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Assessment of Tetracycline, Lead and Cadmium Residues in Frozen Chicken Vended in Lagos and Ibadan, Nigeria

Adetunji Victoria Olusola, Belleh Efie Diana and Odetokun Ismail Ayoade
Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: This study determined the levels of tetracycline and heavy metals (lead and cadmium) levels in frozen chicken. One hundred frozen chicken muscle samples were sourced from major markets in Lagos and Ibadan (fifty samples each). The samples were analyzed using High Power Liquid Chromatography (HPLC) for tetracycline residue determination while Atomic Absorption Spectroscopy (AAS) was used to determine the levels of lead and cadmium residues in the samples. Mean concentrations of tetracycline residue levels in the frozen chicken sampled ranged from 1.1589-1.0463ppm which is higher than the maximum residue limit set by international food safety agencies. Pb contents were higher in chicken muscles sampled from markets in Ibadan (0.0227±0.0069 μg dL^{-1}) than Lagos (0.0207±0.0082 μg dL^{-1}), while Cd levels were 0.0013 μg dL^{-1} higher than in the Lagos samples (0.0065±0.0026 μg dL^{-1}). These values were within maximum residue limits. There were no significant differences ($p<0.05$) in levels of tetracycline, lead and cadmium levels from the two market locations (Lagos and Ibadan) and parts (wings and thigh muscles). However, significant differences occurred in tetracycline and Pb levels in frozen chicken sourced from Cotonou. Though not significant, tetracycline contents in the thigh muscles of the frozen chicken samples was higher than that of the wings muscles and this was attributed to the site of administration of antibiotic injection and failure to observe the pre-slaughter withdrawal period by the farmers. This study is of public health importance as the presence of these residues above the maximum residue limit in frozen chicken predisposes consumers to drug resistance, allergic reactions and poisoning as a result of toxicity.

Key words: Tetracycline, lead, cadmium, frozen chicken

INTRODUCTION

Chicken meat is a very important and good source of essential amino acids, vitamins and minerals for human consumption. Antibiotics are widely used in veterinary medicine and subsequently drug residues may persist in foods derived from animals, which may pose an adverse health effect for the consumer (Pikkemaat, 2009). The presence of antibiotic residues in the foodstuffs of animal origin is one of the most important indexes for their safety (Pavlov *et al*., 2008). Use of antibiotics in food animals has led to concerns regarding residues which might be present in food, as well as potential increases in microbial resistance (Schneider *et al*., 2007) in both food animals and humans. Tetracyclines, over the years, have remained one of the most commonly used groups of antibiotics in poultry production.

Tetracyclines (TCs) produced by *Streptomyces* spp. are broad-spectrum antibiotics active against most Gram-positive and Gram-negative bacteria (Cherlet *et al*., 2003). The tetracycline group includes tetracycline, chlortetracycline (CTC), oxytetracycline (OTC), methacycline, demeclocycline, doxycycline and minocycline. The entire group are derivatives of the polycyclic compound naphthacenecarboxamide. They are used in the prevention and treatment of disease conditions in livestock and poultry industry. Sometimes, they are utilized as growth promoters. In Nigeria like most developing countries, antibiotics are used in animals indiscriminately for the prevention and treatment of bacterial infection (Dina and Arowolo, 1991; Kabir *et al*., 2004) and withdrawal periods in slaughtered poultry are not observed thereby resulting in residue deposition in meat tissues and other animal derived products. Tetracycline levels exceeding the tolerance limits could be of toxicological, microbiological or immunological concern (Booth, 1978; Shahid *et al*., 2007). Various human health conditions have been associated with consumption of food contaminated with tetracycline residues especially at sub-chronic levels. These include: gastrointestinal disturbances, teratogenic risk to fetus, allergic reactions, bone and teething problems and the emergence of resistance bacteria strains in humans and animals (Woodward, 1991; Walton *et al*., 1994; Czeizel *et al*., 1998; Schenk and Collery, 1998; Van de Bogaard and Stobberingh, 2000; Larkin *et al*., 2004; Mesgari Abasi *et al*., 2009).

Corresponding Author: Adetunji Victoria Olusola, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria Tel: +234 (0)704 097 9193

Heavy metals from man-made pollution sources such as industrial waste are continuously released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystems is growing (Iwegbue *et al*., 2008). The presence of heavy metal residues in meat and meat products also constitute a serious public threat because of their toxicity, bioaccumulation and biomagnifications along the food chain (Demirezen and Uruc, 2006). This has led to an increasing concern as regards their consumption in foods (Akan *et al*., 2010).

Lead and cadmium are environmental contaminants which are present in almost all living organisms and are non-essential for plants, animals and humans. Lead belongs to heavy metal group characterized by high accumulation in the organs and tissues of the animals and people can be exposed through inhalation or orally (Cunningham and Saigo, 1997; Zraly *et al*., 2008). Though it has been discovered that lead levels among human populations in industrial countries have a decreasing trend (Cerna *et al*., 2001), a study to determine their presence in chicken meat sold locally in the Nigeria markets is essential due to the toxic effect of this metal on the central and peripheral nervous systems, damage of tubular cells in kidneys, immune system and carcinogenesis (Flegal and Smith, 1995; Lee *et al*., 2001; Lurie *et al*., 2006).

Cadmium is a highly reactive and toxic element, which is sparsely distributed in most agricultural ecosystems (Herzig *et al*., 2007). Exposure to cadmium for the nonsmoking population occurs mainly via the food (Rahimi and Rokni, 2008). Meat and offal are the most important sources of cadmium after vegetables (Coni *et al*., 1992) and is poorly excreted when consumed in foods by animals or humans (Underwood and Suttle, 1999). Toxic effects of cadmium are kidney dysfunction; hypertension, hepatic injury and lung damage arise as a result of the toxic effects of cadmium (John and Jeanne, 1994). Reddy and Yellamma (1996) also, reported that some cadmium compounds such as Cadmium chloride at teratogenic dose induced significant alterations in the detoxification enzymes in the liver and kidney.

Efficient methods are required for monitoring residue levels to ensure safety of the food supply (Schneider *et al*., 2007). In Nigeria, no reports on lead and cadmium residues exist on frozen chicken meats sold in the markets while several research has been conducted to assay antibiotics administration and deposit in meats and various food animal products (Kabir *et al*., 2004; Dipeolu and Alonge, 2002). These studies were either carried out using microbiological screening methods (having low specificity and sensitivity) which do not classify and quantify antibiotics. Investigation using the High Performance Liquid Chromatography (HPLC) procedures for oxytetraxycline residues in cattle tissues has been reported (Olatoye and Ehinmowo, 2010). Published data of residues of frozen chicken muscle in circulation and on sale in the Nigerian market is scares. We therefore, assayed the muscle tissue of thighs and wings of frozen chicken, which are the most frequent parts of frozen chicken meat sold for human consumption, for the presence of tetracycline, lead and cadmium residues.

MATERIALS AND METHODS

Study area: The 2 study areas are Ibadan, the capital of Oyo state and Lagos state. Ibadan is located on latitude 7°26'N 3°54'E while Lagos is located on 6°27'N 3°24'E both in Southwestern Nigeria. A preliminary survey was done to identify sources and points of sale for frozen chicken. Samples were collected from markets in Lagos (Agboju market, Igando market and 23rd market) and Ibadan (Bodija and Ojoo markets) and a major frozen chicken outlet in Ibadan.

Sampling: In this study, one hundred frozen chicken samples were sourced across the major markets. Fifty samples each were collected at random from Lagos and Ibadan. Replicate samples of wing and thigh muscles were collected aseptically from each retailer.

Antibiotic residue analysis: High performance liquid chromatography standard methods were used for antibiotic residue analysis.

Determination of tetracycline

Extraction: 2.5 g of the blended samples in solution was weighed into a set of centrifuge tubes. 1.8 mL of SN HCl was dispensed and mixed properly. 20 mL of a mixture of phosphate buffer solutions + sodium hydroxide+sodium metabisulphite solution in the ratio of 2:1:4, respectively into the samples and covered properly, shaken for 15 min and then centrifuged for 20 min at 4500 rpm. The supernatant was collected into plastic vinyls for the determination on HPLC (Wang *et al*., 2008).

Extraction: Weigh 2.5 g of the blended sample solutions into a set of centrifuge tubes. Dispense 1.6 mL of 5N HCL and mix properly. Dispense 2.0 mL of a mixture of phosphate buffer solutions plus sodium hydroxide plus sodium metabisulphate solution in the ratio of 2:1:4, respectively into the samples and cover properly. Shake for 15 min. Transfer to a centrifuge machine and centrifuge for 20 min at 4500 rpm. Sample the supernatant in a set of sterile vials (plastic vials) for determination of the analyte on HPLC.

Heavy metal analysis: Atomic absorption spectrophotometer (AAS Perkin Elmer AAnalyst, USA) was used for the determination lead and cadmium in chicken tissue. The samples were freeze dried for 24 h after which it was crushed in a mortar and pestle to homogenize it, variable weight of each sample was then taken between 0.1-0.5 g into 75.0 mL glass digestion tubes. The combined stock standard (Pb and Cd, 1000 ppm each) was prepared from the reference standards and stored in the refrigerator at 7-10°C until use. The certified sensitivity check of standard solutions for each element was used in optimizing the efficiency of the AAS. The working standards were first determined to create the standard curve, this was followed by the measurement of the unknown analytes. The atomic absorption spectrophotometer was adjusted to specific wavelength corresponding to each of the metals to be measured. Measurements were done using the hollow cathode lamps at the wavelength of 283.3 and 228.8 for Pb and Cd, respectively. Concentrations of the metals were determined from the standard curve and reported in μg dL^{-1}:

$$\mu g\ dL^{-1} \times 100 = mg\ kg^{-1},\ mg\ kg^{-1} = ppm$$

Data analysis: Means of replicate samples were analyzed using t-test on SPSS 17. Significant values were noted at 95% confidence limit. Data were also presented in charts using Microsoft excel 2007.

RESULTS

Pb contents were higher in chicken meat sampled from markets in Ibadan (0.0227±0.0069 μg dL^{-1}) than Lagos (0.0207±0.0082 μg dL^{-1}) (Table 1). Also, Cd content in the Ibadan chicken samples was 0.0013 μg dL^{-1} higher than the Lagos samples (0.0065±0.0026 μg dL^{-1}). The differences noted in the heavy metal residue levels in the two regions were not significant ($p<0.05$). However, chicken samples from Lagos had higher antibiotics (tetracycline) residues (1.1589±0.4677 ppm) than noted in the Ibadan samples (1.0463±0.3877 ppm) (Table 1).

Tracing the chicken samples sold in Lagos and Ibadan to their sources (Fig. 1), the mean Pb contents assayed in the frozen chicken samples sourced from Cotonou (0.0222 μg dL^{-1}) higher than the 0.0215 and 0.0193 μg dL^{-1} determined in the samples sourced from a major poultry outlet in Ibadan and Seme, respectively. Furthermore, average Cd residue levels was highest from the Ibadan source (0.0089±0.0042 μg dL^{-1}) but not significantly different from chicken imported from Cotonou (0.0068±0.0036 μg dL^{-1}) and Seme

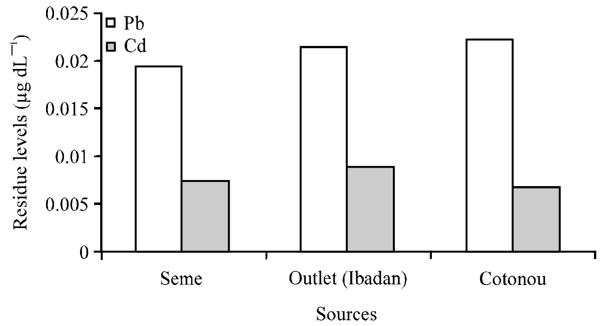

Fig. 1: Heavy metal residue levels assayed in the chicken samples based on sources

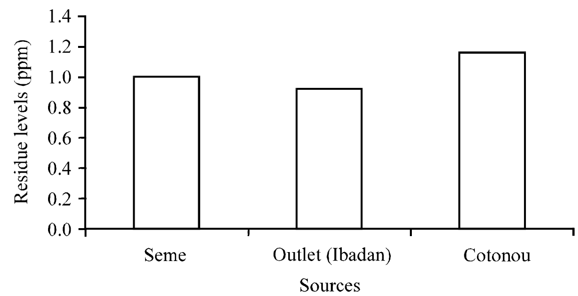

Fig. 2: Tetracycline residue levels assayed in the chicken samples based on sources

Table 1: Mean contents of lead, cadmium and tetracycline in wings and thigh muscles based on markets in two states

Residues	Markets	Mean±SD
Pb (μg dL^{-1})	Lagos	0.0207±0.0082
	Ibadan	0.0227±0.0069
Cd (μg dL^{-1})	Lagos	0.0065±0.0026
	Ibadan	0.0078±0.0042
Tetracycline (ppm)	Lagos	1.1589±0.4677
	Ibadan	1.0463±0.3877

Means with similar superscripts are not significant at $p<0.05$

(0.0073±0.0021 μg dL^{-1}) while Tetracycline levels (Fig. 2) were significantly higher ($p<0.05$) in the sample imported into the country (1.0041±0.4518-1.1631±0.4348 ppm) than produced at the major poultry production plant in Ibadan (0.9114±0.3250 ppm).

The concentration of heavy metals (Pb and Cd) residues vary in the wing and thigh muscles of the chicken samples (Table 2). Pb levels were significantly higher at $p<0.05$ in the wings (0.0242±0.0085 μg dL^{-1}) than the thigh (0.0205±0.0069 μg dL^{-1}) of the chicken. Though, Cd residue level was higher in the thigh muscles (0.0074±0.0037 μg dL^{-1}) than in the wings (0.0067±0.0033 μg dL^{-1}), this was not significantly different. Obviously, the concentration of Tetracycline residue (Table 2) is significantly higher

Table 2: Mean lead, cadmium and tetracycline contents of wing and thigh muscles of frozen chicken

Residue	Parts	Mean±SD
Pb (μg dL^{-1}) (MRL:10 μg dL^{-1})	Wings	0.0242±0.0085[b]
	Thighs	0.0205±0.0069[a]
Cd (μg dL^{-1}) (MRL:50 μg dL^{-1})	Wings	0.0067±0.0033[a]
	Thighs	0.0074±0.0037[a]
Tetracycline (ppm) (MRL:0.2 ppm)	Wings	1.0083±0.3165[a]
	Thighs	1.1454±0.4703[a]

Means with similar superscripts are not significant at $p<0.05$, MRL: Maximum residue limits

($p<0.05$) in the thigh muscles (1.1454±0.4703 ppm) of the chicken sampled than in the wings (1.0083±0.3165 ppm).

DISCUSSION

The contents of tetracycline residues which was significantly higher in samples sourced from Cotonou and in the thigh muscles analyzed could be attributed to the injection site (since the thigh is the major site of injection). A much lower concentration of tetraxycline was reported in thigh muscles by Dipeolu and Dada (2005) in frozen chicken. This indicates an increase over a 7 year period due to none adherence to withdrawal periods prior to slaughter of the chicken by poultry meat producers possibly leading to bioaccumulation of tetracycline in the thigh muscles. This can predispose to development of drug resistant bacteria and allergic reactions in consumers. The levels of contamination of tetracycline noted in this study were higher than the stipulated Maximum Residue Limit of 200 μg kg^{-1} (0.2 ppm). Similar reports of higher tetracycline levels have been discovered in edible tissues of slaughtered animal in Nairobi slaughterhouses in Kenya (Muriuki *et al*., 2001). The results in this study were also higher than tetracycline residues reported in previous works by Kabir *et al.* (2004) in poultry and in cattle by Oboegbulem and Fedelis (1996) and Dipeolu and Alonge (2002). However, our findings were comparable with an earlier report on antibiotics residues in cattle tissues by Olatoye and Ehinmowo (2010). This is an indication that consumers are potentially at risk. Regulatory authorities should constantly conduct surveillance to monitor the antibiotic levels in order to safeguard human health (Shahid *et al*., 2007).

The frozen chicken sold at the various markets in both Lagos and Ibadan contains both heavy metal residues in detectable levels. Lead levels in frozen chickens sold in both cities did not exceeded the international maximum residue limit of 0.1 ppm (10.0 μg dL^{-1}) stipulated by international food standard agencies (Australia Government Comlaw, 2011). This finding is in agreement with reports by Akan *et al.* (2010) in Northeastern Nigeria who recorded detectable values lesser then the stipulated residue limit in poultry meat. In contracts to values reported in this study, Iwegbue *et al.* (2008) reported high Pb residue levels in some chicken meat sampled in some parts of southern Nigeria. In the same trend, Trampel *et al.* (2003) and Mariam *et al.* (2004) in their studies also recorded Pb residue values in chicken muscle greater than the maximum permissible limits. These variations in report may be due to differences in sample source, time of sampling. and industrial activities at the time of sampling. The insignificant differences noted between the frozen chicken meats sold at the two cities (Lagos and Ibadan) and the sourced from Seme, a major outlet in Ibadan and Cotonou suggest that the trend at which frozen chicken meats were being contaminated was the same. Pb is widespread in the environment and its presence is mostly airborne facilitated by industrial emissions and combustion of fuel containing lead additives. Significantly higher levels of Pb residues present in the wings than the thigh of the frozen chickens could have arisen as a result of their feeding habit and behavior. High levels of metals in poultry products also emanate from contamination of feeds and water sources (Iwegbue *et al*., 2008). A very high level of Pb has been discovered in feeds fed to chicken (Okoye *et al*., 2011). Other sources are untreated waste effluents of industry, which find their way to irrigation channels and hence pollute the fodder through soil (Mariam *et al*., 2004).

Cadmium residue levels which were not significantly different in frozen chicken meats sold in Lagos and Ibadan, the sources (Seme, major outlet in Ibadan and Cotonou) as well as the parts (wings and thigh) as noticed in this study were below the set maximum residue limits (0.5ppm or 50.0 μg dL^{-1}) (FAO/WHO, 2000; 2001). The results obtained for Cd in this study is far below that reported by Mariam *et al.* (2004) (0.31 mg kg^{-1}). Gonzalez-Weller *et al.* (2006) and Iwegbue *et al.* (2008) reported 1.68 μg kg^{-1} and 0.01–5.68 mg kg^{-1}, respectively which were higher than the maximum residue limits. Since toxic Cd residues were detected in lower levels in the frozen chicken meats, regular surveillance and monitoring should be employed as its bioaccumulation and biomagnifications could lead to serious human health problems in consumers.

CONCLUSION AND RECOMMENDATIONS

Food is the main source of consumers to antibiotic and toxic heavy metals residue, who are not occupationally exposed. Since some of the samples tested exceeded international limits for tetracycline and Pb, consumers of such products are predisposed to drug resistance and poisoning or toxicity from Pb contaminants. Problems of bioaccumulation and biomagnifications could also arise from continuous consumption of the frozen chickens with levels below maximum limits if not excreted from the system. Veterinary officers should ensure the judicious use of antibiotics in combating bacteria infections in poultry with under/over-dosage put into consideration. Furthermore, the observance of the pre-slaughter withdrawal periods after antibiotic usage should be emphasized. The Government through the National Agency for Food, Drug Administration and Control (NAFDAC), Consumer Protection Council (CPC) and Standard Organization of Nigeria (SON) should employ veterinarians that would set standards for antibiotics, heavy metals and other food additives as there exist no standards for the country. They should also ensure continuous monitoring of residues in food animal products produced locally or imported and set mechanisms for the constant detoxification or chelation of residues in the environment to reduce public health risks.

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