



## EVALUATING DRUG WITHDRAWAL TIME OF IN-FED ANTIBIOTIC CHLORTETRACYCLINE IN LAYER CHICKEN

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### ABSTRACT

A biological trial was carried out on 60 week old culled layers. Thirty 60 week old laying Rhodo white hens, weighing 1.1-1.3 kg were kept in layer cages and were provided with feed free of pesticide and other contaminants for seven days. The first day the feed was analysed for Chlortetracycline (CTC) residue was taken as 0 day. Feed was analysed using HPTLC to identify any traces of CTC. After confirming the feed to be free of CTC residue, CTC was added at 1000 mg / kg. Layer birds were sacrificed on the zero day (pre treatment), 5<sup>th</sup> day during treatment and 4<sup>th</sup> day after treatment (9<sup>th</sup> day of trial). No residual level of CTC was detected at 0 day in egg, dropping, feather, blood, liver, ovary, follicle, breast and thigh. CTC levels on the 5<sup>th</sup> day in the liver, muscle (breast and thigh) and egg was  $205 \pm 2.24$ ,  $107 \pm 1.22$  and  $106 \pm 1.87$  µg/kg respectively, and in plasma, large ovarian follicle, small ovarian follicle and poultry dropping was,  $114 \pm 5.10$ ,  $74 \pm 3.67$ ,  $68 \pm 1.22$  µg/kg and  $49 \pm 2.45$  µg/kg respectively. Fourth day, after chlortetracycline was withdrawn (9<sup>th</sup> day) no residue of CTC was detected in any of the organs, egg and droppings. CTC was not detected at any time in feathers.

**KEY WORDS** – Chicken- antibiotic- chlortetracycline- feed- withdrawal.

### INTRODUCTION

Poultry plays a pivotal role in bridging the protein gap of animal origin in most countries of the world (Mumtaz *et al.*, 2000), its significance is even greater in developing countries where poultry are relatively cheap and can be kept in a small enclosure, usually providing both protein and income for a family. The use of modern systems of planning, organisation along with new technologies has enabled a steady growth in poultry production. Among these technologies are antibiotics that can be used as growth promoters (low doses), treatment (high doses), preventing diseases (intermediate doses) in food producing species (Gomes, 2004). Growth promoter's account for 42 % of the mass of veterinary pharmaceuticals used worldwide and therapeutic antibiotics at 18 % (Mitchell and Yee, 1995). Chlortetracycline (CTC) is the first member of tetracycline family. This was used act as a broad spectrum antibiotic and to treat and control a variety of bacterial infections and as growth promoter (De Ruyck *et al.*, 1999). Chlortetracycline is approved by the European Union (EU) for therapeutic use and United States Food and Drug Administration (USFDA) has approved for therapeutic, nutritional and prophylactic use in poultry (Nikolaidou *et al.*, 2008; Tanner, 1993).

Antibiotic residues can result from any of the following : improper use, extra label use or over use, lack of veterinary control and lack of adherence to withdrawal times which is a major cause of violation worldwide (Adesiyun *et al.*, 2005) . This harmful health effect of residues on humans makes the control of veterinary drug residue an important measure in ensuring consumer protection.

An attempt has been made in this study to assess the transfer of antibiotics from feed to liver, meat, eggs ovary, plasma, feathers and droppings. A Study was taken up to when this antibiotic namely Chlortetracycline (CTC) must be withdrawn from the feed so that the meat consumed by humans would be free of CTC and also where feather meal and poultry droppings are being used as feed for other animals it needs to be safe for those animals.

### MATERIALS & METHODS

Biological trial was conducted for fixing a withdrawal period for Chlortetracycline antibiotic in layer chicken egg, dropping, feather, blood, liver, ovary, follicle, muscle (breast and thigh). Birds were reared at University Research Farm, Madhavaram Milk Colony, Chennai. The experimental design is given in Table 1.

**TABLE 1:** Experimental design for biological trial

Sl.No	Treatment	No of birds
1.	Control (T <sub>1</sub> ) Birds were fed without antibiotics in feed	15
2	Chlortetracycline (CTC) birds were fed with commercial CTC preparation for five days - (T <sub>2</sub> )	15
2.	Chlortetracycline (T <sub>3</sub> )- 1000 mg / kg	15

Thirty 60 week old laying Rhodo white hens from the Institute of Poultry Production and Management, Madhavaram milk colony, Chennai. Weighing 1.1-1.3 kg, were kept in layer cages and fed with feed free of pesticide and other contaminants (analyzed at Pharmacovigilance Laboratory for Animal Feed and Food Safety, TANUVAS)

Layer birds were scientifically slaughtered on the zero day (pre treatment), 5<sup>th</sup> day during treatment and 4<sup>th</sup> day after treatment (9<sup>th</sup> day of trial). Six birds from each treatment were sacrificed and the egg, dropping, feather, blood, liver, ovary, follicle, breast and thigh muscle were collected on 0, 5<sup>th</sup> and 9<sup>th</sup> day. The samples were labelled with unique identification and brought to laboratory and stored in deep freezer (-20 °C) until analysis.

#### Preparation of Chicken liver Sample

Analytical sample of 25 g was drawn separately from each laboratory sample already collected and to this 50ml acetonitrile and 10 ml 5N HCl were added. It was shaken for 20 minutes in a shaker and filtered through Whatman No.1 filter paper (125 mm). The complete filtrate was taken in 500 ml separating funnel and to this 25 ml dichloromethane was added. It was manually shaken well and then 10 ml distilled water was added with this and allowed to settle down. The lower layer so formed was made to pass through anhydrous sodium sulphate kept over cotton in a funnel and the extract was collected in a beaker and concentrated to near dryness on the hot plate (100 °C). Concentrated sample in the beaker was subjected to High Performance Thin Layer Chromatography (HPTLC).

#### Preparation of Chicken Muscle (Breast and thigh) / Ovary / Follicle Sample

Analytical sample of 25 g was drawn separately from each laboratory sample already collected. The preparation of extraction from muscle / ovary / follicle was similar to the procedure of preparation of extraction from liver as per the method described by Shareef *et al.* (2009), but 10 ml distilled water was not mixed with dichloromethane.

#### Preparation of chicken plasma Sample

Analytical sample was drawn separately from each laboratory sample already collected. Equal volume of plasma and acidified acetonitrile was centrifuged and supernatant volume was taken for HPTLC analysis.

#### HPTLC

A two µl volume of the standard solution was applied to a TLC plate. The precision of the retention factor (R<sub>f</sub>) value was evaluated. Extracted sample was diluted with 200 µl of dichloromethane and from this 10 µl of diluted sample was used for spotting on the aluminium oxide TLC plates. The plate was developed first in ether and then developed in dichloromethane, methanol and water (5.8: 3.5:0.7) for chlortetracycline residue detection.

Chromogen dipped aluminium oxide plates were air dried and exposed to fluorescence densitometry (400 nm and 380 nm for chlortetracycline and tylosin respectively). The limit of detection for chlortetracycline was 10ppb. All the procedures were performed in triplicate. The limit of detection (LOD) was validated appropriately using spiked samples with known standards as part of laboratory quality control measures. The recovery percentage of spiked standards varied from 85-90 %. The limit of quantification was 15ng / g. The results were obtained by comparing the chromatogram from the HPTLC using a scanner. Results were expressed as µg/kg (ng / g or ppb).

The collected data was statistically analysed by one way ANOVA by using SPSS ver. 17 for windows. The significance was tested by using Duncan's multiple range test (Duncan, 1955). The procedure described by Snedecor and Cochran (1994) were adopted on this purpose.

## RESULTS & DISCUSSION

### Chlortetracycline residue in liver, muscle (breast and thigh) and egg

Residual level of Chlortetracycline (CTC) in liver, muscle (breast and thigh) and egg are presented in Table 2. No residual level of CTC was detected at 0 day in liver, muscle (breast and thigh) and egg. On feeding 1000 mg / kg of feed for 5 days, on the 5<sup>th</sup> day CTC levels in the liver were 205 ± 2.24 µg / kg. In muscle (breast and thigh) the residue level was 107 ± 1.22 µg / kg. CTC residual level in egg was 106 ± 1.87 µg / kg. Fourth day, after chlortetracycline was withdrawn (9<sup>th</sup> day) no residue of CTC was detected in the liver, muscle and egg. Four days after withdrawal (9<sup>th</sup> day) Chlortetracycline (CTC) residue in liver was not detected. Alhendi *et al.* (2000) had recommended five day withdrawal for OTC in chicken. Trypenou and Frangiadaki (1986) recommended one day withdrawal period for liver.

Wells (1996) had observed that two day after withdrawal, CTC level in liver was 0.09 mg / kg, but in turkeys even after three days of withdrawal residue level in liver was 0.1mg / kg. The withdrawal period depended on the concentration of antibiotic administered (Omija *et al.*, 1994), Physiology of the bird, climatic conditions and calcium level in feed. In muscle on the 9<sup>th</sup> day, four days after withdrawal of the drug, no CTC residue was detected. Gingham (1979) had also observed the same. While Trypenou and Frangiadaki (1986) had stated that one day withdrawal period was enough for muscle to be free of antibiotic residues. However, De Ruyck *et al.* (1999) and Anadon *et al.* (2012) had detected CTC residue in muscle after four and five days withdrawals respectively. De Ruyck *et al.* (1999) recommended seven days withdrawal period for chicken meat.

Chlortetracycline residue was not detected in egg, four days after withdrawal of medicated feed. Gingham (1989 a

and b) and Wells (1996) agreed with the present finding where CTC was not been detected in eggs two days after withdrawal of medicated feed. However, Roudaut *et al.* (1989) found measurable CTC residues in albumen for five days, in Yolk nine days after withdrawal of medication; while Yoshimura *et al.* (1991) working with OTC observed even after two days withdrawal peak residue was detected and only gradually decreased. However, in this biological trial it was observed that on

the Fourth day, after chlortetracycline was withdrawn (9<sup>th</sup> day) no residue of CTC was detected in the liver, muscle and egg.

#### **Chlortetracycline residue in plasma, large and small ovarian follicle, dropping and feather**

Residual level of Chlortetracycline (CTC) in plasma, large ovarian follicle, small ovarian follicle, dropping and feather are presented in Table 3.

**TABLE 2.** Mean  $\pm$  (S.E) of Chlortetracycline residue in liver, muscle and egg at 0, 5<sup>th</sup> and 9<sup>th</sup> day- controlled experiment, n=6

Sl.no	Sample	0 day ( $\mu\text{g} / \text{kg}$ )	5 <sup>th</sup> day ( $\mu\text{g} / \text{kg}$ )	9 <sup>th</sup> day ( $\mu\text{g} / \text{kg}$ )
1	Liver	ND	205 $\pm$ 2.24	ND
2	Muscle	ND	107 $\pm$ 1.22	ND
3	Egg	ND	106 $\pm$ 1.87	ND

ND-Not detected

**TABLE 3.** Mean  $\pm$  (S.E) Chlortetracycline residue in plasma, large and small ovarian follicle, dropping and feather at 0, 5<sup>th</sup> and 9<sup>th</sup> day n=6

Sl.no	Sample	Days		
		0	5	9
1	Plasma ( $\mu\text{g/L}$ )	ND	114 $\pm$ 5.10	ND
2	Large ovarian follicle ( $\mu\text{g/kg}$ )	ND	74 $\pm$ 3.67	ND
3	Small ovarian follicle ( $\mu\text{g/kg}$ )	ND	68 $\pm$ 1.22	ND
4	Feather ( $\mu\text{g/kg}$ )	ND	ND	ND

ND-Not detected

No residual level of CTC was detected at 0 day in plasma, large ovarian follicle, small ovarian follicle, poultry dropping and feather. On feeding 1000 mg / kg of feed for five days, on the 5<sup>th</sup> day residual CTC levels in the plasma was 114  $\pm$  5.10  $\mu\text{g} / \text{kg}$ . CTC residual level in large ovarian follicle, small ovarian follicle and poultry dropping was, 74  $\pm$  3.67, 68  $\pm$  1.22  $\mu\text{g} / \text{kg}$  and 49  $\pm$  2.45  $\mu\text{g} / \text{kg}$  respectively. Parent CTC residue was not detected in feather at 5<sup>th</sup> day of treatment. On the 4<sup>th</sup> day after withdrawal of feed mixed with chlortetracycline (9<sup>th</sup> day), no residue of CTC was detected in plasma, large ovarian follicle, small ovarian follicle, poultry dropping and feather.

In plasma, on the 9<sup>th</sup> day, four days after withdrawal of the drug no Chlortetracycline (CTC) residue was detected. Schumacer (1968) recommended one day withdrawal period for plasma CTC level. Berger (1971) detected no CTC residue in plasma 24 hours after withdrawal of the medicated water. Also Alhendi *et al.* (2000) observed that one day after withdrawal of medicated feed, concentration of OTC fell significantly in plasma.

After four days of medicated feed withdrawal (9<sup>th</sup> day) large ovarian follicle and small ovarian had no CTC residue. No withdrawal period has been established for CTC residue in large or small ovarian follicle in chicken. No literature could be traced for withdrawal period for chlortetracycline residue in dropping and feather. As per this trial a four day withdrawal of medicated feed had removed chlortetracycline residue from poultry dropping. No literature could be traced concerning withdrawal time of CTC for chicken droppings, ovary and feather.

#### **CONCLUSION**

Literature couldn't be traced for withdrawal period for chlortetracycline residue in dropping and feather. As per this trial a four day withdrawal of medicated feed had removed chlortetracycline residue from poultry dropping. No literature could be traced concerning withdrawal time of CTC for chicken droppings, ovary and feather.

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