



## Determination of Tetracycline Residues in Broilers Reared in Makurdi Metropolis

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### SUMMARY

Tetracycline residues in broiler tissues were assayed using a microbiological assay method. A total of four hundred samples representing one hundred samples of liver, kidney, lungs and breast muscles each, were collected from five different farms within Makurdi Metropolis. The analysis showed a total of 70 % residue incidence with the liver having 60 %, Kidney 31 %, lungs 14 % and muscles 5 % of the total. The highest concentration, of 6 µg/kg, was seen in the liver from farm 1, which had an incidence of 50 %, with the liver contributing 90 % of the incidence. Farm 2 had an incidence of 75 %, in which the liver contributed with 50 % of the cases. Farm 3 had a 100 % incidence, with the liver contributing with 75 % of the cases. Farm 4 had 75 % incidence, with the liver involved in all cases. Farm 5 had a 50 % incidence, all from the liver and kidney. Despite the higher incidence of 70 %, all the values were significantly ( $p < 0.01$ ), lower than the recommended Maximum Residue Limits (MRL) or Tolerance of 600, 300 and 200 µg/kg for the liver, kidney and muscle respectively. The values were also in accordance within the Acceptable Daily Intake (ADI) of 0-30 µg/kg, recommended by the Codex Alimentarius. This may be an indication of the rational use of antibiotics in good agricultural management in the selected farms in Makurdi Metropolis. Significantly, it shows that this method can be used mathematically to quantify drug residues in lower income areas.

**Key words:** Tetracyclines, Residues, Broilers, Makurdi, Determination.

### INTRODUCTION

The demand for good quality food is universal and has driven human activities such as gathering of edible plants, haunting, domestication etc. in early civilization up to the present day

agriculture, science and technology. Good quality food and its availability is presently being referred to as food security. Food security according to the Food and Agricultural Organization (FAO) of the United Nations,

ensures a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO, 2003).

One key component of good quality food is animal protein which usually comes from various sources of livestock such as cattle, pigs, poultry, sheep, goats etc. (Moller, 2015; Oyedipe, 2013). Among all these sources of animal protein, poultry is gaining popularity, because it's a major source of white meat (Hakem *et al.*, 2013), which is widely accepted by many people as opposed to red meat because of its high cholesterol content and other health related issues (Hakemet *al.*, 2013). Another factor that contributes to the acceptability of poultry meat is its acceptability by the major religions (Christianity, Islam and African Traditional), which do not prohibit the consumption or rearing of poultry. In addition to the above, the domestic chicken, especially the broiler, grows very fast and can be reared for table between 35 -70 days (Jordan and Randell, 1982). It also requires a little space compared to other livestock, this has encouraged backyard farming (Abdul, 2014). This is so because people can easily convert their car parks or kitchen into poultry houses. The bulk of poultry farming in Nigeria is through this means (Jajere *et al.*, 2015; Abdul, 2014). This is also true in Makurdi metropolis.

Good agricultural management practice (GAMP) in animal husbandry is needed to produce safe and high quality meat, milk, eggs, and other animal by-products for human consumption (Mamman, 2013). The GAMP involves giving appropriate and timely treatment to sick animals, when necessary, by using drugs recommended or

prescribed by qualified veterinarians (WHO, 1987).

In order to enhance productivity and control of diseases a variety of veterinary drugs are administered for therapy or prevention (Jajere *et al.*, 2015; Donoghue *et al.*, 2013). Veterinary drugs are pharmacologically and biologically active chemical agents, specifically designed for diagnosis, treatment and prevention of animal diseases, correction or restoration of organic function or control of vermin, insect or pest (WHO, 1985).

In poultry practice, antibiotics are among the most widely used veterinary drugs (Jajere *et al.*, 2015; Donoghue *et al.*, 2013; Simon and Baht 2006). Antibiotics have been used since the 1950s in poultry for combating bacterial infections (Donoghue *et al.*, 2013; Olatayo and Ehinmowo 2007; Simon and Baht, 2006). In low doses, they are used to enhance feed efficiency, achieve prophylaxis or as probiotics (Nisha, 2008), growth promoters (Al-Barhy 2013, Alaboudi *et al.*, 2013), for egg production (Kabir *et al.*, 2004). Among the antibiotics, tetracyclines are the most widely used antibiotics in poultry (Al-Bahry, *et al.*, 2013; Adetunji, *et al.*, 2013; Alaboudi *et al.*, 2013; Donoghue *et al.*, 2013).

In most countries of the world, only those drugs that are officially registered and approved for food producing animals are used on them (Moller, 2015; Thomas, 2004; Anonymous, 2004). Usually, drug withdrawal periods are prescribed by the manufacturers and if they are observed, during this time period the quantity of drug/residue in food stuffs of animal origin (meat, milk, egg), should be reduced to a level that does not threaten the consumers' health (Codex Alimentarius, 2015; Anonymous, 2004).

This is based on rational use of drugs, observance of withdrawal periods, ADI, MRL and safe concentration (Codex Alimentarius, 2015; 2004; Junaidu, 2014; Mamman, 2013).

Therefore this study was carried out to assay the tetracycline residues in the liver, kidney, lungs and breast muscles of broilers reared in Makurdi metropolis, using the microbiological assay or Agar Diffusion Test. The aim was to establish if the residues were above the MRL set out by regulatory agencies, and also to use this method, which is usually used for screening purposes to quantify the amount of residues found in these tissues.

## MATERIALS AND METHODS

Samples from twenty birds were collected from farms 1,2,3,4 and 5 at the point of slaughter, where the birds were being processed. Thus, 2-3g of the liver, kidney, lungs and breast muscle were collected with a sharp knife from each bird and kept in a clean sample bottle. Each organ was placed in a separate bottle well labeled with the identity of the bird and the farm. The samples were immediately transported to the Ahmadu Ali Centre for Public Health and Comparative Medicine, where they were refrigerated at 4°C until analyzed. The agar diffusion test (four plate method) was carried out as described by Ibrahim *et al.*, (2015). Briefly, the four plate method employed different pH and temperatures in which some antibiotic families responds better to specific microorganisms than others (Hakem *et al.* 2013).

The manufacturer instruction (Tulip Diagnostics (P) Ltd, India) was followed in preparing the media and the pH of the media was adjusted to 6.0 with a phosphate buffer. The agar was

allowed to cool and 20 ml was poured out into the Petri dishes and was allowed to settle at room temperature. From the stock culture, a suspension of *Bacillus subtilis* ATCC 3366 was prepared and standardized using McFarland standard to obtain a solution of  $10^8$  cell / ml. A drop of 0.1ml of the suspension was uniformly spread on the nutrient agar surface using swap stick. The inoculum was allowed to settle for 15 minutes. A meat puncture was used to cut 2mm of tissue sample which was placed on the seeded agar surface in the Petri dish with the help of a sterile forceps. This was done in triplicate on each Petri dish.

The agar diffusion procedure was used to access the inhibitory activity of the antibiotic tetracycline. Two (2) mm Whatman's filter papers were impregnated with tetracycline (0.4, 0.3, 0.2, 0.1, 0.05, 0.025, and 0.0125) mg/ml. The filter papers were placed on seeded agar plates and both were incubated at 37°C overnight. A meter rule was used to measure the zones of inhibition around the tissues. The average of the zones of inhibition for the tissues and tetracycline were determined and a graph of zones of inhibition against concentration was drawn and the curve determined. This was used to determine the amount of tetracycline in the tissues by comparing the zones of inhibition of the various tissues to those of the tetracycline.

## DATA ANALYSIS

The data obtained was presented as means  $\pm$  standard error of mean (SEM) in tables, graph and percentages. The data was analyzed using student  $t^2$  test to compare the results. Significance was accepted at  $p < 0.05$ .

**RESULTS**

Out of the 100 birds, liver, kidney, lungs and muscle were collected from each bird making a total of 400 samples. The total incidence of tissue residues in the five farms was 70 %. The breakdown based on organs and not number of birds showed that the Liver has the highest incidence (60 %), followed by the Kidney (31%), Lungs (14 %) and muscle (2 %) (Table 1).

**TABLE I: The tetracycline residues in the various tissues of the birds sampled.**

s/n	TISSUE	POSITIVE CASES	%	% +VE CASES
1	Total residue	70	70	100
2	Liver	60	60	85.71
3	Kidney	31	31	44.29
4	Lungs	14	14	20.0
5	Muscle	2	2	2.86

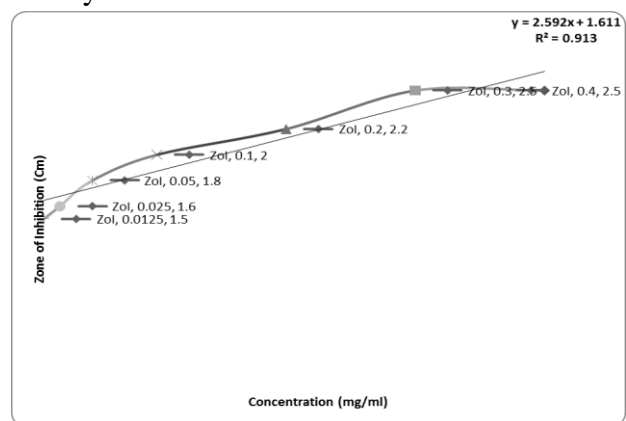
The result of the standard tetracycline in which the inhibition zones from the tissues were compared to establish their quantities in mg is presented in Table 2 below.

The results of analysis of the various tissues residue in the five farms are presented in tables 3-7.

**TABLE II: Inhibition Zones Of Tetracycline (Standard)**

CONCENTRATION (mg)	INHIBITION ZONE (mean±SEM, cm)
0.4	2.5 ± 0.00
0.3	2.5 ± 0.01
0.2	2.2 ± 0.01
0.1	2.0 ± 0.01
0.05	1.8 ± 0.02
0.025	1.6 ± 0.00
0.0125	1.5 ± 0.01

The result from farm 1 is presented in Table 3. There were a total of ten (10) positive cases representing 50%. The highest level of tetracycline residue was found in the liver, while the lowest level was found in the lungs. The result from farm 2 is depicted in Table 4. The total number of positive cases was 15, representing 75 %. The liver only had 10 cases (50 %), the lungs and the kidney had 20 %, respectively 5 %. The highest residue of 3.13µg/kg was found in the liver, while the lowest residue was observed in the lungs. The result from farm 3 is presented in Table 5. It showed that the total residue was 100 %, with liver alone having 50 %. The kidney had 20 %, while the lungs had 5 %. The highest residue was also found in the liver and the lowest in the kidney. The result from farm 4 is presented in Table 6. The total residues was 75 %, with the liver alone having 30 %. Liver and lungs had 20 %, while liver and kidney had 25 %. The highest residue was found in the liver, while the lowest was found in the lungs. The result from farm 5 is presented in Table 7. It revealed a total residue of 75 %, with the entire incidence in liver and kidney.



**Figure 1: Graph of inhibitions of tetracycline (standards) against concentration**

The highest residue was found in the liver while the lowest in the kidney.

**TABLE III: Result of Residue Incidence from Farm 1**

Organs	Incidence	Percentage
Total Positive	10	50
Liver only	1	5
Kidney only	0	0
Muscles only	0	0
Lungs only	0	0
All organs (same bird)	0	0
Liver/kidney	2	10
Liver/Lungs	2	10
Liver/Kidney/Lungs	3	15
Liver/Kidney/muscles	1	5
Lungs/Muscles	1	5
Highest residue (liver)	6.0 µg/kg	
Lowest residue (lungs)-	2.0 µg/kg	

**TABLE IV: Result of Residue Incidence from Farm 2**

Organs	Incidence	Percentage
Total Positive	15	75
Liver only	10	50
Kidney	1	5
Muscles	0	0
Lungs	4	20
Highest residue (liver)	4.13 µg/kg	
Lowest residue (lungs)	2.46 µg/kg	

**TABLE V: Result of Residue Incidence from Farm 3**

Organs	Incidence	Percentage
Total Positive	20	100
Liver only	10	50
Kidney only	4	20
Lungs only	1	5
Liver/Kidney	5	25
Highest residue (liver)	4.02 µg/kg	
Lowest residue (lungs)	2.13 µg/kg	

**DISCUSSION**

The result of our earlier study indicated that the poultry business is on the increase in Makurdi

metropolis (Bosha *et al.*, 2019). Despite the increase in poultry activities in Makurdi metropolis, the demand for poultry products (meat and eggs) in Makurdi cannot be realized by the local farmers. A sizeable part of poultry products is provided by neighboring states of Nasarawa, Plateau, Enugu and Abuja. Since a

**TABLE VI: Result of Residue Incidence from Farm 4**

Organs	Incidence	Percentage
Total Positive	15	75
Liver only	6	30
Kidney only	0	0
Lungs only	0	0
Liver/Lungs	4	20
Liver/Kidney	5	25
Highest residue (liver)	4.13 µg/kg	
Lowest residue (lungs)	2.13 µg/kg	

sizeable part of the poultry products comes from other states, we decided to evaluate the antibiotic residue level in the tissues of those birds that are reared in Makurdi metropolis, as a preliminary step in launching a full screening of the birds marketed and consumed in Makurdi metropolis.

The choice of broilers that are reared in Makurdi metropolis was informed on the fact that it is possible to trace back and forward any findings easily at the end of the study. This is so because the researchers know the owners of these farms. Some are colleagues of the researchers and they will be more open to them and they are also in the picture of what the researchers are doing so there is more confidence in each other. So, the issue of suspicion that usually exists between

researchers and farmers with regards to sample collection was eliminated. Broilers were chosen

**TABLE VII: Result of Residue Incidence from Farm 5**

Organs	Incidence	Percentage
Total Positive	10	50
Liver only	0	0
Kidney only	0	0
Lungs only	0	0
Liver/Kidney	0	50
Highest residue (liver)	10	
Lowest residue (lungs)	3.68 µg/kg	
Lowest residue (kidney)	2.38 µg/kg	

because of their short generation time 35-75 days (Jordan and Randell, 1982). They are always slaughtered en-mass especially during festivities. This enables us to collect large samples within a short period of time and reduces the period in which the samples were stored.

Antibiotics are the most widely used veterinary drugs in poultry (Jajere *et al.*, 2015; Simon and Baxter, 2006). They are used for various purposes, including combating bacterial infections (Olatayo and Ehinnowo, 2009; Abdul, 2014), and in low doses to aid feed efficiency and prophylaxis and probiotics (Nisha, 2008, Kabir *et al.*, 2004), growth promoters (Al-Bahry *et al.*, 2013; Alaboudi *et al.*, 2013; Jajere *et al.*, 2015), enhance egg production (Abdul, 2014; Kabir *et al.*, 2004).

Tetracycline was also chosen as an antibiotic to assess its residue because it is one of the most common antibiotics used in poultry (Al-Bahry *et al.*, 2013; Adetunji *et al.*, 2013; Alaboudi *et al.*, 2013). Also, the Agar Diffusion Test or Four Plate method used in this study, favor's Tetracycline because the microorganism used (*Bacillus subtilis*, ATCC 3366) is sensitive to it at a particular temperature and pH (Ibrahim *et al.*, 2015; Hakem *et al.* 2013). All these factors

can easily be manipulated in a low income laboratory such as ours and elsewhere. This contributed to the choice and adaptation of this method for the study. In addition to the benefits of antibiotics generally in animal production, tetracycline has been reported to be relatively safer than other antibiotics like penicillins and aminoglycosides (Aliyu, 2007). They have anti-inflammatory (Al-Bahry *et al.*, 2013) and antitumor properties (Wang-Gillam *et al.*, 2007; Fuco, 2012). This may also be a contributory factor to the popularity of tetracyclines, even though in the present situation, most antibiotics meant for poultry use, comes in a combination of one or more antibiotics. For example, antibiotics like Neoceryl or NCO, which is a combination of Neomycin, Colistin and Oxytetracycline; Keproceryl is a combination of Colistin, Oxytetracycline, Erythromycin and Streptomycin; Neo-Dox 2020 is a combination of Neomycin and Doxycycline; Doxy-Gen is a combination of Doxycycline and Gentamycin while Tylo-Dox extra is a combination of Tylosin and Doxycycline. In most, if not all, of these combinations of antibiotics, tetracycline is common (personal observation). This still underscore its popularity in poultry (Adetunji *et al.*, 2013; Alaboudi *et al.*, 2013).

The fact that antibiotics in general and tetracycline in particular are important in poultry production, they are not without some negative effects, especially when they are not properly used. The most common problems associated with the overuse of antibiotics include the effect

on the normal flora of the birds, which affects the metabolism of nutrients and non-nutrients substances (WHO,1987). This can also result to antimicrobial resistance (Al-Bahry *et al.*, 2013; Alaboudi *et al* 2013; Jajere *et al.*, 2015). The spread of resistant microorganisms to the environment and other animal species is of serious public health importance (WHO, 1987; Berry, 2016). Tetracyclines are known to affect the teeth and bones development in young children (Aliu, 2007; Karimi *et al.*, 2006, Al-Bahry *et al.*, 2013).

Because of the importance of antibiotics in poultry production, as well as their undesirable effects, regulatory agencies have set out tolerance or maximum residue levels, that are not supposed to be exceeded in all tissues of animal origin for consumption, including poultry products (meat and eggs).For tetracycline it is 600 µg/kg for liver, 300 µg/kg for kidney and 100 µg/kg for muscle and fats (JECFA, 2006; Codex Alimentarius, 2015).

There are various methods that are used in screening these antibiotics. Some of the most common ones include microbial assays. Thin-layer chromatography (TLC), High performance liquid chromatography (HPLC), Gas-Chromatography-mass spectrometry (GC-MS) as well as immunoassays (Ibrahim *et al.*, 2015; Khan, 2010).All the various methods have their merits and demerits. In the present study we used microbial assays, as described by Ibrahim *et al.*(2015) and Hakeem *et al.* (2013).The microbial assay rely on the residues of the antibiotics to inhibit the growth of the test organism, and the pH to promote or inhibit the activities of some antibiotics. Tetracycline activities increase in acidic pH (Ibrahim *et al*, 2015; Hakem *et al.*, 2013). It is generally used

for screening purposes, where large samples are involved (Ibrahim *et al.*, 2015; Hakem *et al.*, 2013; Kabir *et al.*, 2004). Other merits of the method include the fact that it's inexpensive and simple and can reveal some information on the nature or type of residue (Ibrahim *et al.*, 2015, Hakem *et al.*, 2013). In our study, we also used an equation ( $Y = 2.592x + 1.611$ ) to quantify the tetracycline in different tissues. This is an improvement over the previous practice, which hitherto shows only the presence or absence of antibiotics but the actual quantity can be assayed using the equation. Its major demerit is low specificity. The other methods have high specificity but are expensive (Hakem *et al.*, 2013; Ibrahim *et al.*, 2015). In the present study, tetracycline residue was found to be 70 %, mean while other researchers have reported various percentages in different countries. Hakem *et al.* (2013) reported 85.52 % in Algeria, Abiola *et al.* (2005) reported 69.7 % for Senegal and Kabir *et al.* (2004) reported 4.8% in Nigeria, while Olatoye *et al.* (2013) reported 51.1% in Nigeria, in different types of birds. The variation in the amount of antibiotics in poultry tissues can be explained by many factors. The behavior of the producers in a particular region and their personal experiences with diseases and drugs can influence their use of drugs (Hakem *et al.*, 2013).The type of birds kept also influence the frequency of use of antibiotics. Olatoye *et al.* (2013) stated that the use of antibiotics in layers as egg boosters by farmers results in higher residues in layers and their eggs, than broilers. Despite the high percentage incidence of antibiotics level in this study 70 %, the level was far below the MRL for the various organs. The highest residue level of 6 µg/kg was seen in the liver; meanwhile the MRL for the liver is 600

µg/kg. This is significantly ( $p \leq 0.01$ ) lower than the MRL. Al-Bahry *et al.* (2013) reported a similar finding in Oman. In the present study the low level of tetracycline residue, below MRL, may be as a result of the type of birds used, the broilers, as was explained above (Olatoye *et al.*, 2013). Most farmers avoid too much use of antibiotics in broilers. Another likely reason that may be responsible for the low level of residue in our study is that most of the birds were kept by veterinarians and they were been slaughtered for their own occasions, like weddings, graduations, etc. For this purpose, we consider they were more careful with the birds in terms of drug administration. Some of these veterinarians had been advocating for the less use of antibiotics, but more biosecurity in husbandry management. Berry (2016), reported that antibiotics used in agriculture are responsible for more than half of the antibiotics resistance cases in the world. Also, the study reported that over 700,000 people die annually due to antimicrobial resistant bacteria (super bugs). Another factor that can contribute to these low levels of tetracycline residues is the storage factor, as has been stated that low and high temperatures as in refrigeration and cooking can reduce the quantity of tetracycline residues in poultry samples. Alaboudi *et al.* (2013) and Hakem *et al.* (2013), stated that cooking at high temperatures or refrigeration at temperatures of  $-18^{\circ}\text{C}$  can reduce up to 90% of antibiotic residues. In the present study, the samples were refrigerated at  $0-4^{\circ}\text{C}$  for a period of 1 month. This might have affected the quantity of the tetracycline residues. Despite the residue been below the MRL, it was observed that the concentration was more and higher in the liver (26%) followed by liver and kidney (20%), liver and lungs (6%), liver, kidney and lungs

(2%), muscle (0%). It is known that tetracycline is widely distributed in the body, especially excretory organs (Al-Bahry, 2013; Aliu, 2007). The lack of tetracycline residues in the muscles alone may be due to the common route of administration which is the oral route in poultry. Despite the low level of residues as seen in the present study, it can still pose a health challenge. Low levels of antibiotics act as persistent pressure for the selection of resistant bacteria which may eventually affect the normal flora (Al-Bahry *et al.*, 2013). The transfer of these resistant bacteria strain to other species and humans is an important public health concern (JECFA, 2006; WHO, 1987). Tetracyclines are known to undergo extensive enterohepatic circulation and stay longer in the body, even after the drug is withdrawn (Al-Bahry, 2013; Aliu, 2007). This increases their chances to contaminate animals and food of animal origin including poultry products (Al-Bahry, 2013; Fuoco, 2012).

The low level of tetracycline observed in the present study also fall within the ADI of 0-30 µg/kg (Codex Alimentarius, 2015).

## CONCLUSION

In conclusion, the assessment of tetracycline residues using the microbiological assay as carried out in the present study, revealed that the level of tetracycline residues in broilers reared in Makurdi metropolis is below the recommended minimum residue limits stipulated by the regulatory agencies and is within the acceptable daily intake. Most importantly, the results of this study revealed that the microbiological method can be used quantitatively to estimate tetracycline levels in the tissues of broilers, and chicken in general.



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