Original Paper

Microbial and Heavy Metal Assessment of Meat Samples from Ranched and Non-ranched Domestic Animals Sold at Gariki Market, Enugu State, Nigeria

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Received: April 9, 2023	Accepted: May 2, 2023	Online Published: May 26, 2023			
doi:10.22158/rhs.v8n2p22	URL: http://dx.doi.org/10.2	22158/rhs.v8n2p22			

Abstract

Studies were carried out to evaluate the microbial and heavy metal contamination of meat samples from ranched and non-ranched domestic animals sold at Gariki market, Enugu State, using standard analytical and biochemical procedures and instrumentation.

The meat samples from the ranched and non-ranched domestic animals (goat, cow, pig and chicken) were procured at slaughter from the Gariki market and under ice condition transported to the laboratory for microbial and heavy metal analysis. Ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, indoor-reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples had mean Cd values ranging from 0.110- 0.292 µg/g; Pb, 0.176 -0.505 µg/g and Cu, 1.303 -5.972 µg/g.

The mean levels of the investigated heavy metals in the meat samples were statistically significant. Meat samples procured from the non- ranched domestic animals and some ranched or in-door reared ones (cow and pig) had mean Cd levels at toxic level. Mean Pb value in the non –ranched cow meat samples followed the same trend. The pathogenic bacteria of interest to food scientists namely, S. aureus, Streptococcus spp. and Salmonella enteritidis were isolated in the meat samples from the domestic animals. The range of mean bacterial counts of S.aureus, Streptococcus spp. and Salmonella enteritidis in the ranched goat meat, non-ranched goat meat, non –ranched cow meat, ranched cow meat, in-door-reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples were $1.04 \times 10^2 - 1.73 \times 10^8$ Cfu/g. $1.14 \times 10^2 - 2.64 \times 10^6$ Cfu/g and $0.82 \times 10^2 - 3.92 \times 10^5$ Cfu/g respectively. With the inclusion of ranched cow meat and in-door reared pork meat samples, the meat samples from the non-ranched domestic animals had mean bacterial counts of the isolated pathogenic organisms above threshold limits. The quality of meat samples from the non-ranched (out-door reared) domestic animals sold at Gariki market, could be said to be more burdened by heavy metal and microbial contamination, which apart from decreasing the nutritive quality of the meats, also portends food safety challenges to the consumers.

Keywords

Heavy metals, meat samples, non ranched (out –door reared) domestic animals, ranched (in-door reared) domestic animals and pathogenic bacteria

1. Introduction

Meat is a very rich and convenient source of nutrient, especially the macro and trace elements. Meat from domestic as well as wild animals are a good source of animal protein, with high biological value since it contains essential amino acids, vitamins and minerals, required for human nutrition, growth and replenishment of worn-out tissues (Bendeddouche et al., 2014). The chemical and nutrient composition of meats and availability for consumption depends essentially on the quality of feeds fed to the animals, water consumed and size or kinds of the feeding animal. Because of economy of space and to maintain the sanctity of man's environment and to avoid loss of economic to free-ranging (out-door reared) animals, majority of domestic animals reared for both economic and personal reasons, follow the in-door (ranching) rearing system. Domestic animals like cow, though ranched by relevant Nigerian laws, are still mainly reared through the open -grazing or non-ranched rearing system (Sabuwa et al., 2019). The majority of animal protein requirement especially in Sub-Saharan Africa and, Nigeria in particular are supplied from domestic animals such as cow, goat, chicken, pig and sheep etc. The production efficiency of animals meant for human consumption is mainly due to their health status, which is often impacted by the level of undesirable contaminants they are exposed to via water, food and deposition (Gupta et al., 2021). According to Okeke and Okeke (2015), the quality and nutrient content of meats from domestic animals is dependent on the quality of its feeds, water and air around the reared environment. Many chemical and biological pollutants follow anthropogenic processes and could enter the food chain of animals as well as humans through food, water or air (Sabuwa et al., 2019). Undesirable contaminants such as heavy metals and harmful micro-organisms impact negatively on animal and as well as human health even at very low concentrations (Dorne & Firk- Gemmels,

2013). Climate change, economic status and cultural practices influences the rearing system adopted by farmers and invariably, the biological and chemical contaminants in the meats from the reared animals. In other words, domestic animals share environment with man and are exposed to heavy metals mainly through ingestion of contaminated vegetation, feed, water and soil and to some extent particles present in the air due to industrial and vehicular emissions (Fennie et al., 2011). According to Okeke et al. (2016); Aniobi et al. (2019), the anthropogenic heavy metal pollution that follow the composition of feeds fed to domestic animals reared especially in many Nigerian communities constitute a major source of heavy metal exposure to the animals. Ezeh et al. (2019) observed that the improper discharge of industrial effluents, very common in major African cities and rural centres, has led to the contamination of fresh water sources with heavy metals and micro-organisms, reducing their safety for agriculture, irrigation and drinking purposes. Pesticides, insecticides and fertilizers used in agricultural field constitute the secondary sources of heavy metal pollution to the domestic animals as well as humans in the food chain sequence. Once in the body, heavy metals generally bind to one or more bio-active ligands such as -OH, -COO⁻, OPO, P, -C=O, -SH, -S-S, - NH₂ and -NH, that are essential for nutritional and physiological function and disrupts several enzyme reaction and persisting in such condition for a considerable time (Liu et al., 2018). Toxicity effects of heavy metals on animals depends on extent of exposure, type of metal, its form, age, sex, physiological and nutritional status of the exposed animal and route of contamination (Gupta et al., 2021). General symptoms of toxicity include cardio-toxicity, tetratogenicity, oxidative stress, immune-toxicity, enzyme inhibition, reproductive defects and endocrine disruption (Okeke & Okeke, 2015; Ezeh et al., 2018; Ezeh et al., 2019; Aniobi et al., 2019).

Consumption of unsafe water constitutes one of the major sources of pathogenic organisms that trigger acute and severe tropical food and water-borne diseases that plague domestic animals and human beings (Okeke et al., 2021). Because most farmers pay less attention to the safety and quality of foods and water consumed by domestic animals under their care, exposure to the biological contaminants by these animals is usually widespread and high. Equally, most domestic animals reared through in-door (ranched) or out-door (non –ranched) system rely on fermented, soured, and discarded food remnants to satisfy their food needs and therefore becomes unduely exposed to pathogenic organisms. Pathogenic organisms such as *S.aureus, Streptococcus spp., Salmonella enteritidis, E.coli* and *Bacillus cereus* are ubiquitous in dirty and contaminated food materials and humans by extension (Okeke et al., 2020). Increased exposure to these pathogenic organisms impacts negatively on the growth, development, quality and well being of domestic animals.

Meats from domestic animals such as goat, cow, chicken and pig serve the animal protein needs of people of all ages and classes in our food-conscious society today, the various systems of rearing these meats at the point of sale are immaterial to the willing buyer and consumer, however, food safety from food contaminants such as heavy metals and pathogenic bacteria are important in safeguarding the well being of the meat consumers especially in a city centre like Gariki, that serves as abattoir and meat selling depot to surrounding communities, hence this research.

2. Materials and Methods

2.1 Sample Collection and Preparation

Fresh meat samples from non-ranched cows, ranched cows, non-ranched goats, in-door reared chickens, out-door reared chickens and in-door reared pigs were purchased immediately after slaughter from meat sellers at Gariki market, Enugu State. The meat samples were separated from bone attachments and washed separately with distilled water until all the blood stains and sand particles have been removed. The samples were stored under ice in well labeled polyethene containers and taken to the laboratory for analysis.

2.2 Heavy Metal Analysis

5g of each meat sample was digested in 9ml of 65% Conc. HNO_3 and 6ml of 60 % $HCIO_4$. The solution was transferred to hot plate of 110°C and the heating lasted for 180min. Next the samples were introduced into an oven under a temperature that was gradually adjusted to 100°C every 60min, until a temperature of 450°C was reached and white ash seen.

The white ashes were then dissolved in 5ml of 1.5% HNO₃ and a final volume of 25ml were made by adding de-ionized water. The resulting solution was filtered using Whatmann filter paper and transferred into tightly sealed containers. The digests were subsequently aspirated into the atomic absorption spectrophotometer (Perkin-Elmer Analyst 200, USA) to determine the levels of Pb, Cu and Cd.

All necessary precautions were taken to avoid any possible contamination of the samples in accordance with AOAC guidelines (Ezeh et al., 2019).

2.3 Microbiological / Biochemical Analysis and Identification of Bacteria Isolates.

In order to identify bacteria species, the microbiological techniques employed included inoculation, gram staining, colony and morphological characterization for physical and structural features of the organisms as described by (Janseen, 2006). Pure cultures of the isolates were subjected to various biochemical tests to determine the identity of the bacteria species. The result of each test was recorded and the probable identity of the isolates was determined by the use of Bergey's manual of determinative bacteriology (Forbes et al., 2002). Bacteria colonies were counted to obtain the viable cell count.

3. Results and Discussion

Table 1. Mean Heavy Metal Levels in the Meat Samples from the Ranched (in-door Reared) and

Meat	Ranched	Non-ranched	Non-ranched	Ranched	In-door	Out-door	In-door	F	WHO
samples	goat meat	goat meat	cow meat	cow meat	reared pork	reared	reared	test	(2017)
Heavy					meat	chicken	chicken	Р	STD
Metal						meat	meat	value	
(µg/g)									
Cd	0.136±0.012	0.224±0.031	0.292±0.052	0.206±0.044	0.211±0.052	0.181±0.034	0.110±0.020	0.01	0.20
Pb	0.272±0.050	0.361±0.064	0.504 ± 0.082	0.371±0.067	0.455±0.039	0.311±0.052	0.176±0.043	0.01	0.50
Cu	3.713±0.171	2.344±0.031	3.084 ±0.230	5.972±0.108	4.131±0.157	1.306±0.043	1.945±0.027	0.01	100

non-ranched (out-door reared) Domestic Animals Sold at Gariki, Enugu State

Table 2. Biochemical Characteristics of Bacterial Isolates from the Meat Samples

Cultural	Morphology	Gram	Glucose	Indole	Coogulata	Catalase	Citrate	Most probable identity	
characteristics	Worphology	staining			Coagulate	Catalase	Cluate		
Yellowish	Cocci in	+	+	-	-	+	+	Staphylococcus	
orange and	pairs							aureus (S.	
slimy								aureus)	
Cream,	Cocci raised	+	+	-	+	+	-	Streptococcus	
circular raised	in clusters							spp.	
Creamy and	Pods	+	-	-	-	+	-	Salmonella	
irregular	scattered							enteritidis	

Table 3. Mean Bacterial Counts (Cfu/g) of the Meat Samples from the Ranched (in-door reared)

and non-ranched (out-door Reared) Domestic Animals Sold at Gariki Market, Enugu State

Mean bacterial counts (Cfu/g) Bacterial isolate	Ranched goat meat	Non-ranched goat meat	Non-ranched cow meat	Ranched cow meat	In-door reared pork meat	Out-door reared chicken meat	In-door reared chicken meat	F test P value	WHO (2011) STD
S.aureus	4.32×10^{2}	2.16×10^5	1.73×10^{8}	2.86×10^{5}	2.41×10^{5}	1.22×10^{5}	1.04×10^2	0.01	$\leq 10^3$
Streptoco ccus spp.	1.66 ×10 ²	1.57 ×10 ⁴	2.64×10^{6}	1.01×10 ³	2.06×10 ⁵	1.08×10^4	1.14×10 ²	0.01	≤10 ³
Salmonella enteritidis	1.88×10^{2}	3.41 ×10 ⁴	3.92 ×10 ⁵	2.51 ×10 ³	1.59 ×10 ⁴	1.35 ×10 ³	0.82×10^{2}	0.01	≤10 ³

3.1 Cadmium

The mean Cd levels in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples were 0.136 ± 0.012 , 0.224 ± 0.031 , 0.292 ± 0.052 , 0.206 ± 0.041 , 0.201 ± 0.052 , 0.181 ± 0.034 and $0.110 \pm 0.020 \ \mu\text{g/g}$ respectively as shown in Table 1. The mean Cd level in the meat samples from the domestic animals were statistically significant (p<0.05). Considering the mode or system of rearing of the studied animals, their age and size, the observed variation in the mean Cd levels in their meat samples was therefore expected. The meat samples from the domestic animals had mean Cd levels in the following decreasing order; non-ranched cow meat> non-ranched goat meat > in-door reared pork meat > ranched cow meat > out-door reared chicken meat > ranched goat meat > in-door reared chicken meat samples as shown in Figure 1.



Figure 1. Bar Chart Representation of the Mean Cd Levels in the Meat Samples from the Studied Domestic Animals

From Table 1, it was observed that the non-ranched cow meat, non-ranched goat meat, in-door reared pork meat and ranched cow meat samples had mean Cd levels above the threshold limits. Considering the fact that the meats from these animals serve the animal protein requirement of communities around Gariki market on daily basis, and the fact that cadmium is a very toxic metal even at low concentrations, this observation is therefore of a public health concern, especially at consistent dietary exposure to the metal by the meat consumers. It was equally observed that the mean Cd levels in the meat samples from the out –door reared (non- ranched domestic animals) were consistently higher than the metal's level in the meat samples from the in-door reared (ranched) domestic animals. This observation was in agreement with the findings of Mukesh et al. (2010), who stated that ranched animals usually have a profiled and regulated feeding regime, hence have minimal level of exposure to toxic heavy metals

compared to non –ranched animals. The mean Cd levels in the non-ranched cow meat, non-ranched goat meat, ranched cow meat and in-door reared pork meat samples were at toxic level probably because of the age and size of these animals, anthropogenic pollution that follows their feeding pattern (especially for cow and goat) based on climatic considerations and non-profiling of foods fed to the animals, since the farmer's concern is usually to have his/her animals grown rapidly to maturity to meet economic and sundry needs. Considering the toxicity associated with dietary exposure to Cd even at low concentrations, the toxicity effects that it exerts either acutely or chronically to animals and humans are already well documented (Okeke & Okeke, 2015; Okeke et al., 2016; Ezeh et al., 2018; Gupta et al., 2021).

Okeke and Okeke (2015) had a lower mean Cd levels of 0.053 ± 0.008 and 0.0035 ± 0.012 µg/g respectively for the out-door reared chicken meat and in –door reared chicken meat samples sold within Enugu metropolis than what was reported as mean Cd levels in the studied chicken meat samples sold at Gariki market, Enugu State.. Both research agreed that the meat samples from the in-door reared chickens were less polluted with Cd than the meat samples from the out-door reared chickens.

3.2 Lead

The result of Table 1 shows that the mean Pb levels in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples were 0.272 ± 0.050 , 0.361 ± 0.064 , 0.504 ± 0.082 , 0.371 ± 0.067 , 0.455 ± 0.039 , 0.311 ± 0.052 and 0.176 ± 0.043 µg/g respectively. The meat samples had mean Pb levels in the following decreasing order; non-ranched cow meat > ranched cow meat > in-door reared pork meat > non-ranched goat meat > out-door reared chicken meat > ranched goat meat > in-door reared chicken meat samples as shown in Figure 2.



Figure 2. Bar Chart Representation of the Mean Pb Levels in the Meat Samples from the Studied

Domestic Animals

The mean Pb levels in the meat samples from the domestic animals were statistically significant (p<0.05). Just as observed for the Cd levels in the domestic animal meat samples, the varying mode of rearing the animals, the degree of the consciousness of the farmers in profiling the foods fed to the animals, the levels of contamination with the Pb and duration of exposure could be possible reasons for this significant variation. Of all the studied meat samples, only non-ranched cow meat samples had its mean Pb level above the permissible limits. Although cow meat is a red meat, its availability and consumption rate by people of all ages in Nigerian communities is still very high and therefore, it becomes a food safety risk for consumers to be exposed to Pb at this observed level, especially over a consistent period of time. Okeke and Okeke (2015) reported a lower mean Pb values of 0.101 \pm 0.009 and 0.187 \pm 0.043 µg/g respectively for the in-door reared chicken meat and out-door chicken meat samples raised within Enugu metropolis than what this study got for the metal in the investigated chicken meat samples.

The toxic effects of Pb to humans and animals even at low concentrations are well documented (Okeke et al., 2016; Ezeh et al., 2019; Aniobi et al., 2021; Gupta et al., 2021).

3.3 Copper

The result of Table 1 shows that the mean Cu levels in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples were 3.713 ± 0.171 , 2.344 ± 0.031 , 3.084 ± 0.230 , 5.972 ± 0.108 , 4.131 ± 0.157 , 1.306 ± 0.043 and 1.945 ± 0.027 µg/g respectively. The meat samples had mean Cu levels in the following decreasing order; ranched cow meat > in-door reared pork meat >ranched goat meat > non-ranched goat meat > in-door reared chicken meat > out-door reared chicken meat samples as shown in Figure 3.



Figure 3. Bar Chart Representation of the Mean Cu Levels in the Meat Samples from the Studied

Domestic Animals

The mean Cu levels in the meat samples from the domestic animals were statistically significant, however, were within its recommended threshold limits for a food substance meant for consumption. It was further observed from Table 1 that the mean Cu levels in the meat samples from the ranched or indoor reared domestic animals were consistently higher than it was in the meat samples from the non-ranched or out-door reared domestic animals and this could be due to the more conscious profiling of foods fed to the in-door reared or ranched domestic animals based on quality and knowledge of nutrient feed composition by the rearers. Feedstock, forages or prepared foods fed to the ranched domestic animals are usually done based on their nutrient composition and quality, in order to trigger their rapid growth and wellbeing and hence are therefore richer in mineral trace elements such as Ca, Mg, Na, K, Cu and Zn etc. Bhat et al., (2010) observed that many farmers' non-profiling and less consciousness on the food and feedstock consumed especially by non -ranched domestic animals could be the main reason for the increased level of toxic metals as well as decreased level of trace elements that these animals are exposed to. Although copper is a trace element required by animals and humans for optimum enzyme and biochemical function, at excess levels can exert toxicities to vital organs such as the liver and kidney (Okeke et al., 2016; Ezeh et al., 2019; Aniobi et al., 2019; Okeke et al., 2021). The result of Table 2 shows that S.aureus, Streptococcus spp. and Salmonella enteritidis were isolated

and identified in the meat samples from both the ranched (in-door reared) and non-ranched (out-door reared) domestic animals studied. This is instructive in the sense that the mode or system of rearing of the domestic animals does not guarantee that the meats derived from the animals would be free from pathogenic bacteria contamination. The result of Table 3 shows that the mean S.aureus counts in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, out-door reared chicken meat and in-door reared chicken meat samples were 4.32×10^2 , 2.16 $\times 10^{5}$, 1.73 $\times 10^{8}$, 2.86 $\times 10^{5}$, 2.41 $\times 10^{5}$, 1.22 $\times 10^{5}$ and 1.04 $\times 10^{2}$ Cfu/g respectively. The meat samples had mean *S.aureus* counts in the following decreasing order; non-ranched cow meat > ranched cow meat > in-door reared pork meat > non-ranched goat meat > out-door reared chicken meat > ranched goat meat > in-door reared chicken meat. From result of Table 3 it was observed that the meat samples from the non-ranched (out-door reared) domestic animals were more contaminated with S.aureus than it was obtained for the bacteria in the meat samples from the ranched (in-door reared) domestic animals. S.aureus is an ubiquitous pathogenic bacteria that thrives very well in dirty environment, unclean water and fermented food materials (Okeke et al., 2019; Okeke et al., 2020; Okeke et al., 2021). The mean *S. aureus* counts in the meat samples from the domestic animals were significant. It was equally observed from Table 3 that the non-ranched cow meat, non-ranched goat meat, ranched cow meat, in-door reared pork meat and out-door reared chicken meat samples had mean S.aureus counts above the recommended threshold limits for a consumable food material.

The result of Table 3 shows that the mean *Streptococcus spp.* counts in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, in-door reared chicken meat and out-door reared chicken meat samples were 1.66×10^2 , 1.57×10^4 , 2.64×10^6 ,

 1.01×10^3 , 2.05×10^5 , 1.14×10^2 and 1.08×10^4 Cfu/g respectively. The mean Streptococcus spp. counts of the meat samples from the studied domestic animals were statistically significant. Non-ranched cow meat, ranched cow meat, in-door reared pork meat, non-ranched goat meat and out-door reared chicken meat samples had mean Streptococcus spp. counts above the permissible limits. The meat samples had mean counts of the bacteria in the following decreasing order; non-ranched cow meat > in-door reared pork meat > non-ranched goat meat > out-door reared chicken meat > ranched goat meat > in-door reared chicken meat samples. Streptococcus spp., just as S.aureus is a pathogenic bacteria that is predominantly found in dirty environment, water and contaminated food and have been implicated in food poisoning, food intoxication and epidemic diarrhea of its host (WHO, 2011; Okeke et al., 2021). Salmonella enteritidis as shown in Table 3 had mean counts of 1.88×10^2 , 3.41×10^4 , 3.92×10^6 , 2.51×10^6 , 2. 10^3 , 1.59×10^4 , 0.82×10^2 and 1.35×10^3 Cfu/g in the ranched goat meat, non-ranched goat meat, non-ranched cow meat, ranched cow meat, in-door reared pork meat, in-door reared chicken meat and out-door reared chicken meat samples respectively. The meat samples had mean Salmonella enteritidis counts in the following decreasing order; non-ranched cow meat > non-ranched goat meat > in-door reared pork meat > ranched cow meat > out-door reared chicken meat > ranched goat meat > in-door reared chicken meat samples. The mean Salmonella enteritidis counts in the investigated meat samples were statistically significant. The non-ranched cow meat, non-ranched goat meat, ranched cow meat, in-door reared pork meat and out-door reared chicken meat samples had mean Salmonella entertitidis counts above the recommended threshold limits for a consumable food substance.

4. Conclusion

The three investigated heavy metals (Pb, Cd and Cu) were at detectable levels in the meat samples from the studied domestic animals (goat, cow, chicken and pig), reared to maturity using different systems. With the exception of the mean Cd levels in the non-ranched cow meat, non-ranched goat meat, ranched cow meat and in-door reared pork meat samples and the mean Pb levels in the non-ranched cow meat samples; the mean levels of the investigated metals in the meat samples from the domestic animals were within permissible limits. The result of the study clearly shows that the system upon which a domestic animal is reared to maturity significantly influences its level of exposure to pollutants such as heavy metals. Meat samples from non-ranched animals significantly had higher mean values of the investigated metals than meat samples from the ranched or in-door reared domestic animals. Three disease causing bacteria, namely, S.aureus, Streptococcus spp.and Salmonella enteritidis were isolated and identified in the meat samples from the studied domestic animals. With the inclusion of the ranched cow meat and in-door reared pork meat samples, the meat samples from the studied domestic animals reared out-door or through non - ranching system, had mean counts of the three isolated pathogenic bacteria above the permissible limits for a consumable food substance. The implication of this finding is that poor cooking of the meat samples from these domestic animals before consumption could increase the consumers' dietary exposure to these pathogenic bacteria and the health implications can

be huge. It is very important that regulations be put in place, mandating farmers to ranch or rear in-door domestic animals under their care, which are meant for human consumption, in order to reduce the health risk of undue dietary exposure to food contaminants such as heavy metals and pathogenic bacteria by the meat consumers.

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