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Lead (Pb) and Cadmium (Cd) levels in fresh and smoke-dried grasscutter (*Thryonomys swinderianus* Temminck) meat

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Muscle, liver and kidney of wild and domesticated grasscutters were analyzed in order to determine the Lead (Pb) and Cadmium (Cd) concentrations. Meat samples were obtained from four different origin. A total of twenty-four (24) samples were used. An atomic absorption spectrophotometer (AAS) was used for the analysis after wet digestion of the samples with 1:3 Perchloric acid and Nitric acid. Data obtained were statistically analyzed using SAS. A randomized complete block design was used, with all treatments arranged in a 4×3×2 factorial. The levels (mg/kg, d.w.) of Pb and Cd obtained in (fresh and smoke-dried) muscle, liver and kidney samples were as follows: Pb (ND-0.513) (ND-0.154 and Cd (0.186-7.516) (0.277-2.723). Heavy metal concentrations were higher ($p < 0.05$) in fresh and smoke-dried muscle, liver and kidney of wild grasscutters than those levels found in domesticated grasscutters. However, Pb and Cd levels in fresh grasscutter samples were higher ($p < 0.05$) than those levels found in smoke-dried grasscutter samples. Cd concentration levels were higher than the recommended limits set by international regulations. Pb was not detected in domesticated grasscutters. Proper knowledge of public and health workers regarding hygienic and safe handling of bush meat as well as cutting off of infected parts, are highly recommended.

Key words: Cadmium, trace element, domestication, grasscutter, heavy metal, lead.

INTRODUCTION

Issues of food security and poverty have since been recognized as necessary conditions for the creation of a stable socio-political environment for sustainable economic development (Jibrin, 2004). Human nutrition requires that food be combined in an unbound, reduced and wholesome form so as to facilitate digestion, absorption and excretion as well as promote good health and not constitute any form of health hazard or such nutrition disorders as obesity, underweight, iron deficiency, dental caries and allergies (Mahan and Escott-Stump, 2004). Food borne-diseases pose more risk than vector-borne diseases (malaria, yellow-fever, plague, etc), water contact diseases (leptosipirosis, schistosomiasis), aerosolized or soil contact diseases (Lassa fever), respiratory diseases (meningococcal meningitis), and animal contact diseases such as rabies

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(Cia, 2011).

A common source of meat in West Africa is grasscutter, which is obtained from the wild. The grasscutter is a wild hystricomorphic rodent, widely distributed in most areas and constitutes an important source of animal protein (Asibey, 1974a, b; NRC, 1991). It is the second largest rodent after the porcupine. The average adult weight is 3 kg for females and 4.5 kg for males (Eben, 2004). The grasscutter has thickset body, measuring 40 to 60 cm in addition to a 20 to 25 cm tail (Fitzinger, 1995). Its furs comprise a mixture of brown-reddish and grey hairs that vary depending on its habitat (Jori and Chardonnet, 2001). Some other authors reported that the skin and hair (fur) as well as limbs and tails are easily torn out (Kingdom, 1974). This makes the animal very difficult to catch and even more difficult to handle after capture. The grasscutter is also a prominent and steady source of alternative dietary animal protein in many rural areas in Nigeria and other West African countries like Benin, Ghana, Togo, Cote D'voire (Ogunsanmi et al., 2002; Tettey et al., 2004; Ebabhamiegbebho and Ohanaka, 2012; Owen and Dike, 2012). However, the method of hunting is becoming a growing concern due to bullets containing Pb and other metals (Irschik et al., 2012; Doborowolska and Melosik, 2008; Hunt et al., 2009; ASEAN, 2012).

Heavy metals are ubiquitous in soil, water and air. These elements can be transferred into the food chain, thus presenting a risk for human health. Trace elements are toxic depending on dosis, and meat can be contaminated as those chemicals can be accumulated in tissues, such as Cd (Beiglbock et al., 2001) and Pb (Karita et al., 2000). Pb is a metabolic poison and a neurotoxin that binds to essential enzymes and several other cellular components and inactivates them (Cunningham and Saigo, 1997). Toxic effects of Pb are seen on haemopoietic, nervous, gastrointestinal and renal systems (Baykov et al., 1996). In humans, high Pb concentrations can cause damage to internal organs, especially to the central nervous system (CNS), and can even reduce the ability to create new blood cells. In adults, the kidneys are the most sensitive to chronic exposure to Pb. In children up to the age of 7, the nervous system is the most sensitive and such a threat is even greater in infants and small children (BfR, 2011). Humans can be exposed to Pb through foods, water, the air and dust but food is the main source of exposure (ASEAN, 2012). Cd levels have also been implicated in renal tube dysfunction (WHO, 2003). The accumulation of Cd and Pb increases with age and very high values have been registered in the muscle over surrounding the bullet pathway (Falandysz et al., 2005).

Determining heavy metal contamination in wild animal tissue, such as Pb and Cd can be the first step on knowing the levels of these elements, both in overall population and specific groups, such as hunters and their families. Consumption of meat from hunted wild ungulates is strictly associated with the issue of quality and hygiene assurance. The steps from hunting to market to assure meat quality are very difficult to control (Casoli et al., 2005; Coburn et al., 2005). Again, literatures on the level of these heavy metals in organs of various bush meat species particularly in Nigeria are largely unavailable. The consumers of grasscutter meat are unaware of the level of heavy metal occurrence. Moreover, heavy metal concentrations in grasscutter is a critical issue for consumer safety. The toxicity of heavy metal is one of the major environment problems and is potentially dangerous to animal and human health, because metals are able to bioaccumulate through food chain (Aycicek et al., 2008; Aschner, 2002). There is also the possibility that the heavy metal concentration levels in grasscutter varies with location/origin and thus requires investigation. Again, studies on heavy metals levels in organs of various bush meat species are scarce, particularly in Nigeria, and more research is required moreover, concentration of heavy metals in grasscutter is important for consumer safety as many grasscutter consumers are unaware of the level of heavy metal contamination in them. There is the need to improve the grasscutter meat to the status of a viable industry capable of meeting both local and foreign demands without depending largely on hunting from the wild (Adomah, 2009). The objective of this study was to evaluate the levels of Pb and Cd in muscle, liver and kidney of fresh and smoke-dried carcasses of wild and domesticated grasscutters.

MATERIALS AND METHODS

Sample collection

Fresh and smoke-dried meats of wild grasscutter were obtained from game meat processing centres in Uwa, New Benin and Arbico markets at Benin City, Edo State, Nigeria. Moreover, fresh and smoke-dried meats of domesticated grasscutters were obtained from Makarios graduate grasscutter farmers society at Benin City, Edo State, Nigeria. The sex, age and feeding habits of grasscutter were not taken into consideration during the sampling. A total of 24 random grasscutter samples were collected between March and September 2013. A randomized complete block design was used in a 4×3×2 factorial arrangement to assess whether heavy metals varied significantly between liver, kidney and muscle of the animal. The samples were collected in separate polyethylene bags and transported to the laboratory for their analysis.

Sample preparation

All collected samples were decomposed by means of wet digestion. About 1 g of the samples (liver, kidney and muscle) was weighed into the digestion flask. Then, 5 ml of 0.1 N perchloric acid and 15 ml of 0.1 N concentrated HNO_3 in a ratio 1:3 were added and then heated in an electric plate until clarification. Following, 5 ml of 20% HCl (0.1 N) was added to the content. The content of the flask was filtered using Whatman filter NO 42 paper into a 100 ml volumetric flask and was made up to the mark with a distilled water and then stored in a plastic reagent bottle, ready for Atomic Absorption Spectroscopy (AAS) analysis. Parallelly, 5 g of the fresh and smoke-dried samples (liver, kidney and muscle) was also weighed

Table 1. Mean concentrations of lead (mg/kg) in fresh kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	ND	ND	ND	ND
	NB	$0.165 \pm 0.005^{b}_{C}$	$0.369 \pm 0.002^{a}_{B}$	$0.119 \pm 0.008^{c}_{B}$	0.0000146
Pb	U	$0.276 \pm 0.005^{a}_{B}$	$0.214 \pm 0.006^{b}_{C}$	$0.089 \pm 0.002^{c}_{C}$	0.00000989
	AB	$0.326 \pm 0.107^{b}_{A}$	$0.513 \pm 0.022^{a}_{A}$	$0.141 \pm 0.008^{c}_{A}$	0.0000929
	SEM	0.0000108	0.0000608	0.000016	

ND: Not Detected; Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market (AB) Arbico Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at $P < 0.05$; Mean with the same scripts are not significantly different at $P > 0.05$.

in separate beakers and oven dried at 100°C to constant weight for determination of moisture content.

Sample analysis

Pb and Cd in fresh and smoke-dried liver, kidney and muscle samples were analyzed by using an atomic absorption spectrophotometer (AAS) MODEL-SOLAAR 969 UNICAM Series () at 217 nm for Pb and 228.8 nm for Cd. The allowed amounts of heavy metals (HMs) in food vary from country to country and are based on the World Health Organization (WHO) recommendations and local requirements (Oluyemi and Olabanji, 2011).

Statistical analysis

Data obtained from this study were statistically analyzed using Genstat software (2009) to establish the results. Data are shown as the mean ± standard error. Metal concentrations in fresh and smoke-dried samples were analyzed with respect to locations/origin. A statistical level of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Lead (Pb)

The results obtained in Table 1 showed that Pb concentration was significantly higher in the liver of fresh grasscutters from Arbico market, than those levels from New Benin and Uwa markets. On the other hand, Pb concentration (mg/kg) in kidney of smoke-dried grasscutters was higher ($P<0.05$) at Uwa market than Pb levels at Arbico and New Benin markets (Table 2). Pb concentration was significantly higher in fresh liver, kidney and muscle than in the smoke-dried samples irrespective of the market location.

The highest Pb levels (0.513 and 0.154 mg/kg) were observed in fresh liver and smoke-dried kidney at Arbico and Uwa market, respectively. The lowest concentrations in fresh and smoke-dried muscle were found at Uwa (0.089 mg/kg) and New Benin (0.043 mg/kg) market. This finding indicates that Pb concentrations in fresh and smoke-dried samples from all markets were lower than the permissible limit (1 mg/kg) set by the Australia-New Zealand Food Authority (ANZFA, 2001). Pb levels showed that fresh and smoke-dried samples were also lower than 1 mg/kg reported by Galadima and Garba (2011), and approved by the Federal Environmental Protection Agency of Nigeria (FEPA). Similar Pb values were also observed by Falandysz (1994) in liver and kidney, ranging from 0.090 to 0.240 mg/kg and 0.080 to 0.360 mg/kg, respectively. In agreement, Jarzynska and Falandysz (2011) reported Pb contents in liver and kidney of deer as 0.17 mg/kg and 0.30 mg/kg (dry weight), respectively. An earlier study by Doganoc and Gacnik (1995) reported similar Pb levels (0.16 mg/kg) in liver of roe, fallow deer, deer, pheasant, wild duck and hare in Slovenia.

On the contrary, Martelli (2005) found higher Pb levels in liver of wild boars as exceeding regulatory limit, even though he did not observe high values in kidney. Meanwhile, Piskorova et al. (2003) observed higher Pb levels in kidney than Pb levels in liver (0.39 and 0.24 mg/kg, respectively) of wild hunted boars in the Slovak Republic. Martelli (2005) observed similar Pb concentrations in liver (0.302 to 0.674 mg/kg, w.w.) and in kidney (0.401 to 0.774 mg/kg, w.w.) of wild boars reared in Pisa, Italy. Similarly, Flanjak and Lee (1979) reported Pb concentrations up to 0.85 mg/kg and 2.25 mg/kg in liver and kidney of cattle at New South Wales, Australia. An earlier study showed that Pb levels in liver of birds (turkey, pheasant and partridge) were as high as 25 mg/kg (Kreager et al., 2008). Similarly, Maldonado et al. (1996) studied Pb related to its intestinal mobilization during lactation in rats and found significantly higher Pb levels in liver and kidney.

In agreement with that, Mariam et al. (2004) reported Pb levels of 2.18, 4.25 and 3.15 mg/kg in beef, mutton and poultry, respectively. Hunt et al. (2009) stated that high Pb levels could be related to the metal dispersion

Table 2. Mean concentrations of lead (mg/kg) in dried kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	ND	ND	ND	ND
	NB	$0.111 \pm 0.003^{b}_{B}$	$0.129 \pm 0.002^{a}_{C}$	$0.043 \pm 0.003^{c}_{C}$	0.00000246
Pb	U	$0.154 \pm 0.004^{a}_{A}$	$0.146 \pm 0.006^{a}_{B}$	$0.068 \pm 0.003^{b}_{A}$	0.0000085
	AB	$0.098 \pm 0.002^{b}_{C}$	$0.153 \pm 0.003^{a}_{A}$	$0.057 \pm 0.002^{c}_{B}$	0.00000219
	SEM	0.00000255	0.0000047	0.0000025	

ND: Not Detected; Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbico Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at P < 0.05; Mean with the same scripts are not significantly different at P > 0.05.

Table 3. Mean concentrations of cadmium (mg/kg) in fresh kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	$0.477 \pm 0.035^{a}_{D}$	$0.360 \pm 0.021^{b}_{D}$	$0.186 \pm 0.016^{c}_{D}$	0.00029
	NB	$1.071 \pm 0.026^{b}_{C}$	$3.923 \pm 0.071^{a}_{B}$	$0.636 \pm 0.055^{c}_{C}$	0.00137
Cd	U	$4.045 \pm 0.074^{a}_{A}$	$3.452 \pm 0.059^{b}_{C}$	$1.873 \pm 0.031^{c}_{B}$	0.00156
	AB	$3.592 \pm 0.020.^{b}_{B}$	$7.516 \pm 0.079^{a}_{A}$	$2.459 \pm 0.041^{c}_{A}$	0.00131
	SEM	0.000917	0.00179	0.000694	

Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbicu Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within locations indicated locations are statistically significant; Mean with different scripts are significantly different at P < 0.05; Mean with the same scripts are not significantly different at P > 0.05.

after bullet fragmentation into animal body. The levels of Pb in animals depends on various factors such as the frequency of the quantity of meat consumed, the degree of leaded-bullet fragmentation, the path that the ammunition has taken and the level of care taken to remove the flesh surrounding the wounds (Hunt et al., 2009). The results revealed that Pb levels in fresh and smoke-dried kidney, liver and meat of grasscutters were at safe levels and were of no danger to health (ANZFA, 2001). As shown in Tables 1 and 2, Pb concentrations were significantly higher in fresh tissues than in smoke-dried tissues, in agreement with the findings of Ajani et al. (2013) and Eboh et al. (2006).

Cadmium (Cd)

As shown in Table 3, Cd concentrations were significantly higher in liver, kidney and muscle of fresh grasscutters at Arbico, Uwa, New Benin and Domesticated. On the contrary, Cd concentrations were lower (P<0.05) in liver, kidney and muscle of smoke-dried grasscutters at Arbico, Uwa, New Benin and Domesticated location (Table 4). Cd concentration was the highest in fresh liver at Arbico market, whereas Cd level was the highest in smoke-dried muscle at Uwa market. In general, Cd levels in samples of kidney, liver and muscle of fresh grasscutters were significantly higher than Cd levels detected in samples of smoke-dried grasscutters.

The highest Cd concentrations were observed in fresh liver (7.516 mg/kg) and smoke-dried muscle (2.723 mg/kg) from Arbico and Uwa market. Those Cd levels are above the permissible limit (0.5 mg/kg) stated in meat and liver (FAO/WHO, 2000). On the contrary, the lowest Cd concentrations were found in fresh muscle (0.186 mg/kg) and smoke-dried muscle from domesticated samples. The highest Cd levels in fresh kidney (4.045 mg/kg) and smoke-dried kidney (1.75 mg/kg) were detected in samples from Uwa market. These Cd values in kidneys are above the limit (1.0 mg/kg) accepted (FAO/WHO, 2000). Aranha et al. (1994) and Roga-Franc et al. (1996) found also Cd levels above threshold values (FAO/WHO, 2000) in liver and kidney of cattle in Poland. Doganoc (1996) found Cd levels in liver and kidney of chicken above official tolerance levels.

In contrast, Mariam et al. (2004) reported Cd

Table 4. Mean concentrations of cadmium (mg/kg) in smoke-dried kidney, liver and muscle of grasscutters from different origen (dry matter basis).

		Organs			
Variable	**Origen**	**Kidney**	**Liver**	**Muscle**	**SEM**
	DC	$0.795 \pm 0.032^{a}_{C}$	$0.636 \pm 0.019^{b}_{D}$	$0.277 \pm 0.019^{c}_{D}$	0.00027
	NB	$1.002 \pm 0.016^{b}_{B}$	$1.957 \pm 0.022^{a}_{B}$	$0.331 \pm 0.017^{c}_{C}$	0.000157
Cd	U	$1.750 \pm 0.053^{b}_{A}$	$1.690 \pm 0.017^{b}_{C}$	$2.723 \pm 0.040^{a}_{A}$	0.000744
	AB	$1.060 \pm 0.011^{b}_{B}$	$2.205 \pm 0.033^{a}_{A}$	$0.871 \pm 0.007^{c}_{B}$	0.00019
	SEM	0.00049	0.000258	0.00027	

Location: (DC) Domesticated; (NB) New Benin Market; (U) Uwa Market; (AB) Arbicu Market; SEM: Standard Error of Mean; a, b, c, superscripts within rows indicated organs are statistically significant; A, B, C, D subscripts within columns indicated locations are statistically significant; Mean with different scripts are significantly different at P < 0.05 and Mean with the same scripts are not significantly different at P > 0.05.

Table 5. Mean concentrations of heavy metals (mg/kg) in fresh kidney, liver and muscle of grasscutters (dry matter basis).

Organ	Pb	Cd	SEM
Kidney	0.1918^{b}	2.2965^{a}	0.1838
Liver	0.2738^{b}	3.8127^{a}	0.299
Muscle	0.0873^{bc}	1.2886^{a}	0.1319

ND: Not detected; SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at p < 0.05

concentrations of 0.33, 0.37and 0.31 mg/kg in lean meat of beef, mutton and poultry, respectively. Previously, a study conducted at Poland by Zmudzki and Szkoda (1995) found 0.146 mg Cd/kg in liver and 0.580 mg Cd/kg in kidney of young cattle, while in older animals Cd levels were 0.204 and 0.829 mg/kg in liver and kidney, respectively. Similarly, Falandysz (1994) reported 0.10 mg Cd/kg and 0.450 mg Cd/kg in liver and kidney of cattle in northern Poland. Tahvonen and Kumpulainen (1994) reported in cattle liver, values of 0.061 mg Cd/kg from Finland, 0.070 mg Cd/kg from Sweden and 0.105 mg Cd/kg from Netherlands. Those values probably are reflecting a major environmental pollution, as also suggested by Gasparik et al. (2012), because Cd is one of the most toxic elements to humans and animals (Naja and Volesky, 2009). Cd is toxic virtually to every system in the normal body. The high levels of Cd found in both fresh and smoke-dried tissues are significantly higher than the limits set by FAO/WHO (2000). Chemically, the consumption of grasscutter meat could present a risk for human health.

Interactions

The levels of Pb and Cd found in the fresh and smoke-dried liver samples from the different locations were generally higher than the concentration levels of these heavy metals in the fresh and smoke-dried kidney and muscle samples (Tables, 5, 6 and 7). However, Cd levels were lower in muscle samples than kidney and liver levels in both fresh and smoke-dried samples. This is an indication that these heavy metals accumulate more in the liver than in the kidney and muscle. However, there are a few exceptions, where the concentrations of these heavy metals are higher in the kidney and in the muscle.

It is a favorable condition that Pb was not detected in all the samples of the domesticated grasscutter location. This may probably be attributed to method of capture or capture procedure, good monitoring and uncontaminated feed or drinking water as well as low environmental pollution near the breeding location or area. Overall, the results of this study show that the levels of (Pb) was generally low compared to Cd in the kidney, liver and muscle of fresh and smoke-dried grasscutters studied. Generally low Pb and Cd concentrations observed in the smoke-dried kidney, liver and muscle reduced. This agrees with Ahmed et al. (2011) who suggested heavy metal as being water borne which could explain a dripping deposition resulting from the washing of the meat prior to smoking. The drop in concentration of heavy metal in the smoked samples was also corroborated by Ajani et al. (2013) and Eboh et al. (2006) who attributed it to heat having a significant effect on heavy metals and a possibility of the heavy metal being converted to other compounds.

Table 6. Mean concentrations of heavy metals (mg/kg) in smoke-dried kidney, liver and muscle of grasscutters dry matter basis).

Organ	Pb	Cd	SEM
Kidney	0.0909[bc]	1.1517[a]	0.0427
Liver	0.1071[a]	1.5532[a]	0.0773
Muscle	0.0419[b]	1.0508[a]	0.1248

ND: Not detected; SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at $p < 0.05$

Table 7. Interactive comparisons of heavy metal concentrations (mg/kg) between fresh and smoke-dried tissues of grasscutters (dry matter basis).

	Fresh		Dried		
Organ	Pb	Cd	Pb	Cd	SEM
Kidney	0.1918[c]	2.2965[a]	0.0909[c]	1.1517[b]	0.240
Liver	0.2738[c]	3.8127[a]	0.1071[c]	1.5532[b]	0.394
Muscle	0.0876[b]	1.2886[a]	0.0419[b]	1.0508[a]	0.204

ND: Not Detected, SEM: Standard Error of Mean; Means with different superscripts (a, b, c) within rows are significantly different at $p < 0.05$.

It is imperative to enforce actions to ensure an adequate health education to people, public health personnel and bush meat handlers through media. Grasscutter meat must be safe and suitable for human consumption, therefore all stakeholders including scientists, government, industries and consumers have a role in achieving this outcome. Sadly, West Africa has depleted stocks of wildlife, and farmers have to be financially supported by both public and private sectors toward the domestication of grasscutter. These measures could guaranty a continuous provision of meat of good quality to the growing population. In the meantime, it is highly recommended to cut off animal parts which have bullet wounds before meat processing.

Conclusion

This study has shown that Pb levels were significantly higher in liver, kidney and muscle of fresh grasscutters than smoke-dried grasscutters. Pb was not detected in fresh and smoke-dried organs of domesticated grasscutter samples. Cd levels were highest in fresh liver and smoke-dried muscles of meat obtained from local markets even though Cd levels were lower from samples of domesticated grasscutters. Education of people and public health officials on proper bush meat handling is also highly required.

Conflict of Interest

We declare that we have no conflict of interest.

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