

RESIDUE DEPLETION OF TILMICOSIN IN CHICKEN TISSUES**Abdelrahman Mostafa*¹, Nuha M. A. Agbna², Samia A. Wahab³ and Sania Shaddad⁴**¹PhD Student Faculty of Vet. U of K.²Pharmacologist Faculty of Medicine U of K (Collaborator).³Pharmacologist Vet. Research Admin. Soba (Collaborator).⁴Prof of Pharmacology Faculty of Medicine U of K (Supervisor).**SUMMARY**Article Received on
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The study aimed to assess depletion of residues of tilmicosin in chicken tissues following oral administration of the drug via drinking water. Thirty broiler chickens four weeks old were administered tilmicosin in drinking water for five consecutive days at a dose of 75.0 mg/l. Six birds were sacrificed at day 1, 3, 5, 7, 9, and 12 after withdrawal of the drug. Tissue samples from muscle, liver and kidney were collected in sterile plastic containers and stored at -20°C for analysis. The concentration of the drug in different tissues was evaluated using LC-MS/MS. High concentrations were noted in the

muscle 401.8 µg/Kg, in the liver 3817.5 µg/Kg and in the kidney 1523.0 µg/Kg. at day one after withdrawal of the treatment. The concentration decreased to MRL (75 µg/Kg) in the muscle on day 7 from withdrawal, while in the liver it decreased to 604.9 µg/Kg and 180.8 µg/Kg in kidney which are below MRL set by EU. On day 9 the concentration in all tissues decreased to below the MRL that set by EU. This study indicates that withdrawal period of tilmicosin in broiler chickens could be 9 days.

KEYWORD: Residue, Depletion, Tilmicosin, chicken tissues.**INTRODUCTION**

Tilmicosin is a chemical modification of the naturally occurring macrolide tylosin.^[1] It is chemically closely related to erythromycin. Tilmicosin is rapidly absorbed and slowly eliminated after oral administration of single dose of aqueous and powder formulations.^[2] All macrolide drugs have a high volume of distribution. Tilmicosin accumulates in lung tissue and phagocytes at concentrations many times those seen in plasma. Tilmicosin persists in

lung tissue for at least 72 hours following a single dose in cattle.^[3] The macrolide drugs are mainly excreted by the liver with a small proportion of the drug excreted unchanged in urine.^[4]

The spectrum of activity is mainly Gram positive aerobes, but includes the Gram negative respiratory pathogens *Pasteurella* spp., *Mannheimia* spp. and *Histophilus* spp. The spectrum also includes some *Mycoplasma* spp.^[5] (NCCLS 1999).

Tilmicosin is licensed for the treatment of respiratory disease in cattle and sheep at a single dose of 10 mg/kg subcutaneous, it is one of the most widely used medications for the treatment and control of feedlot pneumonia in cattle.^[6,7,8] Tilmicosin has mainly been used for the treatment of respiratory infections in cattle and sheep^[9,10] in its injectable form. More recently, the drug has been formulated as a feed additive for the treatment of respiratory disease in swine (Pulmotil).^[11] The product is licensed for use in growing swine at a dose of 200 ppm in feed.

The main advantages of tilmicosin are the high concentrations of the drug that accumulate in the lung tissue and its persistence at this site. It has been widely used in the treatment of BRD complex^[12], for the treatment of individual animals, metaphylaxis and also prophylaxis.^[13]

The efficacy of tilmicosin for treating bacterial pneumonia in food producing animals is beyond question; tilmicosin has been widely studied in cattle feedlots and is effective^[7] as or more effective than other commonly used medications such as oxytetracycline, florfenicol or ceftiofur.^[14,15] Tilmicosin is also effective in the treatment of enzootic pneumonia of calves.^[8]

For the use of tilmicosin in poultry, tilmicosin is indicated for the treatment and control of respiratory disease associated with mycoplasma^[16,17,18] and other organisms susceptible to tilmicosin in broiler chickens at a dose of 75 mg/l of drinking water for 3 days (equivalent with approximately 15mg tilmicosin/kg bw/day).^[19] Tilmicosin is not indicated for laying hens.

The main advantage of tilmicosin over tylosin is its pharmacokinetics. Tilmicosin has a longer elimination half-life and accumulates in lung tissue at higher concentrations.

MATERIALS AND METHOD

Animals

Thirty chicks-days old (ARBER-AKERS) were obtained from a local hatchery in Khartoum and moved to a poultry farm in Soba. All chicken were fed antibiotic-free diet, and received antibiotic-free drinking water ad libitum. After 4 weeks acclimatization, the chickens became weighing 1.0 – 1.05 kg.

Chemicals

Mycojat (Tilmicosin solution 250 mg/ml) Muntajat for veterinary drugs. Saudi Arabia.

Tilmicosin standard from Fluka (33864), potency 85%.

Instrument and materials

Analysis was performed on LC-MS/MS

Equipment: Agilent 1260 HPLC, Agilent 6460 LC-MS/MS (Triple Quad. LC-MS), Degasser, Pump, Autosampler, Column oven.

Column used Zorbax SB C18 100X4.6mm 3.5 micrometer from Agilent.

A gradient containing Acetonitrile (ACN) 100% from Merk and 0.2% formic acid from Merk

Experiment

The chickens were administered tilmicosin orally in drinking water at concentration of 75.0 mg/l for 5 consecutive days. Six birds were sacrificed at day 1, 3,5,7,9, and 12 from withdrawal of the drug. Tissue samples from muscle, liver and kidney were collected in sterile plastic containers and stored at –20°C for analysis by chemical method (LC-MS/MS).

Sample preparation

After thawing, homogenized tissue (3 ± 0.03 g) was weighed in a 50 ml polypropylene test tube and 200 μ l 0.1M EDTA (ethylene diamine tetraacetic acid) was added. Samples were mixed and allowed to stand in dark for 15 min. The antibiotics were extracted from the tissues using 15 ml of 70% methanol and 10 min of shaking. After extraction the samples were centrifuged at 3800 rpm for 5 min. The extracts were then diluted with water by adding 100 μ l extract to a polypropylene vial containing 400 μ l water. The samples were mixed, filtered on 0.45 μ m and injected in the LC-MS/MS.

Analysis

The samples were analyzed by LC-MS/MS following Granelli & Branzell method.^[20]

RESULTS

After treatment with 75.0 mg/l tilmicosin in drinking water for five days, the concentration of the drug in tissues after one day from withdrawal was 401.8 μ g/Kg in the muscle, 3817.5 μ g/Kg in the liver and 1523.0 μ g/Kg in the kidney. On day 7 the concentration in the muscle was 75.0 μ g/Kg which is equal to the MRL that was set by EU while the conc. in the liver was 780.2 μ g/Kg and in the kidney was 232.5 μ g/Kg which are below the EU MRL.^[19] On day 9 the concentration in all tissues decreased to below the MRL that set by EU.

Different tissues concentrations versus time were recorded in table one.

Table (1): Tissue residues of Tilmicosin (mean \pm SD μ g/Kg) after oral administration of 75.0 mg/l. for five consecutive days.

Tissue	Concerteration mean \pm SD μ g/Kg					
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 12
Muscle	401.8\pm277	310.0\pm164.08	174.5\pm51.53	75.0\pm43.53	50.0\pm22.32	21.6\pm5.45
Liver	3817.5\pm980.5	2811.1\pm306.84	1857.4\pm355.3	780.2\pm32.7	604.9\pm124.7	218.6\pm80.96
Kidney	1523.0\pm25.03	826.3\pm16.01	458.1\pm28.25	232.5\pm21.5	180.81\pm3.01	59.4\pm5.51

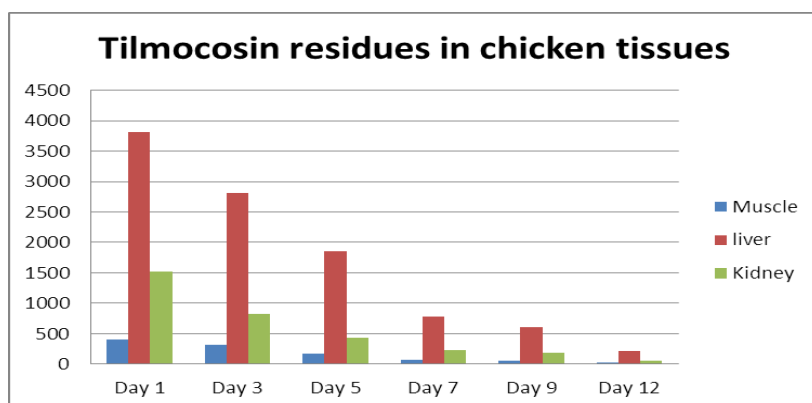


Fig 1: Tilmocodin residues in chicken tissues.

DISCUSSION

Tilmicosin concentrations in muscle, liver and kidney were highest on day 1 after withdrawal of medication. The residue levels were significantly higher in liver than in kidney or muscle. This finding was similar to those obtained by Zhang *et al.*^[21] Likewise EMA 1998 in a study found that the highest total radioactive residues were observed in liver (6.6 mg/kg at day 3).

According to the veterinary drug residue regulations of European Union, the maximum residue levels (MRLs) of tilmicosin in broiler chicken muscle, liver, and kidney are 75, 1000, and 250 μ g/Kg respectively.^[19] The recommended withdrawal time is 10 days. In this study

tilmicosin residue in all tissues (muscle, liver and kidney) decreased to the approved level after 7 days of withdrawal. These findings were slightly different from those obtained by Zhang et al.^[21] who found that tilmicosin residues in muscle decreased to the approved level after 2 days of withdrawal, in liver after 9 days, and in kidney after 5 days.

In this study tilmicosin residue in the muscle decreased to 75µg/Kg on day 7, but a concentration higher than 75µg/Kg was detected in one. In day nine the residue in the muscle decreased to 50 µg/Kg. Accordingly the withdrawal period of tilmicosin in broiler chickens under Sudan conditions could be 9 days.

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