004 mg/kg minimum blood Cd value detected in our study tally with the value obtained by Nwude *et al.* (2010) when they sampled the blood of cows in Awka, Southern Nigeria. However, they obtained 0.02mg/kg as maximum blood Cd concentration, a value that is four times lower than the 0.093mg/kg obtained in this study. Cd was detected in the blood of all the dairy cows sampled in the study area, this is also in-line with the findings of Tomza-Marciniak *et al.* (2011) where they showed that the blood of all Friesian cows sampled under similar management system contained some levels of Cd. Cattle can have elevated levels of Pb in their blood without showing any outward signs of clinical disease (Lucky & Temitayo, 2017). Toxic effects of Pb and Cd were reported in cattle and human due to ingestion of contaminated products containing heavy metals (Cai *et al.*, 2009). The environment where Ogabiela *et al.* (2011) conducted their study using blood of cows in Northern Nigeria is highly contaminated as a result of agricultural and tannery activities. They recorded a blood mean Cd concentration (mg/kg) of 0.14 in Kano and a higher value of 0.17 in Zaria, these levels of blood Cd are higher than the mean obtained in this study conducted in north central Nigeria. According to Swarup *et al.* (2005), lactating cows had increased blood levels of heavy metals as a result of grazing in metal polluted environment. Fe is an essential element and play vital role in biological process. Pandey & Madhuri (2014) have reported that Fe can be toxic in high amounts and the metabolic role of essential elements can be changed by the toxic metals, resulting into toxicity. Fe is considered as a micronutrient (Reeves & Baker,

2000) but can be toxic when an elevated level in excess of requirements is observed in tissues, organs and blood during feeding. The mean blood Fe detected in this study is above the recommended levels for tissues. It is also above the aggregate value of 1.21mg/kg detected in the blood of cows by Nwude *et al.* (2010) in Southern Nigeria and ten times higher than the mean blood Fe levels of 4.346mg/kg reported by Tomza-Marciniak *et al.* (2011) from an inorganic and intensively managed Holstein-Friesian cows in Poland. The high levels of Fe recorded in this study may likely be from the irrigation water or from bore hall water which the study of Coup & Campbell (1964) shows that water sources from borehole contain high levels of Fe and can contaminate Pasture and crops when used for irrigation.

Table 2 shows the concentration of heavy metals according to different age groups of the animals. The results show no significant difference ($P>0.05$) on the concentration of Pb, Fe and Cd among the different age groups. Although, younger animals had an elevated value of Pb (1.340mg/kg) and Fe (48.335mg/kg) when compared to 0.019mg/kg and 43.54mg/kg values observed in animals above 3.5 years of age. Cd concentration (0.053 mg/kg) was higher in animals above 3.5 years than the concentration of 0.050 mg/kg observed in those below that age category. The possible reason for a lower Fe levels in older age groups could be increased mineral demand during milk production, which could reduce the concentration of the essential metals in their blood. Since Cd is not degradable in tissues of cattle, the high levels recorded from the older age group could be the results of prolong bioaccumulation over time. As the animal ages, more levels of Cd accumulate in blood and tissues. This is in-line with the saying of Mukesh *et al.* (2010) that Cd is almost absent in the body at birth, but it accumulates with age.

The results of concentration of heavy metals according to sex are presented in Table 3. Male and female cattle had no significant different values in the concentrations of Pb, Fe and Cd in their blood. In this study, mean Pb, Fe and Cd concentrations (mg/kg) were 0.341, 55.415 and 0.048 respectively in males and 1.232, 47.313 and 0.051 respectively in females. A higher concentration of Fe was recorded in male than females. The lower concentration in females could be as a result of high mineral demand in females for milk production especially during lactation in high milk producing cows.

In conclusion, the results of this study showed that Holstein-Friesian cattle in the study area are exposed to Pb, Cd and Fe and the mean concentrations Pb is above permissible level recommended by FAO/WHO. Age and sex have no

significant difference ($P > 0.05$) in bioaccumulation of heavy metals in cattle. In order to curtail the cases of accumulation of heavy metals in blood, tissues, organs and other animal products, organic farming should be encouraged and the use of agricultural pesticides, herbicides and Phosphorous fertilizers should be avoided. Also, sources of irrigation water for pastures and crops must be free of heavy metals to reduce anthropogenic contamination of soil and pasture. Plastic pipes instead of iron steel pipes should be used for irrigation channels. Feed and water containing toxic heavy metals should not be given to animals. All sources of heavy metals such as battery, cement, paints, lubricating oils etc should be kept away from animal sources of feed and water. Continuous monitoring and evaluation of the environment and animal tissues should be adopted.

Conflicts of Interest

The authors declare no conflicts of interest.

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