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Comparison of Premi® test, Four Plate Test, ELISA and HPLC tests in the detection of tylosin residue and its depletion time in chickens treated with tylosin by intramuscular injection and oral routes

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Abstract

Tylosin residue (TR) occurs in tissues of animals treated with tylosin, and this is of public health concern. This study compared the levels of detection of tylosin residue in muscle and liver tissues of chickens treated with tylosin, using four testing techniques: Premi® test (PT), Four plate test (FPT), enzyme linked immunosorbent assay (ELISA) and high performance liquid chromatography (HPLC)-based tests. Sixty chickens randomly assigned to two groups (A and B) of 30 each were used for the study. Group A chickens were given 10 mg/kg tylosin by intramuscular injection, while Group B chickens were given 2g/L tylosin orally for five days. Group A birds were sacrificed ½, 1, 2, 4, 8, 12, 24, 48 and 72 hours post injection (PI) of tylosin, while Group B birds were sacrificed 24, 36, 48, 60, 72, 84, 96, 120 and 144 hours post completion of oral dosing (PCOD). Muscle and liver tissues were collected for assay from both groups, following standard procedures for the four tests. Results showed that PT did not detect TR in liver of both Groups A and B chickens but detection in muscle using PT was inconsistent. FPT detected relatively low levels of TR in both muscle and liver of Group A and B chickens with no detection at 84 hour PCOD for muscle and 120 hours PCOD for liver. ELISA and HPLC-based tests detected relatively higher levels of TR all through, in both Groups A and B chickens, though the levels were below the 100 µg/kg maximum permissible limit, except slightly higher results recorded with muscle tissue of Group B chickens tested with ELISA at 24 and 36 hours PCOD. The PT and FPT showed varied sensitivities and specificities when compared to the HPLC. There was a strong (high) positive and significant correlation between the levels of TR detected by ELISA and HPLC for both muscle and liver tissue in both groups, but the correlation between TR levels detected by FPT and HPLC was low and not significant in Group A chickens.

Keywords: Tylosin residue; Chicken; Muscles; Liver; Premi® test; Four plate test; ELISA; HPLC.

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Introduction

Tylosin is a macrolide antibiotic that is used against Gram positive bacteria and mycoplasma infection in animals, including poultry (Huang *et al*., 2021). The mechanism of action of tylosin is not fully understood, but it has been posited that it disrupts protein synthesis in susceptible organisms (Suchodolski *et al.,* 2009). It has a molecular weight of 916 g/mol. It is water soluble, with a low oral bioavailability. Tylosin is moderately bound to plasma proteins and it has been reported to be widely distributed in body fluids and tissues of treated birds (Suchodolski*et al.,* 2009). It may be administered to birds via drinking water at 0.5 g/litre, a dose equivalent to 75 mg/kg body weight per day. It can also be given intramuscularly at a dose of 20 – 120 mg/kg per day (Locke *et al*., 1982).

Farmers routinely use tylosin administered for 3 to 7 days in the management of poultry diseases, depending on the severity of the disease (Tijani *et al*., 2023). However, its use could result to the residue accumulation in the tissues and organs, especially if the withdrawal period is not observed before slaughter. Its concentration in excess of the established maximum permissible limit (MPL) of 100 µg/kg in meat is of public health concern. It can lead to the development of antibiotic resistant strains of microbes, hypersensitivity reaction in sensitized individuals, distortion of intestinal micro flora as well as carcinogenic and mutagenic effects (Serwecińska, 2020). This is more worrisome in areas where the poultry farmers and processors do not have a good knowledge of the possible implications of tylosin residue (TR) in tissues and organs of slaughtered poultry and where the drug is wrongly used as a growth promoter without adhering to its stipulated withdrawal period (Barros *et al*., 2021).

In order to guarantee meat safety, most countries have established their official standard MPL of veterinary drugs including tylosin (USDA, 2020). Countries like Korea and Japan have agreed to the internationally established MPL even though they have their own MPL values (Lee *et al*., 2022). In fact, the USA has approved more permissive tolerable limits of 200 ng/g in the muscle tissues of meat, whereas, the European community has set an MPL of 100 ng/g for the muscle of all food animals (FDA, 2022).

The antibiotic residue detection assays that are currently available employ the use of different methods and test microorganisms. Among these is the Four Plate test (FPT), a simple microbiological method that utilizes bacteria such as *Bacillus subtilis* because of its high sensitivity. FPT can assay multiple antibiotic substances (Heitzman, 1994). Premi® Test (PT) is a microbial screening test that contains viable spores of a strain of *Bacillus stearothermophilus* which is sensitive to antimicrobial residues, such as beta-lactams, tetracyclines, macrolides and sulphonamides. The growth of the strain is inhibited by the presence of antimicrobial residues in muscle tissue samples. PT has been reported to have the capacity to detect tylosin at 90 µg/kg (Gaudin *et al*., 2008). The enzyme linked immunosorbent assay (ELISA)-based tests has been widely used in the evaluation of antimicrobial residues in the food chain. ELISA-based tests are generally carried out in 96 well plates, allowing multiple samples to be measured in a single test of antigen-antibody reaction (Ramatla *et al*., 2017). ELISA-based tests has been reported to provide an excellent sensitivity and selectivity for tylosin at maximum residue limit of 100 µg/kg, both in liver and muscle tissue of chickens (Ramatla *et al*., 2017). The introduction of high performance liquid chromatography (HPLC) based tests has surpassed microbiological assays, and its use in the determination and quantification of TR in tissues has been reported (Prats *et al*., 2001). According to the guidelines for the approval of analytical

techniques for residues analysis, each method must detect residues at the MPL and also at half and twice the MPL (EC, 2017). However, limited data still exist on the comparative analysis of the test methods in TR assay in poultry tissues. The aim of this study was to comparatively detect and quantify TR and its depletion time in tissues of tylosin treated birds using four detection methods: Premi® test, Four Plate Test, ELISA and HPLC-based tests.

Materials and Methods

Study Location: The study was conducted in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

Experimental Animals and Design of the study: The study adopted an experimental design. One hundred (100) day old broiler chicks were purchased from a reputable hatchery (Agrited Limited Ibadan, Nigeria) and reared for the purpose of the study. They were given drinking water and fed *ad libitum* with antimicrobial free commercially available feed for 8 weeks before commencement of the experiment. Sixty four of the chickens were randomly selected for the experiment. Four out of the 64 chickens were further randomly selected and slaughtered before commencement of treatment; samples of the breast muscle, liver and kidney were collected after slaughter and screened for antimicrobial residues (pre-treatment screening). The remaining 60 chickens were randomly assigned to two equal groups (A and B) of 30 each. Birds in group A were injected once with Tylosin (Kepro®) intramuscularly at the dose of 10 mg/kg body weight while those of group B were given Tylosin (Kepro®) orally at the dose of 2 g/l for 5 days. Three birds were randomly selected from each group and humanely sacrificed at different time intervals: the group A birds were slaughtered at ½, 1, 2, 4, 6, 8, 12, 24, 48 and 72 hours post-completion of treatment while group B birds were sacrificed at 24, 36, 48, 60, 72, 84, 96, 120 and 144 hours post-completion of treatment. Samples of the breast muscle, liver and kidney were collected for the determination of the presence and concentration of TR in the tissues using standard protocols of FPT, Premi® Test, ELISA and HPLC. The birds were cared for and handled humanely during the study, following stipulated institutional guidelines for the care and use of animals for research*.*

Specimen preparation and Detection of TR with Four Plate Test: Briefly, two grams of each organ were macerated with equal volume of sterile water in porcelain mortar and pestle, centrifuged at 3000 revolutions per minute (rpm) in a test tube for 10 minutes after which the supernatant was decanted and stored for analysis. Three batches of Muller Hinton agar were prepared according to manufacturer's recommendations and autoclaved. After cooling to 45 – 50°C, they were adjusted to pH 6, 7.2 and 8 using NaOH (base) and HCL (acid). Ten millilitre of the media was poured on sterile Petri dishes and allowed to solidify. Each plate with pH 6, 7.2 and 8 was seeded with a ready to use suspension of *Bacillus subtilis* (Merck Darmstadt, Germany) and another media at pH 8 was seeded with 24-hour culture of *Micrococuss luteus* bacterial suspension (ATCC® 10240). Four wells were bored, and 80 µl of each tissue extract was inoculated into each of the wells and the fourth well was inoculated with tissue extract from oxytetracycline treated bird as positive control. After, the plates were incubated for 24 hours at 37°C. They were observed for clear zone of inhibition with annular diameter ≥ 2 mm, which indicated positive test for antimicrobial residues (Onwumere-Idolor *et al*., 2022).

Specimen preparation and detection of TR with Using Premi® Test: The extraction method was done as described above for FPT.

The Premi® test ampoules (*DSM Venturing* B.V. Geleen, *Netherland)* containing nutrient agar embedded with standardized number of spores of *Bacillus stearothermophilus var. calidolactis,* organism and a color indicator were used for the test. Firstly, the incubator containing ten wells for the ampoules was pre-heated for 20 minutes while using the thermometer provided to ensure optimum temp of 64 ± 1°C. Each extracted sample (100 µl) was inoculated into the ampoule, and was used for the test series. The negative control was the ampoule containing 100µl of distilled water (which is the meat extract diluent) while the positive control was the meat extract spiked with tylosin. The ampoules were incubated for 3 – 4 hours until the negative control changed from purple to yellow while the positive control retained its purple coloration. After the incubation, the ampoules that remained purple were recorded as positive for antimicrobial residues, while those that turned yellow were recorded as negative.

Tylosin residues detection by ELISA-based test: The microtitre plates of ELISA test kit and the reagents were sourced from Shenzhen Lvshiyuan Biotechnology Company Limited, Shenzhen, China. The ELISA test kit was adjusted to room temperature before use. The lyophilized conjugate was reconstituted first with 1 ml of conjugate diluents, vortexed and diluted with the same conjugate diluents at 1:10 ratio. The standards and the control were reconstituted with 1 ml of deionized water. The wash buffer (5×) concentrate was diluted at a ratio of 1:5. For each plate, a working scheme was prepared, the standards and samples were run in duplicates as described below: Twelve strips, each containing 8 wells, were fixed on the plate. Each of the six provided 50 µl standard solutions (0, 2, 6, 18, 54, and 162 ng/kg) was added in duplicate wells according to the working scheme. 50 µl of each tissue extract sample was added in duplicate wells following the standards according to the working scheme. The antibody conjugate (50 µl) was added to each of the wells. The plate (wells) was covered with a paraffin tape and the content mixed by circular motion on the bench for several seconds and then incubated at 30°C for 30 seconds. It was tapped from time to time to remove bubbles. The microtitre wells were further washed with a wash solution five times and tapped to remove bubbles completely. Solution A (50 µl) color was added followed by solution B color immediately and mixed thoroughly by shaking. The microtitre plate was incubated at 37°C for 10 minutes. Stop solution (50 µl) was then added. The absorbance was read at 450 nm wavelength, within 5 min of adding the Stop solution.

Tylosin Residue Detection by HPLC-based test: This was done using modified method as described (Prats *et al*., 2001). Briefly, tylosin stock solution was obtained from Sigma (St Louis, MO, USA). One mg/ml tylosin was prepared by dissolving 10 mg tylosin tartrate in 10 ml methanol and stored at -18°C. Working standard solutions for calibration curve was prepared by appropriate diluting of the stock solution, using dilution factor. The kidney, liver and muscle samples that were tested and confirmed to be free of macrolide antibiotic residues (control) were used as blanks for the preparation of matrix matched calibration curves. For fortification, standard solutions were prepared by dissolving standard substance in methanol at concentrations 40, 20, 10, 5, and 2.5 mg/ml. Two grams each of the kidney, liver and muscle samples of birds were weighed and macerated with mortar and pestle. 2 ml of distilled water was added, followed by 10 ml HPLC grade acetonitrile. It was then mixed with vortex mixer to homogenize for 1minute. Then, the sample was centrifuged for 15 minutes at 3,000 rpm. The clear extracted solvent layer was then collected using disposable pasture pipettes and diluted to 50 ml with distilled water. The SPE Cartridges

Bond Elute C_{18} 500 mg/3ml were activated with 2 ml of methanol and 5 ml of distilled water. The cartridge was washed with 20 ml of distilled water and allowed to dry. The extracted sample solution was loaded and allowed to elute from the cartridge with 3 ml of HPLC grade methanol. The solution was then filtered using 0.45 micromilllipore syringe filter. The samples were manually injected into the HPLC. Chromatographic analysis was performed with isocratic elution on Zorbax Eclipse XDB - C_{18} (150 x 4.6mm, 5 µm) analytical column at 30°C. The mobile phase composed of HPLC grade acetonitrile and water (90:10), at the flow rate 1.00 ml/min, 20µl was injected. The chromatogram was monitored at wavelength 292 nm.

Data analysis: Graphpad Prism 5 statistical software version 5.02 was used for the analysis of the data generated. Chi square was used to determine association between occurrence of TR and the tissue type while correlation analysis was done on the detected levels of TR and the detection methods. Significance was accepted at $p < 0.05$. The results were presented in tables and bar charts.

Results

In the tylosin-injected chickens, TR was detected in the muscle tissues from 30 minutes up to 12 hours post-injection (PI) with Premi® test, while the detection using the FPT, ELISA and HPLC based tests continued till 72 hours PI, with values below the 100 µg/kg MPL (Table 1). For the liver tissue of the tylosin-injected chickens, TR detection using Premi® test was inconsistent at 30 minutes and i1 hour (Table 1). Detection of TR in liver tissue of the tylosin-injected chickens using FPT started from 2 hours PI, peaked at 12 hours PI, and the levels detected gradually decreased towards 72 hours PI (Table 1). In the liver tissues of the tylosin-injected chickens, TR was further detected using ELISA and HPLC-based tests from 30 minutes PI till 72 minutes PI in values less than the 100 µg/kg MPL, but the concentrations of TR with HPLC were lower than those detected with ELISA (Table 1). However, for the purposes of calculating the validity of the test, the values at 72 hours PI for both ELISA and HPLC-based tests were termed negative (Table 1).

Table 1: Levels of tylosin residue in the muscle and liver tissues of chickens given 10 mg/kg intramuscular injection of tylosin, as detected using Premi® test, four plate test (FPT), ELISA and HPLC-based tests.

Test Method	Tissues	Time, post-intramuscular injection (hours)								
		½	1	2	4	8	12	24	48	72
Premi® test	Muscle	+	+	+	+	+	+	-	-	-
	Liver	+(-)	+(-)	-	-	-	-	-	-	-
FPT(IZ)	Muscle	9.0	9.0	9.0	9.0	10.0	10	9.0	9.0	9.0
	Liver	-	-	9.7	11	13	13	10	8	7
ELISA (µg/kg)	Muscle	56.49	68.41	61.90	44.82	32.90	26.99	16.86	16.58	10.44
	Liver	18.65	26.37	48.94	86.33	63.57	42.16	32.14	28.21	22.00
HPLC (µg/kg)	Muscle	50.00	72.67	57.00	39.67	30.00	28.67	18.67	15.67	9.0
	Liver	12.00	18.00	37.30	75.30	51.67	26.00	22.00	19.67	16.67

[+ = Detected; - = Not detected; +(-) = Inconsistent]

The concentration of TR in the muscles of the chickens that were intramuscularly injected with tylosin peaked at 1 hour PI and from then decreased steadily till 72 hours PI (Figure 1). On the other hand, for the liver tissue, TR was detected using only ELISA and HPLC-based tests from 30 minutes to 1 hour PI, but from two hours PI it was also detected using FPT (Figure 2). At 4 hours PI, the levels of TR detected using ELISA and HPLC-based tests peaked, and then later started declining from 8 hours PI to 72 hours PI (Figure 2). The trend of rise and decline in TR levels detected by both ELISA and HPLC-based tests were similar (Figure 2).

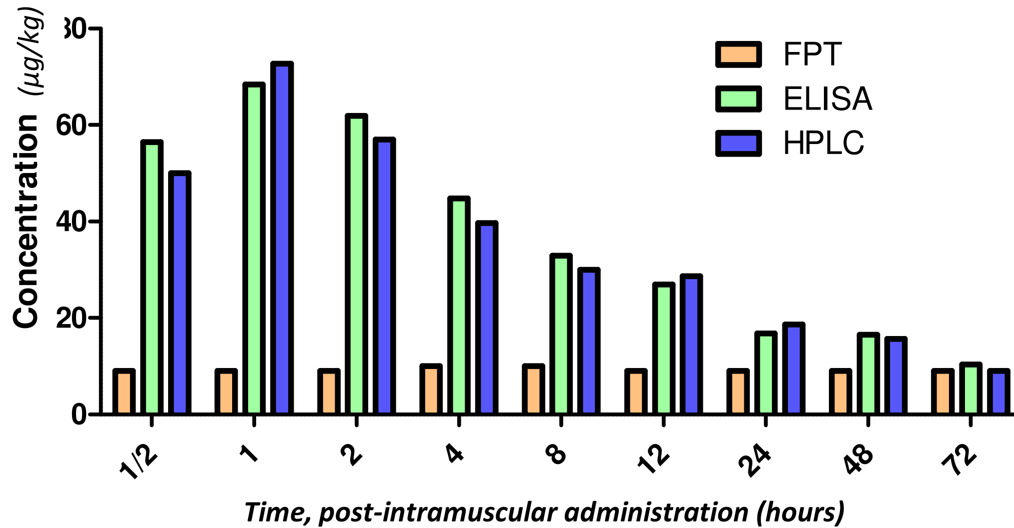

Figure 1. Levels of tylosin residue in chicken muscle tissue after 10 mg/kg intramuscular tylosin injection, as detected with Four Plate test, ELISA and HPLC based tests.

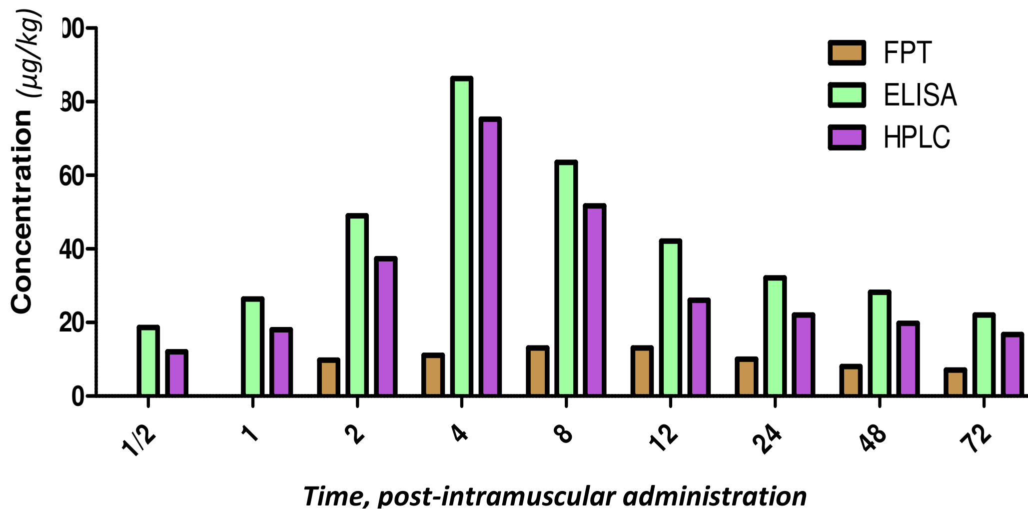

Figure 2. Levels of tylosin residue in chicken liver after 10 mg/kg intramuscular tylosin injection, as detected with Four Plate test, ELISA and HPLC based tests.

The validity of Premi® Test in detection of TR in muscle tissues of the tylosin injected chickens using HPLC as a gold standard indicated five samples as positive for both Premi® test and HPLC; only one was Premi® test positive and HPLC negative (false positive); and three were both Premi® and HPLC negative. Premi® test therefore had a sensitivity of 62.5% and specificity of 0%. On the other hand, the sensitivity and specificity of FPT in TR detection for liver of seven samples were both FPT and HPLC positive and no FPT positive and HPLC negative (no false positive). One of the samples was negative for both FPT and HPLC. The FPT therefore, had a sensitivity of 87.5% while both tests have 100% specificity.

There was a strong (high) and significant positive correlation ($r = 0.9855$; $p < 0.0001$) between TR levels detected in muscle tissues by the ELISA and HPLC-based test, but the correlation between the levels detected by FPT and HPLC-based test was weak (low), negative and not significant ($r = -0.172$; $p = 0.6581$). For the liver tissues, the correlation between the TR levels detected using the ELISA and HPLC based tests was strong (high), positive and significant ($r = 0.9914$; $p < 0.0001$), but the correlation between TR levels detected in liver tissues using FPT and HPLC was moderate, positive and not significant ($r = 0.5827$; $p = 0.0996$).

Table 2: Levels of tylosin residue in the muscle and liver tissues of chickens given 2g/L of tylosin orally for five days, as detected using Premi® test, four plate test (FPT), ELISA and HPLC-based tests.

Test Method	Tissues	Time, post oral administration (hours)								
		24	36	48	60	72	84	96	120	144
Premi® test	Muscle	+	+(-)	+(-)	+	+(-)	+	+(-)	+(-)	+(-)
	Liver	-	-	-	-	-	-	-	-	-
FPT(IZ)	Muscle	7.5	9.5	9.5	8	3	-	-	-	-
	Liver	7.5	9.5	9.5	9.5	7	4	3.5	-	-
ELISA (µg/kg)	Muscle	100.5	109.5	92.0	65.0	62.0	52.0	44.5	34.0	20.5
	Liver	45.82	46.0	51.0	42.0	35.5	32.5	28.5	19.5	18.0
HPLC (µg/kg)	Muscle	83.0	93.0	81.5	51.5	45.0	45.5	30.0	22.5	11.5
	Liver	40.0	42.5	48.9	40.5	37	36.0	27.0	22.0	16.0

[+ = Detected; - = Not detected; +(-) = Inconsistent]

For the chickens orally dosed with tylosin, using FPT, TR was detected in muscle tissue from 24 – 72 hours post-oral dosing (POD) only, but the ELISA and HPLC-based tests detected TR detected from muscle tissue of tylosin dosed chicken as from the 24 hours till 144 hours POD (Table 2). The concentrations detected in muscle tissue using ELISA based test were slightly above the 100 µg/kg MPL 24 – 36 hours POD (Table 2). The detection of TR with Premi® Test in the muscle tissue of the orally dosed chicken was inconsistent throughout the 24 – 144 hours POD (Table 2). For the liver tissue of the chickens dosed orally with tylosin, Premi® Test did not detect TR from the 24 to the 144 hours POD, but the FPT detected levels of TR that increased from its value at 24 hours to a higher value at 36, 48 and 60 hours POD before it gradually started decreasing to the lowest detectable value at 96 hours POD (Table 2). The trend of the TR levels detected using the ELISA and HPLC based tests in the liver tissues of the chickens orally dosed with tylosin followed the same

trend as was recorded for the muscle tissue, increasing from their 24 hour POD value to a peak at 48 hours that then started decreasing till 144 hours POD (Table 2). The levels of TR detection in the liver tissues using ELISA and HPLC-based tests were considered negative at 144 hour POD for the purposes of calculating the validity of the tests (Table 2).

The ELISA and HPLC-based tests detected a slight increase in the TR levels in muscles of the orally dosed chickens from 24 to 36 hours POD, from which point there was gradual decrease till 144 hours POD (Figure 3). However, FPT detected very low concentration of TR in the muscle tissue of the orally dosed chickens up to 72 hours POD, and from 84 – 144 hours POD, FPT did not detect TR in the muscle tissues of the orally dosed chickens (Figure 3). In the liver of the orally dosed chickens, the FPT detected very low levels of TR from 24 – 96 hours POD, but from 120 to 144 hours POD, FPT did not detect TR in liver tissues (Figure 4). The ELISA and HPLC-based tests however detected relatively high levels of TR in the liver tissues of the orally dosed chickens, which peaked at 48 hour POD, and afterwards started decreasing gradually till 144 hours POD (Figure 4).

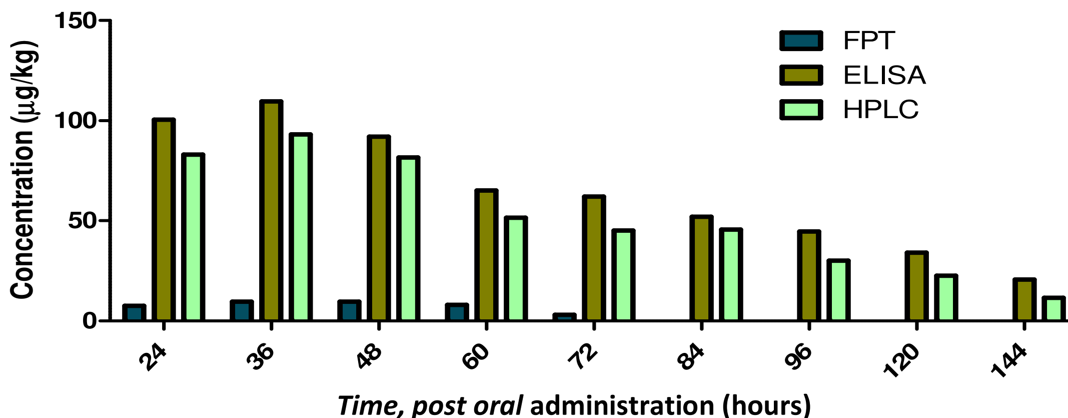

Figure 3. Levels of tylosin residue in chicken muscle tissues after oral dosing with tylosin (2 g/L) for five days, as detected by Four Plate test, ELISA and HPLC based tests.

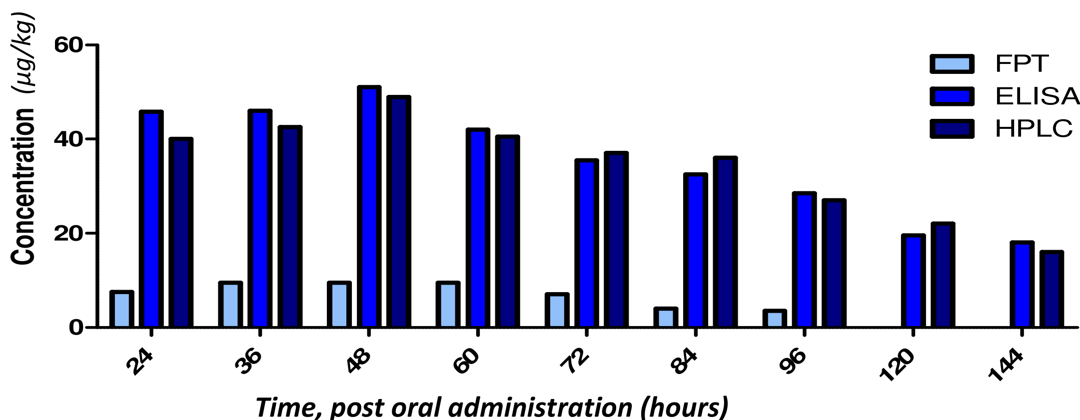

Figure 4. Levels of tylosin residue in chicken liver after oral dosing with tylosin (2 g/L) for five days, as detected by Four Plate test, ELISA and HPLC based tests.

When the sensitivity and specificity of TR detection from the muscle tissue of the orally treated birds was assessed using HPLC as a gold standard, six samples were positive for both FPT and HPLC-based test; one sample was negative for both FPT and HPLC-based test, while two samples were HPLC positive but FPT negative. The sensitivity of TR detection in muscle tissues of chicken using FPT showed a sensitivity of 75% and 100% specificity.

There was a strong (high) positive and significant correlation between levels of TR detected in muscle tissue of orally dosed chickens using ELISA and HPLC-based tests ($r = 0.9937$; $p < 0.0001$), also HPLC-based test and FPT ($r = 0.8905$; $p = 0.0013$). In the same vein, there was also a strong (high) positive and significant correlation between levels of TR detected in liver tissue of orally dosed chickens using ELISA and HPLC-based tests ($r = 0.9706$; $p < 0.0001$), and also HPLC-based test and FPT ($r = 0.9444$; $p = 0.0001$).

Discussion

Tylosin is a hydro soluble compound with reported low oral bioavailability and apparently low volume of distribution (Elbadawy *et al*., 2019) It has been reported that the rate of its accumulation in the muscle and liver tissues is strongly influenced by the route of administration (oral or intramuscular), with higher concentrations reported in orally dosed birds than the intramuscular injected ones (Soliman and Sedeik, 2016)

The higher values of detection in the muscles than the liver tissues in the present study could be associated with well-known biotransformation and detoxification activities of the liver (Gu and Manautou 2012). The results of the present study which showed that peak concentration of TR occurs one hour post-intramuscular administration with a steady gradual decrease till 72 hours PI indicates that the tylosin bio-accumulates at high concentration levels within the first one hour of intramuscular administration compared to 36 – 48 hours in orally dosed birds where it decreased to <50 µg/kg at day 4 and < 25 µg/kg at day 6. This finding is in agreement with other earlier reports where edible tissues exhibited fast depletion for TR to concentrations < 50 µg/kg after day 4 of oral treatment (Lewicki, 2006). The faster accumulation in chickens treated by intramuscular administration could be attributed to tylosin's reported high solubility in lipids (Lee *et al*., 2021).

The low levels of TR detected with the use of FPT and the non-detection later at 72 – 96 hours POD in muscle and liver tissues using FPT compared to the higher values detected using HPLC-based test validates HPLC as the best analytical method for the quantification of TR concentrations in meat according to the European Commission Decision 2002/657/EC guidelines (EC, 2002). However, results of the ELISA-based test demonstrated comparability to the results of the HPLC-based test and thus could complement conventional detection tools in regular monitoring program particularly in developing countries. Both the ELISA and HPLC-based tests used in this study have revealed their capacities in terms of efficacy and reliability for the detection of TR in meat as had earlier been reported in their use in the detection of chlorpyrifos ethyl residues in water and sediments (Otieno *et al*., 2013). This finding in this present study of the reasonable comparability of results of ELISA and HPLC-based tests is in agreement with similar earlier reports on the use of the same techniques in the detection and quantification of aflatoxin B_1 in feed samples (Beyene *et al*., 2019; Maggira *et al*., 2022).

The use of Premi®Test has earlier been reported to be associated with false-positive results (Pikkemaat *et al.,* 2009) and this has been corroborated by finding in this present study. The finding in this present study that

levels of TR detected by ELISA-based tests were slightly higher than the 100 µg/kg MPL when compared with relatively lower levels below the MPL detected using HPLC-based test does not concur with earlier reports of concurrence between ELISA and HPLC-based results in the detection of sulfamethazine residues in tissues (Pikkemaat *et al*., 2011).

Conclusion: Tylosin residue occurs in varying levels in the liver and muscle tissues of treated chickens mostly below the 100 µg/kg MPL. The Four Plate test (FPT) was found to be a more sensitive and specific method than the Premi® test, while ELISA-based test compared favorably with the HPLC-based test in the quantification of TR in muscle and liver tissues of chicken. Strict adherence to TR withdrawal time of between 3 – 5 days at minimum depending on the route of the drug administration is recommended before the slaughtering of treated chickens.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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