DETERMINATION OF OXYTETRACYCLINE RESIDUE LEVELS IN EGGS AND MEAT OF CHICKEN USING MICROBIOLOGICAL ASSAY

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A thesis submitted in partial fulfillment for the degree of Master of Science in Pharmacology and Toxicology in the University of Nairobi.

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1991.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ACKNOWLEDGEMENTS

First and foremost, I am very grateful to my supervisors, Drs. E.S. Mitema and T.E. Maitho for their guidance and constructive criticism that helped make this thesis a reality.

My sincere gratitude also goes to Drs. E. Kangethe, D. Gacheru and Mr. H. Kaburia whose invaluable help and suggestions in the laboratory contributed to the production of this thesis. I am grateful for the help accorded to me by Miss J. Kamau, Messrs. C. Macharia, A. Githua and the other technicians of Public Health laboratory. I am also grateful to Miss D. Chege and Mr. C. Matere for their help with the computer work. I am indebted to Mr. Chebe who assisted in the acquisition of chickens and the assistance of Drs. M. Wanyoike, Baptist and Ms. K. Kiugu is also appreciated.

My thanks also go to Prof. Gathuma, Chairman of the Department of Public Health, Pharmacology and Toxicology, University of Nairobi, for allowing me to carry out my research in the Public Health laboratory. My very special gratitude goes to Miss Agnes Mwende Kioko whose encouragement kept me going.

I am very thankful to NORAD for the scholarship which enabled me undertake this project. I also would not forget the Norwegian Veterinary Association for raising funds to enable us complete the research projects successfully.

Last but not least, my appreciation goes to Professors Nafstad, Løkken and Ølsvik for considering our case in Norway for further funding when the scholarships were temporarily terminated.

DEDICATION

This thesis is dedicated to my mother, Mrs. Margaret Mary Awiti Oloo.

ABSTRACT

Tetracyclines are some of the most widely used antibiotics in the world for human and veterinary purposes. They are effective against both gram positive and gram negative bacteria as well as mycoplasmas, some viruses, rickettsiae, spirochaetes Chlamydia and actinomycetes. In chicken, tetracyclines are effective against chronic respiratory disease, infectious synovitis, infectious coryza and fowl typhoid. They are available either as powders, injections, capsules, boluses or infusions.

There has been an alleged misuse of the poultry soluble antibiotics especially tetracyclines, by farmers and other users in Kenya. The main objective of carrying out this study was to determine whether oxytetracycline residues were present in commercial eggs and then compare these levels with the WHO/FAO accepted maximum limit of 0.2 ppm for oxytetracycline residues.The other aim was to determine the concentration of oxytetracycline residues deposited in eggs and chicken meat following administration of 0, 400, 600, and 800 ppm of oxytetracycline via drinking water for 7 days and thereafter determine safe withdrawal periods in eggs and meat of chicken.

Eggs were obtained from Nairobi area (the city and its suburbs), Mombasa and Nakuru. These areas gave a representative sample since they are regions of intensive poultry farming. A total of 712 eggs were analysed, of which 355 were from Nairobi, 298 from Mombasa and 59 from Nakuru areas respectively. A microbiological method of analysis with a limit of detection of 0.1 ppm was employed, using *B. cereus* var. *mycoides*, ATCC 11778 as the test organism. Two assays of the yolk were run, one portion freeze-dried

while the other one was not freeze-dried. The same procedure was applied to the albumen. The analysis involved dispensing 200 µl of each sample into wells of diameter 10 mm, dug out on Mueller Hinton agar and after an incubation of 18 hours at 30°C, the diameters of the zones of inhibition were measured and compared with standards, which were run alongside the samples. The concentration of oxytetracycline in the samples was then extrapolated from the standard curve.

In Nairobi, six eggs had detectable residue levels which were all above the WHO/FAO maximum limit of 0.2 ppm. The residues were only detected in the freeze-dried samples and the mean concentration of the yolk samples was 0.478 ppm. The oxytetracycline residues were only detectable in the yolk apart from one case where both the yolk and albumen had detectable levels of oxytetracycline (0.324 ppm of oxytetracycline in the albumen). None of the eggs from Nakuru had detectable levels of oxytetracycline while from Mombasa, one egg was positive for sulfur and none for oxytetracycline.

In the feeding study, the birds were divided into four groups: the first group (control) received antibiotic-free water, the second group received water containing 400 ppm oxytetracycline, the third group received water containing 600 ppm and the fourth group were offered water containing 800 ppm. Eggs were collected during the 7-day feeding trial administration period and for another 6 days post drug administration. Ten days after the feeding trials were completed, the birds were sacrificed and the breast muscle removed. The meat samples were homogenised with 0.01N hydrochloric acid as the oxytetracycline extractant and after

filtration, the filtrate was handled in the same way as the egg samples. A microbiological method of analysis was used for both the eggs and meat samples. Zones of inhibition were obtained and their diameters were proportional to the concentration of oxytetracycline present. For yolk, 6 out of 38 eggs were positive for group 2, 11 out of 27 for group 3 and 29 out of 43 eggs for group 4. The corresponding values for albumen were zero, one and seventeen for groups 2, 3 and 4 respectively. No residues were detected in the yolk and albumen 6 and 3 days respectively, after medication was stopped whereas no residues were detectable in the breast muscle samples ten days after the termination of drug administration. There was a significant difference ($p<0.05$) among the groups and also between groups 3 (600 ppm of oxytetracycline) and 4 (800 ppm of oxytetracycline) and groups 2 (400 ppm of oxytetracycline) and 4 for both yolk and albumen. However, whereas there was a significant difference ($p<0.05$) between groups 2 and 3 for yolk, there was no significant difference ($p>0.05$) between groups 2 and 3 for albumen.

This study shows that commercial eggs obtained from the areas of study are generally safe for human consumption, despite the possible widespread use of antibiotic feed additives. Less than 1% of the eggs analysed had detectable levels of oxytetracyclines even though all were above the acceptable level of 0.2 ppm. It has also confirmed that oxytetracycline residues in eggs reach a peak faster in the albumen than in the yolk. The residues, however, persist longer in the yolk than in the albumen. Higher levels of oxytetracycline were observed in eggs from birds which received higher dosages.

The study confirms that oxytetracycline is eliminated to safe levels in chicken meat within ten days even when higher dosages, up to 800 ppm, are administered.

This study has also shown that *Bacillus cereus* var. *mycoides* ATCC 11778 is more sensitive to oxytetracycline than to sulfur.

Chapter 1

INTRODUCTION

Antibiotics have been used for a long time in the treatment and prevention of diseases in both man and animals. After administration, they find their way into the bloodstream where they are bound to blood proteins and transported to different parts of the body. After metabolism in the body, some of the drug is excreted while some is stored in the tissues.

In poultry, some of the drug or its metabolites are deposited in eggs, as well as in the meat .

Over the years, there has been much concern about the possible hazards to human health posed by the use of antibiotics, particularly tetracyclines, for animal husbandry purposes. (Anderson, 1968; Drews and Hogenauer, 1977). Such health problems include hypersensitivity, development of resistant strains of bacteria and gastrointestinal disturbances. The WHO/FAO, in this regard, has set standards which have to be adhered in order to safeguard the human health.

Generally, tetracycline levels of 5-20 ppm in feed of farm animals is acceptable. In eggs the acceptable residue level is 0.2 ppm for oxytetracycline and 0.05 ppm for chlortetracycline. In the tissues, the following levels are acceptable: kidney 3 ppm; muscle, liver, fat and skin 1 ppm each. These levels can only be realised when withdrawal periods are observed. For example, in laying hens, a withdrawal period of 4 days is recommended by

public health personnel but this is not usually strictly observed by farmers due to economic considerations.

Several procedures exist for detecting antibiotic residue levels in feeds and animal tissues but each method has its own limitations. To detect oxytetracyclines in feeds, tissues or products such as eggs, the easiest, fastest and cheapest method is the microbiological assay, using *Bacillus cereus* type ATCC 11778 as the test organism. Another advantage of this method is that many samples can be run at once. The method involves the use of analytical grade oxytetracycline to prepare standard curves from which the oxytetracycline level in a given substance is extrapolated after measuring the zones of inhibition.

Several workers have used the microbiological assay in their studies, with slight modifications and these include: Katz et al (1972), Yoshida et al (1973), Roudaut et al (1987 and 1989) and Yoshimura et al (1991) to mention a few. Due to modifications, varying detection limits have been reported e.g. 0.27 µg/g (Yoshida et al, 1973), 0.1 µg/g (Katz et al, 1972) and 0.2 µg/g-yolk (Roudaut et al, 1987 and 1989). The latter authors also found a detection limit of 0.07 µg/g for albumen. Oxytetracycline residues can be detected in albumen as early as one day following oral administration due to its very low detection limit, unlike in the yolk where residues can only be detected after 3-4 days. However, residues can be detected for longer periods in yolk than in albumen after the termination of treatment.

Other methods which have been used for the determination of oxytetracyclines include: fluorimetry (Kohn, 1961; Wilson, et al.,

1972), chromatography (Wagman and Weinstein, 1983; Ryan and Dupont,1974; Bocker and Estler, 1979; Lin, 1985; Ashworth, 1985, Oka et al, 1985 and MacNeil et al., 1989), radioimmunoassay method (Faraj and Ali, 1981; Blomquist and Hanngren, 1966 and Ullberg, 1977).

In Kenya, the tetracyclines are the most widely used group of antibiotics and they have a very wide range of trade names. The reason for concern of oxytetracyclines is because these poultry products have been used indiscriminately by lay people e.g. farmers and also by para-veterinarians. The proper use of oxytetracycline is open to abuse because not all the farmers or producers adhere to the manufacturer's instructions and the resulting effects could be detrimental to humans.

Objectives:

a). To investigate the effect of freeze-drying on the detection of oxytetracycline residues in chicken meat and eggs using B. cereus var mycoides ATCC 11778.

b). To determine the levels of oxytetracycline residues in eggs obtained from areas where intensive poultry farming is carried out, by microbiological techniques.

c). To determine oxytetracycline levels in eggs and meat of layers following oral administration of different doses of the drug.

d). To compare the oxytetracycline levels obtained with the WHO/FAO acceptable residue limit of 0.2 ppm in eggs and thereafter institute any safety or control measures where necessary.

Chapter 2

LITERATURE REVIEW

2.1. Introduction

Tetracyclines are a group of broad spectrum antibiotics which have been used in the treatment of both animal and human diseases for the last forty years. Members of this group include: oxytetracycline, chlortetracycline, tetracycline, doxycycline, dimethylchlortetracycline, minocycline, demeclocycline and methacycline. Of these the first three are the most common and most important. It is also noted that tetracycline is the name given to a specific compound as well as the whole group.

The main source of these antibiotics is the fungus *Streptomyces*. For example chlortetracycline is obtained from *S. aureofaciens* while oxytetracycline is obtained from *S. rimosus*. Chlortetracycline was first described by Duggar in 1948 (Dornbush & Abbey, 1972). Two years later, Finlay et al. (1950) discovered oxytetracycline. Although strains of Streptomyces are capable of producing tetracycline, this drug is produced commercially by hydrogenolysis of chlortetracycline (Aronson, 1980).

2.2. Physical and chemical properties of tetracyclines

Tetracyclines are amphoteric compounds forming salts with acids or bases. The bases are yellow, crystalline compounds which are odourless and slightly bitter. Oxytetracycline, when dissolved in a propylene glycol-water solution is more stable

compared to chlortetracycline . Aqueous solutions show appreciable loss of activity within 24-48 hours, especially when the pH is elevated (Thompson, 1976). Oxytetracycline crystals show no loss in potency on heating for 4 days at 100°C. Aqueous solutions of the hydrochloride at pH 1.0 to 2.5 are stable for at least 30 days at 25°C. Solutions at pH 3.0 to 9.0 show no detectable loss in potency on storage at 5°C for at least 30 days (Thompson, 1976). Hydrochloride salts are commonly used for oral administration and are usually encapsulated. Compared to oxytetracycline, chlortetracycline is very unstable *in vitro*, losing much of its activity in a few hours, so for this reason, it is not widely used clinically at present (Jawetz, 1984). The compounds also form stable chelate complexes with divalent metals such as calcium, magnesium and iron. Tetracyclines are available as capsules, powders, feed additives, parenterals, boluses and ointments for use in Veterinary Medicine and Animal Production.

Under high temperatures, tetracycline and oxytetracycline do not suffer any breakdown. However, they lose their biologic activity as a result of the structural changes in their molecules, as well as the transference into the respective isomer forms (Ionova, 1971). Preparation of eggs by several cooking procedures indicated a 30-50% retention of potency (Katz et al, 1972).

Oxytetracycline residues are totally destroyed by a thermal treatment at 100°C for 5 minutes, through the formation of derivatives, although the presence of antibiotic residues in eggs

might cause some risks for the consumer's health (Siegmann and Neumann, 1984).

Meredith et al (1965) showed that cooking by poaching and scrambling eggs does not destroy chlortetracycline and oxytetracycline in all cases, but autoclaving at 121^{0}C for 20 minutes at 15 psi does. These workers also demonstrated that normal methods of roasting for 90 minutes at 177^{0}C, frying at 149^{0}C for 20 minutes in cooking oil and autoclaving poultry tissue destroys all residual chlortetracycline and oxytetracycline.

2.3. Antibacterial spectrum and pharmacodynamics

Tetracyclines are effective against both gram positive and gram negative bacteria (but more against gram positive). In addition, they are active against rickettsiae, some viruses, mycoplasmas, spirochaetes and actinomycetes. Sustained-release oxytetracycline boluses (20%) at a dosage of 3.0 mg/kg were found to prevent infection by the rickettsia *Anaplasma marginale* (Byford et al., 1981). The drugs also have prophylactic activity against *Theileria parva* during experimental incubation (Brown et al, 1977). They have no activity against yeasts or other higher fungi. Higher concentrations are necessary to kill microorganisms than to prevent multiplication, and they mainly affect rapidly growing organisms.

Tetracyclines are bacteriostatic antibiotics whose action is interference with RNA and bacterial protein synthesis by affecting protein induction at the ribosomes by messenger RNA . They also inhibit bacterial cellular metabolism by blocking

attachment of aminoacyl transfer ribonucleic acid to ribosomes, which interferes with protein synthesis. The drug blocks protein synthesis by binding to the 50S ribosomal subunit of the 70S bacterial ribosome .

The antibacterial activity is only affected to a small extent by the presence of bacterial debris, blood or serum. A shortening of blood coagulation time following the administration of chlortetracycline to some animals has also been seen.

Among the bacteria relatively susceptible to tetracyclines are : *E. coli, Pasteurella, Salmonella* and *B. anthracis* while the most susceptible include: Clostridia, *Klebsiella, Hemophilus* and *Streptococcus. Proteus, Pseudomonas, Shigella, Str. fecalis* and several strains of *Staphylococcus* are relatively resistant.

2.4. Routes of administration

Tetracyclines are administered either orally, parenterally or topically in veterinary and human medicine.

2.4.1. Oral

In herbivores, tetracyclines are orally administered in subtherapeutic doses. Following oral administration, normal bacterial fermentation of plant fibre is initially suppressed. In small animals, the antibiotics are given orally at an average dosage of 27 mg/kg/day twice daily . In poultry, they are fed at 500 gm/900 kg feed in case of chlortetracyclines and 5-200 gm/ 900 kg feed for oxytetracyclines. The recommended dosages via the drinking water in poultry are 0.05-0.2 g/l for doxycycline and 0.03-0.5 g/l for oxytetracycline.

2.4.2 Parenteral

Intravenous (IV) and intramuscular (IM) routes are commonly used for administration of the tetracycline compounds in Veterinary Medicine. Intravenous injection is usually given once daily but in acute illness, it is given twice a day, in divided doses. This is said to reduce the chances of shock or toxaemia from bacterial debris (Huber, 1988).

Intramuscular administration of chlortetracycline is not recommended because it causes severe tissue irritation. Oxytetracycline can be given as a deep IM injection.

The suggested dose for IV and IM injection of tetracycline antibiotics is 4.4-11 mg/kg daily .

2.4.3. Topical

Tetracyclines are available as intramammary infusions for the treatment of mastitis in cow, doe and ewe. In the cow, mastitis is effectively treated with a 440 mg tetracycline hydrochloride preparation. Topically, an ophthalmic ointment containing 1 mg tetracycline per gram ointment may be used on conjunctival membranes. The eye may also be treated with a buffered aqueous solution of tetracycline antibiotic containing 5 mg/ml .

2.5 Pharmacokinetics

2.5.1. Absorption

After oral administration, tetracyclines are absorbed readily, but not completely from the stomach and the first part of the small intestine to give a peak plasma concentration in 2-4 hours, especially in monogastric animals. Hardly any drug is detectable in plasma after 24 hours. An aqueous vehicle reduces the amount of time for peak concentrations of oxytetracycline to be achieved, while an oil suspension vehicle prolongs the rate of absorption. In a study of the absorption of tetracycline from loops of the dog small intestine, Pindell et al., (1959), showed that only about 3% of an administered dose of the drug was absorbed in 1.5 hours. Since the rate of absorption remained constant throughout this period, and the amount of drug absorbed was directly proportional to the concentration over a ten-fold range, it was concluded that absorption occurs by passive diffusion. But according to studies done by Banerjee and Chakrabarti (1976), using mouse ileum, it was found that tetracycline uptake is apparently an active process.

The extent of absorption of oxytetracycline in domestic animals depends on the type of preparations as reported by Maritim et al (1986). These workers compared two oxytetracycline preparations- Aquacycline^{R} and Terramycin^{R}-100 and their findings supported the claim that Aquacycline^{R} has better bioavailability than Terramycin^{R}-100 in domestic species.

The oral absorption and tissue penetration of tetracyclines are largely influenced by their lipophilicity. According to Aronson (1980),Doxycycline, classified as a tetracycline, has the ability to be absorbed well from the intestinal tract and concentrates in tissues to a greater extent.

The concentration of oxytetracycline is about ten times greater in the whole egg if given via IM route than oral. This is due to incomplete intestinal absorption of oxytetracycline in case of oral route (Roudaut et al, 1987).

The absorption will be greatly retarded in the presence of calcium and magnesium ions since these divalent metals form insoluble chelates with the drug. Absorption is also impaired to a variable degree by milk and milk products (Neuvonen, 1976). The percentage of an oral drug that is absorbed on an empty stomach in humans has been found to be high for minocycline (100%) and doxycycline (95%); intermediate for oxytetracycline, demeclocycline and tetracycline (60-80%) and lowest for chlortetracycline (30%) (Barza and Scheife, 1977). Plasma concentrations of tetracycline and oxytetracycline administered IM are detectable within 15 minutes, reach a peak within one hour, maintain significant blood concentrations (about 0.5 mg/ml) for about 12 hours, and then decline to trace amounts approximately 24 hours after injection. For maximum blood concentration in food producing animals, not more than 10 ml should be injected in a single IM site .

In case the drug is prepared together with a local anaesthetic, it should not be administered IV because local anaesthetics interfere with cardiac conduction mechanisms.

Oxytetracycline and chlortetracycline are also absorbed, to a slight extent into the blood stream after intramammary infusion. In humans, it has been found that blood plasma concentrations of 0.5-1 mg/ml are adequate for treatment of disease conditions.

2.5.2. Distribution and Biotransformation

Tetracyclines undergo metabolism to various degrees, up to 70%, with as much as 30% being excreted unchanged in faeces. The most frequently identified substance in urine, faeces and tissue is the parent tetracycline .

Wanner et al (1990) reported that bioavailability of oxytetracycline given in medicated feed to piglets, was increased by 26% when the feed contained 1.5% citric acid.

Due to binding to plasma proteins, the volume of distribution of tetracyclines is less than that for total body water (TBW). Tetracyclines are reversibly bound to plasma proteins and are widely distributed. (Blood or plasma values of most antibacterial drugs are of some value as an index of the amount of drug in the body. However, the quantity of drug in plasma or blood is not necessarily a valid measure of the amount of drug in the tissues.). They are removed from the blood by the liver and high concentrations are observed in the parenchymatous organs (such as the kidney, spleen and lung) and in bile. All the tetracyclines are concentrated by the liver and then excreted by way of bile,

into the intestine from which they are partially reabsorbed. At any one given time, the concentration of the drug in bile is ten times higher compared to the concentration in plasma (Acocella et al, 1968). Bile concentration may be 30 times that of blood. They are thus the antibiotics of choice in the treatment of *Leptospira icterohaemorrhagiae* infection.

The drug is also deposited in bones at the active ossification sites. It has been found that the concentration of the drug in foetal blood is approximately one-half that in maternal blood (Huber, 1988).

Of the three common tetracyclines (oxytetracycline, chlortetracycline and tetracycline) only tetracycline can diffuse into the cerebrospinal fluid (CSF) with ease, but only during infection of the meninges (Huber, 1988).

Some tetracyclines also find their way into the prostatic fluid and this can be helpful in the treatment of microbial diseases of the prostate.

Tetracyclines also accumulate in teeth and this can cause teeth discoloration. They are also incorporated in chicken and turkey egg shells during production of eggs in the abdominal cavity.

The tetracyclines also reach the synovial fluid and the mucosa of the maxillary sinus, where the concentrations are close to those of plasma (Parker and Schmid, 1971; Lunderberg et al, 1974).

Vaughan et al (1981) carried out experiments to evaluate plasma and tissue concentrations of oxytetracycline given IV in

three horses. They found that the drug gained access to pulmonary tissue at approximately the same concentration as did that in plasma. Table 1 shows some pharmacokinetic parameters of tetracyclines:

Table 1: Some pharmacokinetic parameters of tetracyclines.

Drug	Serum half-lives (hr)	Volume of distribution (L/kg)	Clearance (ml/kg/hr)
Oxytetracycline			
Horse	10.5	1.4	89
Cattle	9.7	0.8	57
Dog and cat	6.0	2.1	254
Human	9.5	-	-
Tetracycline			
Dog and cat	5.5	1.2	159
Human	10.6	1.5	102
Minocycline			
Dog	6.9	1.9	198
Human	17.5	0.4	18
Chlortetracycline	5.5	-	-

Adapted from Huber(1988).

2.5.3. Excretion

For most tetracyclines, the primary route of excretion is through the urine. A proportion is eliminated via the faeces (about 10% of the dose). The principal excretory organ for doxycycline is the intestine, where the drug diffuses through the intestinal mucosa into the intestinal tract (Aronson, 1980). This unique characteristic makes this drug useful in cases of pre-existing renal dysfunction and may render this drug superior to other tetracyclines in the treatment of intestinal infections. It has been shown that approximately 60-70% of a single dose can be found in urine. Following oral administration, the highest urine concentration obtained is between 2 and 8 hours, but antibacterial activity can be detected for three days or more after therapy is discontinued (Booth, 1988).

Tetracyclines are also eliminated in milk, the concentration being about one-half that of maternal serum. Orally and parenterally administered tetracyclines are eliminated in bovine milk. In studies done by Mol (1975), it was found that most tetracyclines pass freely from the blood to milk. Slee and Brightling (1981) reported that oxytetracycline boluses placed in the uteruses of cows with puerperial endometritis or retained placenta produce oxytetracycline concentrations in the milk from 66% of the treated animals.

The amount of drug excreted into the egg-yolk mainly depends on the lipid solubility of the drug (Blom, 1975). Thus a drug such as doxycycline which has a high lipid solubility causes important

residual concentrations in the egg (0.73 μg/g) and a long elimination period of 19 days (Archimbault et al, 1983).

2.6. Uses of tetracyclines

In Veterinary Medicine, tetracyclines have been used for the treatment of mastitis, coliform infections, pasteurellosis, leptospirosis, fusiformis infections, actinomycosis and actinobacillosis. The tetracyclines (usually oxytetracycline and chlortetracycline) are the only effective specific compounds approved for use against anaplasmosis in the United States (Kuttler, 1980).

Tetracyclines have also been used in poultry husbandry because of their broad antibacterial spectra. Oxytetracycline, tetracycline and chlortetracycline have been widely used to control poultry diseases such as chronic respiratory disease, infectious synovitis, infectious coryza and fowl typhoid. These tetracyclines are deposited in eggs when given to laying hens via the drinking water or feed. Tetracyclines are usually deposited in higher concentrations than oxytetracycline after medication via drinking water (Nogawa et al, 1981).

To treat the birds, two routes of administration may be considered- the oral route and the intramuscular (IM) one. Mass treatment through the drinking water is favoured in large poultry farms when quick and effective therapy is required.

Chlortetracycline was first added to the feed of laying hens in 1953 and has been used extensively in egg layers because of its reported efficacy in increasing egg production (Katz et al, 1972).

It is used at levels of 50 to 100 ppm of feed to improve feed efficiency related to egg production, to maintain or increase egg production, and to improve hatchability of eggs. It is not fed to laying birds at levels above 100 g/ton and is no longer used in France except for therapeutic purposes (Roudaut et al, 1989).

In human medicine, tetracyclines have been used for the treatment of bacterial infections such as brucellosis, cholera, chancroid and granuloma inguinale. They are also the drugs of choice for rickettsial infections- typhus, scrub typhus and spotted fever (although chloramphenicol also appears to be effective) (Huys et al., 1973). In Q fever endocarditis, tetracyclines are effective, though they may have to be administered for an indefinite period (Wilson et al., 1976). They are also used for the treatment of chlamydial infections such as psittacosis, lymphogranuloma venereum, trachoma and inclusion conjunctivitis. In case of non-gonococcal urethritis a high dose is required (Dunlop, 1977). Tetracyclines are also used as an alternative drug in patients allergic to penicillin, although often, another antibiotic is more specifically indicated.

Table 2 shows a list of diseases treated with tetracycline antibiotics, although the list is not exhaustive.

Table 2: Diseases treated with tetracyclines.

Disease	Aetiological agent
Actinobacillosis	*Actinobacillus lignieresii*
Actinomycosis	*Enterobacter bovis*
Anaplasmosis	*Anaplasma marginale*
Anthrax	*Bacillus anthracis*
Canine brucellosis	*Brucella canis*
Blackleg	*Clostridium chauvoei*
Bacillary hemoglobinuria	*Clostridium hemolyticum*
Infectious necrotic hepatitis	*Clostridium novyi*
B,C,D.Enterotoxaemia	*Clostridium perfringens*
Malignant edema	*Clostridium septicum*
Tetanus	*Clostridium tetani*
Heartwater disease	*Cowdria ruminantium*
Canine bartonellosis	*Haemobartonella canis*
Respiratory infections	*Hemophilus* spp.
Leptospirosis	*Leptospira* spp.
Listeriosis	*Listeria monocytogenes*
Bovine infectious keratitis	*Moraxella bovis*
Mastitis, pasteurellosis	*Pasteurella hemolytica*
Pasteurellosis, fowl cholera, hemorrhagic septicemia	*Pasteurella multocida*
Shigellosis of foals	*Actinobacillus equuli*
Mastitis, synovitis	*Staphylococcus aureus*
Mastitis	*Streptococcus agalactiae, Streptococcus dysagalactiae, Enterobacter aerogenes*
Strangles	*Streptococcus equi*
Ovine vibriosis	*Campylobacter fetus*

Tetracyclines have recently found clinical usage in the following diseases: canine ehrlichiosis and canine pancytopenia (Tetracycline); Bovine hoof diseases, East Coast Fever, porcine atrophic rhinitis and porcine balantidiosis (oxytetracycline) (Booth, 1988). Tetracyclines are also widely used in animal husbandry as growth promoters (also called growth permitters) in poultry and other animals reared under commercial conditions, apart from for prevention of disease. If used this way, they are administered orally as part of the feed or drinking water, at subtherapeutic doses. The result is improved efficiency of feed-conversion or the rate of weight gain or both.

There is a reported case of oxytetracycline helping in uterine involution after 500 gm/tablets were administered intrauterine in a group of 28 cows (Onuma et al, 1990).

2.7. Drugs and Chemical Residues

A residue of a parent drug or chemical and its metabolites may accumulate and be deposited or stored within the cells, tissues or organs or even the other products such as eggs, following the administration of the compound in control and treatment of animal diseases or a feed additive used to promote growth and feed efficiency.

It is very important that unacceptable levels of drug and chemical residues do not occur in either meat, milk or eggs, hence the need to observe withdrawal periods (also called depletion or clearance period). This responsibility lies heavily on veterinarians and livestock producers. As previously mentioned

in the introduction, it is important from a public health point of view, because levels of residues exceeding the legally permitted limit may produce injurious effects when consumed over a long time (Jackson, 1980).

With the great strides achieved in the improvement in production of food crops and animals, the chances of humans being continuously exposed to drug and chemical residues for a long time is unequivocally increased (Booth, 1988).

The amount of work reported in the literature prior to 1970 on tissue residues is not extensive. With the exception of analytical procedures published primarily by drug firms, there has been little work involving studies on drug residues in food-producing animals (Booth, 1988).

Drug residue concentrations vary greatly from tissue to tissue and are generally observed to be higher in storage tissues such as body fat or in organs that actively metabolize and excrete them. It seems man is more adversely affected through occupational exposure and accidents associated with application of agricultural chemicals, than through residues in edible animal tissue. This is exemplified by the poisoning by polychlorinated biphenyls (PCBs) of people in Yusho, Japan in 1968 (Kuratsune et al., 1972) and one famous case where seafoods were heavily contaminated with a carcinogenic substance (methylmercuric sulphide) was the Minamata Bay disaster.

In order to safeguard public health, several countries such as the United States of America, conduct a residue-monitoring programme to determine the frequency of occurrence of

residues from drugs, pesticides and environmental contaminants in the commercial meat and poultry supply from slaughter houses (Engel, 1980).

2.7.1. Antibiotics

The misuse of antibiotics that might result in deposition of residues in meat, milk and eggs must not be permitted in food intended for human consumption. With adherence to the present Food And Drug Administration (FDA) regulations, risk of toxicity from antimicrobial residues in food is so negligible it can be disregarded (Hewitt, 1975). There are several tests that can be used to determine whether tissues such as kidneys and livers are contaminated by antibiotics. Some of these tests have been described by Dubbert (1984).

The World Health Organization Expert Committee on the Public Health Aspects of the Use of Antibiotics in Food and Feedstuffs concluded that an antibiotic concentration of 20 ppm used singly or in combination in feeds (on a dry-matter basis) is adequate for promotion of growth and feed-conversion uses (WHO, 1969).

Addition of antibiotics to feed should generally be restricted to the age spans proposed by the World Health Organization as shown in table 3.

Table 3: Age span recommended for addition of antibiotics to feed.

Species	Age
Poultry, with exception of ducks and geese	8-10 weeks
Swine	4-6 months
Calves	3 months
Beef cattle	3 months
Lambs	2 months
Fur-bearing animals	2-3 months

Source: WHO, 1969.

The periods of elimination of tetracycline (TC) and chlortetracycline (CTC) from eggs after discontinuation of treatment are given in table 4:

Table 4: Elimination period (days) of tetracyclines in eggs.

Antibiotic	Route	Dosage	Albumen	Yolk	Whole egg
TC	Water	0.5 g/l	2	9	2
		0.25 g/l	1	6	0
	Feed	600 ppm	2	11	4
		300 ppm	1	8	2
CTC	Feed	600 ppm	5	9	0

Adapted from Roudaut et al (1989).

The elimination lasts longer for the yolk (up to 11 days) as compared to the albumen, since the antibiotic accumulates in the pool of follicles (Siegmann, 1982).

It has been shown that the age of the hen has no effect on the clearance rate of oxytetracycline from the egg (Roudaut et al, 1987).

Tetracycline withdrawal times should be observed for food producing animals, as should all drugs with withdrawal times (See table 5).

Table 5: Withdrawal periods of tetracyclines in different species.

Species	Type of Tetracycline	Withdrawal time (days)	Limitations for use	Tolerance level (ppm).
Cattle	Oxytetracycline hydrochloride (Injectable)	28	Do not use in lactating dairy cattle	Edible tissue 0.1
	Chlortetracycline 11 mg/kg/day(oral).	10	Use only in mature beef cattle and /or non-lactating dairy cows.	Meat-0.1 Fat & milk-0
	Chlortetracycline hydrochloride	3	Use only in calves.	Liver and kidney-4. Muscle and fat-1.
Sheep, goats	Chlortetracycline	2	-	Kidney-1 Meat and liver-0.1
	Tetracycline	-	-	0.25
Swine	Oxytetracycline (Injectable)	26-28	-	0.1
	Oxytetracycline (Oral)	26		Edible tissues-0.1
	Chlortetracycline hydrochloride	5-10		Muscle-1 Fat-0.2 Kidney-4 Liver-2.

Table 5 (cont'd)

Chickens	Chlortetracycline	1	Do not use in egg-laying birds when fed at 500 g/ 900 kg feed.	Kidney-4 Muscle, fat, liver, skin-1 Eggs-0.
	Oxytetracycline	0	50-200 g/900kg feed.	Kidney-3 Muscle,fat, liver, skin-1
Turkeys	Chlortetracycline	-		Kidney-4 Meat, fat, liver-1 eggs-0.
	Oxytetracycline	5	Do not use in egg-laying birds unless eggs are for hatching only.	Kidney-3 Muscle, fat, liver, skin-1
Ducks	Chlortetracycline	-	-	Kidney-0.4 Muscle, fat, liver,skin-1.

Adapted from Booth (1988).

Note: **Tolerance Level**: (also called Maximum Residue Limit). This is the maximum allowable level or concentration of a drug or chemical in or on feed or food at a specified time of slaughter and harvesting, processing, storage and marketing up to the time of consumption by animal or man (Booth, 1988).

Tetracycline levels in feed in the range of 5-20 ppm does not seem to produce residues in edible tissues (WHO, 1969). However, detectable levels have been found in the osseous tissues of pigs, calves and chickens fed 5-80 ppm. Tetracyclines are present in bones of chickens that are fed only 5 ppm for no longer than 3 days. Tetracycline has also been detected in bones at the level of 5.5 ppm in chickens fed 9.2 ppm for 56 days, 0.52 ppm in swine fed 30 mg/day for 96 days and 1.79 ppm in calves

fed 60 mg/day for 56 days (Booth, 1988). Oxytetracycline is detectable in bones of swine following feeding of 200 ppm after a withdrawal period of 3 weeks (Jones et al., 1977).

Meredith et al (1965) found the largest amounts of chlortetracycline in liver, then breast and then thigh tissue when assayed for chlortetracycline. Tissue assays for oxytetracycline revealed that it was not taken up as extensively as chlortetracycline and the largest amounts were found in the liver, then breast. Oxytetracycline residues were seldom found in the thigh tissue.

In birds fed 200 ppm oxytetracycline or chlortetracycline and a low calcium diet, the residues can be detected when the liver is assayed. The levels in the liver will be even higher than in breast or thigh muscles of chicken fed 1000 ppm and a high calcium diet. This is because in the digestive tract, tetracyclines form complexes with bivalent ions such as Ca^{2+}, which markedly diminishes their absorption (Meredith et al, 1965).

Residue levels of chlortetracycline and oxytetracycline are higher in birds fed a low calcium diet compared to those fed a high calcium diet, even if the high calcium diet birds are fed 5 times more antibiotic (Meredith et al, 1965).

Experiments show that storage at $+4^0C$ of livers for 2 weeks from broilers fed antibiotics lead to a decrease in antibiotic content in all cases, though chlortetracycline livers are more resistant to deterioration than oxytetracycline livers at the refrigeration temperatures (Meredith et al, 1965).

At a residue level of 1 ppm, the tetracyclines are not likely to produce a toxic effect in humans; a level of 5-7 ppm might be toxic (WHO,1969), while the use of 5-20 ppm in the feed of animals may induce resistance to Enterobacteriaceae.

Tetracycline hydrochloride, given IV to lactating dairy cattle in an average dose of 4 mg/kg will result in excretion of the antibiotic in milk for approximately 36 hours after treatment (Booth, 1988).

For public health reasons 50-200 gm/900 kg feed of oxytetracycline must be used. This will give a tolerance level of 3 ppm for the kidney and 1 ppm for the muscle, liver, fat and skin (Booth, 1988). In eggs, the tolerance level as stipulated by WHO/FAO, is 0.2 ppm.

In the 36th report of the Joint FAO/WHO Expert Committee on food additives (WHO, 1990) new Maximum Residue Limits (MRL) were recommended: these are 0.1 mg/kg for milk and muscle, 0.01 mg/kg for fat, 0.2 mg/kg for eggs, 0.3 mg/kg for liver and 0.6 mg/kg for kidney.

After IM injection, Huber (1971) proved that oxytetracycline residues remained for 3 or 4 days after a 44 mg/kg dosage.

Raynaud et al (1976) gave experimental birds 1000 ppm Terramycin® (Pfizer), the amount they found necessary for an intake of 55 mg/kg body weight. From this experiment, they recommended a withdrawal period of 3 days for eggs and 10 days for meat.

Withdrawal periods become unexpectedly longer when antibiotics are given via the drinking water in tropical countries (Yoshimura et al, 1991).

Bjorklund et al (1990) have also studied oxytetracycline residues in wild fish [roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*)]. Oxytetracycline residues were detected up to 13 days post medication.

Methods involving low temperature heating fail to eliminate all antibiotic residues in eggs. Because of the danger of developing hypersensitivity or alteration of normal flora resulting from eating antibiotic-fed poultry, it is advisable that the meat and eggs receive proper and adequate heat treatment (Meredith et al, 1965). Withdrawal times aim to eliminate hazardous residues. Tolerances have been proposed (see table 3) which will take this requirement into account.

The acceptable limit for tetracycline and oxytetracycline, as proposed by the Joint FAO/WHO Expert Committee on Food Additives (1990) is 0.2 µg/g while that for chlortetracycline is 0.05 µg/ml. The difference is due to the fact that the sensitivity threshold of the assay methods is low, not because chlortetracycline is more toxic than the other tetracyclines (Roudaut et al, 1989).

2.7.2. Antibiotic residue detection.

Less work has been reported on drug residues in eggs but several workers have studied the problem of potential residues resulting from feeding tetracyclines to egg layers. Few studies

have been done on the elimination of oxytetracycline from hen egg following treatment through drinking water, though more studies have been done through feed. Both the experimental conditions and the sensitivity of the methods used prevent the generalization of the results. The sensitivity of the analytical method is very important for the estimation of the elimination period of residues.

Yoshimura et al (1991) obtained oxytetracycline threshold sensitivities in yolk and albumen of 0.3 and 0.07 µg/g respectively. For doxycycline, they found 0.15 and 0.04 µg/g. Several detection limits have been forwarded: 0.27 µg/g (Yoshida et al, 1973) and 0.1 µg/g (Katz et al, 1972).

In albumen, residues can be detected as early as the day after administration. In yolk the residues appear 3-4 days after the beginning of the treatment for the oral route and 2 days for the IM one. The concentrations reach a maximum 1-2 days after withdrawal of the treatment and rapidly decrease exponentially according to Yoshida et al (1973). Roudaut et al (1987) could detect oxytetracycline residues in both yolk and albumen after feeding 250 ppm (0.25 g/l).

Experiments to investigate antibiotic residues in eggs were done as early as 1953 by Durbin et al (1954), who were only able to detect antibiotic residues in eggs of hens fed over 500 ppm.

Katz et al (1972) fed chlortetracycline continuously to laying hens at levels of 0, 50, 100, 150 and 200 g/ton (ppm) for a period of four months and after assaying for potency by a

chemical fluorimetric procedure, they obtained clear zones only for those chicken fed 200 g/ton.

After feeding a premix of oxytetracycline containing 81,600 ppm of oxytetracycline and mixed in the basal diet at the level of 0, 20, 500, 1000, 2000 and 4,000 ppm, Yoshida et al (1973) did not detect oxytetracycline in both albumen and yolk of the eggs of hens fed the diet containing 20 ppm of oxytetracycline, which is the maximum dose allowed by WHO/FAO in feed for growth promoting purpose. They could only detect a small amount of oxytetracycline (0.28 μg/g, which is only 0.01 μg above the threshold sensitivity level of 0.27) from eggs of hens fed 500 ppm of the antibiotic. They also found that in the case of birds fed 4,000 ppm, the increase in oxytetracycline content in egg white was very rapid and reached a plateau after 4 days of oxytetracycline feeding. After the withdrawal of dietary oxytetracycline, content of oxytetracycline in egg white decreased rapidly and no oxytetracycline was detected on the third day after the withdrawal. On the other hand, oxytetracycline content in egg yolk increased almost linearly after 3 days on oxytetracycline feeding and decreased also linearly after the withdrawal of dietary oxytetracycline.

Roudaut et al (1987) determined the kinetics of oxytetracycline elimination into eggs separately for albumen and yolk after oral administration through either drinking water (0.1-0.25 and 0.5 g/l for five days) or feed (300 and 600 ppm for 7 days) or after IM injections (3 x 15 mg/kg body weight and 3 x 30 mg/kg body weight) 24 hours apart. They found a detection

threshold of 0.07 µg/g for albumen and 0.2 µg/g for yolk. In all cases, the elimination period lasted longer for yolk and it varied between 0 and 10 days after treatment was discontinued, according to administration routes and dosages.

Roudaut et al (1989) also studied the effect of orally dosing laying hens with both tetracycline and chlortetracycline. They obtained a sensitivity threshold of 0.07 µg/g in albumen and 0.15 µg/g in yolk for tetracycline and 0.01µg/g in albumen and 0.06 µg/g in yolk in the case of chlortetracycline. Drug excretion via egg was 3-fold higher for tetracycline than for chlortetracycline, and 75% of the total amount of drug was excreted preferentially into the yolk. They found an elimination period of 6-11 days for tetracycline and 9 days for chlortetracycline after treatment.

The most recently reported work to detect drug residues in eggs and employing the microbiological assay with *B. cereus* var *mycoides* ATCC 11778 as test organism has been done by Yoshimura et al (1991) who dissolved 0.5 g/l of doxycycline and oxytetracycline in drinking water and supplied it to laying hens for 7 consecutive days. They found that the concentration of both antibiotics increased in yolk daily, reaching a peak 2 days after withdrawal and then declined gradually, while peak concentrations for albumen occurred in the middle stage of medication. Doxycycline was detected in albumen until 24 days after withdrawal and for 2 days more in yolk than in albumen. Oxytetracycline appeared in yolk 3 days after commencing medication and was detected in yolk until 9 days after withdrawal. The depletion period of oxytetracycline was shorter

for the albumen, where the residue disappeared in all eggs 6 days after withdrawal. The workers concluded that doxycycline was deposited in higher concentrations than oxytetracycline and lasted for a longer period in eggs. This was due to the greater lipophilicity of doxycycline.

2.8. Undesirable effects of tetracyclines

Over the years, there has been much concern about the possible hazards to human health posed by the use of antibiotics, particularly tetracyclines, for animal husbandry purposes (Anderson, 1968; Drews and Hogenauer, 1977). Among these are:-

2.8.1. Hypersensitivity

Hypersensitivity reactions have been reported for many antibiotics including tetracyclines. However they are not as common as those due to penicillins. These reactions can be fatal if they are very severe (Anderson, 1968).

2.8.2. Resistance factors

Resistance to drugs develops due to the fact that the antibiotics are present in the body in subtherapeutic levels. It has been shown that resistance to tetracyclines by bacteria develops very readily and persists for a long time. This resistance was first detected around 1950 in staphylococci (Lowbury et al., 1952). Resistance in bacteria is mediated via plasmids which are extra-chromosomal hereditary determinants (In rare instances, the resistant factor may be located on the chromosomes). All those so far isolated from bacteria exist as double-stranded DNA cycles, and are designated R-factors (Broda, 1979). Examples of bacteria with plasmids are: *E. coli*, *Shigella*, *Salmonella typhimurium*, *Pseudomonas* and *S. aureus*.. Plasmid-mediated resistance is usually due to enzymes that

modify the antibiotic. Semi-synthetic drugs are usually designed in such a way that the bacterial enzymes cannot interfere with their structure. In the case of tetracyclines, it has been found that the bacteria alter the transport system such that the drug doesn't enter the bacterial cell (Broda, 1979).

There can be transfer of resistance from a pathogenic micro-organism to a hitherto non-pathogenic one. This transfer is effected in three main ways:-

a). *Conjugation*- This occurs when there is transfer of resistance to another bacterial host of the same or different species.

b). *Transduction*- In this case, donor strains produce transducing phage particles via infection with a lytic bacteriophage or by induction of a prophage.

c). In the third method, the resistant bacterial strain simply passes from one person/animal to another, carrying its plasmids with it.

Apart from staphylococci, resistance has been reported in case of clostridia and *Escherichia* in pigs and poultry (Hudd, 1978) . Several surveys have confirmed that tetracycline-resistant strains of *E. coli* are abundant in animals and poultry produced under intensive rearing conditions (Linton, 1977).

Although there have been claims that it is the use of tetracyclines for growth promotion purposes in farm animals which has had the chief part to play in generating populations of resistant bacteria, this theory is no longer considered valid. But

the claims were so overwhelming that many countries introduced legislation forbidding the addition of tetracyclines in animal feeds for growth promotion purposes. In fact, in 1976, tetracyclines were removed from the list of approved feed additives in EEC countries (Mitema, 1985).

Antibiotic-resistant strains of enteric bacteria have been isolated more frequently from cattle given low feed concentrations of tetracyclines than from those fed drug-free rations. Little has been reported on the development of bacterial resistance in livestock given injectable antibiotics (Stabler et al, 1982). In Minimum Inhibition Concentration (M.I.C.) studies, Kariuki (1991), observed that 93% of human strains of *Salmonella typhimurium* and 83% of bovine strains were resistant to oxytetracycline.

Sprunt (1977) reported that persons have fewer resistant organisms when intermittent doses of antibiotics were injected IM, rather than taken orally.

R-factor tetracycline resistance is caused by defective drug uptake by bacteria. Tetracycline R-factor resistance bacteria have become efficient in excluding the drug and thus have become resistant. Tetracycline resistance factor can be transferred from a resistant organism to a non-resistant one as shown by Latour et al (1981). Cross-resistance exists within the tetracycline group of antibiotics.

2.8.3. Immunosuppression

Recently, it has been reported that tetracyclines can cause immunosuppression in domestic animals. Smith et al (1983) reported this effect in calves vaccinated with *Brucella abortus* strain 19. Chlortetracycline, when added to poultry feed at concentrations of 50-200 gm/kg has reduced the immune response to *Mycoplasma synoviae* (Booth, 1988).

2.8.4. Hepatotoxicity

According to work done by Schultz et al (1963), pregnant women appear to be more susceptible to liver damage due to tetracyclines. A post mortem examination shows a characteristic severe diffuse fatty infiltration of the liver. Of the tetracyclines, oxytetracycline and tetracycline appear to be less hepatotoxic.

2.8.5. Nephrotoxicity

Oxytetracycline has also been implicated in nephrotoxicoses of feedlot calves (Lairmore et al., 1984), although excessive doses were used. Histopathological examination revealed cortical tubular nephrosis.

2.8.6. Gastrointestinal disturbances

In humans, after oral administration, tetracyclines can cause irritation of the gastrointestinal tract although the severity depends on the dosage as well as on the type of tetracycline. There are also individual variations. The clinical signs include nausea, vomiting, abdominal discomfort and epigastric burning.

In animals, the most affected species are cats and horses where the clinical signs include diarrhoea, colic, fever and anorexia (Wilkinson, 1968; Cook, 1973). In horse, the situation can prove fatal.

In ruminants, the main effect of oral administration of tetracyclines is alteration of the normal microflora. This leads to anorexia and diarrhoea.

Apart from the problems above, tetracyclines can also lead to superinfections within the gut. The antibiotics depress the growth of the normal gut microflora, leading to the proliferation of antibiotic resistant micro-organisms such as *Candida albicans*, various strains of *Proteus* and staphylococci.

2.8.7. Miscellaneous effects

Tetracyclines have been reported to cause yellowing or browning of teeth as well as dental hypoplasia, although oxytetracycline is considered the main culprit (Weyman, 1965; Moffit et al., 1974). Studies done by Klingeren (1977) showed that the semi-synthetic derivative of oxytetracycline, doxycycline only affects teeth to a negligible degree.

Tetracycline injections are painful and there can be tissue damage at the site of injection (Immelman et al., 1978). When a single vein is used repeatedly for a long time, for IV administration, this can lead to the development of thrombophlebitis which can be fatal. Oxytetracycline causes tissue irritation as shown by Nouws et al (1990) who injected 20% and 10% oxytetracycline (Terramycin/LA® and

Engemycin®) intramuscularly into calves, sheep and pigs. There was no tissue irritation when Engemycin® was dissolved in polyvinylpyrrolidone.

Rapid IV injection of oxytetracycline in cattle can produce acute collapse (Gross et al., 1979). This may be due to the ability of the tetracycline to chelate divalent ions, especially calcium (Cohen et al., 1970).

It has also been shown that tetracyclines given to infants can cause increase in intracranial pressure, leading to the bulging of the fontanelles. Claims that tetracyclines may cause corneal discoloration and lens opacities in newborn animals if administered during pregnancy have been disproved by Maritim (1985). Use of chlortetracycline for intramammary infusion in dairy cows during the dry period is contraindicated because of tissue irritation (Thompson and Leaver, 1972).

2.9. The Kenyan Situation

As in other countries, tetracyclines are the main types of drugs used in Kenya. In poultry, tetracyclines are either added to feed or drinking water, the main one being oxytetracycline.

Commercially, oxytetracyclines are marketed under some of the following trade names: Poltricin® (Cosmos), Egocin® (Dawa), Bioxin® (TEVA), Super Skajcycline®, and Ngombemycin® (Monks),e.t.c. Table 6 summarizes some facts about the products:

Table 6: Some poultry products containing oxytetracycline sold in Kenya.

Trade name	Active ingredient	Composition	Contents	Indication/dosage
Poltricin ®	OTC-HCl	Each 500 gm contains 20 gm OTC-HCl	125 gm	Growth-one teaspoonful/ 5 litres of water. Stress- as above for one week.
Egocin®	OTC-HCl	Each 250 gm contains 13.78 gm OTC-HCl	125 gm	For increased egg production and protection against disease. Young layers- half 5 ml teaspoonful in 5 litres of water, or 2.5 kg feed at start of laying for 5-7 days.
Bioxin®	OTC	Each 1 kg contains 55 gm OTC	250 gm	2-4 gm per litre of drinking water for 7-10 days.

Table 6 (cont'd).

Super Skajcyclin e®	OTC	Each 100 gm has 5.5 gm OTC	100 gm	One level teaspoonful per 5 litres drinking water for 5-7 days.
Ngombemycin®	OTC	1 gm contains 50 mg OTC	125 gm	Use 1 teaspoonful per 5 litres drinking water for 7-14 days.

OTC-HCl = oxytetracycline hydrochloride.

NB. Concentration of oxytetracycline in the products is about 20 mg/g.

Chapter 3

STANDARDIZATION OF BACTERIOLOGICAL PROCEDURES

3.1 Source and preparation of bacteria.

The bacterium used was *Bacillus cereus* var. *mycoides* (American Type Culture Collection) ATCC 11778 (Dept. of Microbiology & Immunology, Norwegian College of Vet. Med., Oslo, Norway) which had been adapted in our laboratories. It was reactivated according to the manufacturer's instructions with the following modifications: The ampoule was opened asceptically and 0.7 ml of Tryptic Soy Broth (TSB) was added into the ampoule. The bacteria was then inoculated from the ampoule into Mueller Hinton agar (Difco Labs, Detroit, Michigan, U.S.A.), Blood Agar and Todd Hewitt Broth. Isolated colonies were obtained after incubation for 24 hours at 30°C, after which single colonies were inoculated into Cooked Meat Medium (CMM) and also onto Tryptose Soy Agar (TSA) slants for storage as stock culture.

The bacterium was streaked from the stock culture onto Mueller Hinton agar plates and incubated at 30°C for 18 hours. This procedure was repeated for three consecutive days until pure colonies were obtained.

A single colony was then inoculated into 10 ml of Mueller Hinton broth and incubated at 30°C for 18 hours. The number of spores in such a broth was determined, using spectrophotometry, to be approximately 1×10^5 spores/ml.

3.2. Sensitivity of Test Organism.

The sensitivity of *B. cereus* var. *mycoides* type ATCC 11778 (Dept. of Microbiology & Immunology, Norwegian College of Vet. Med., Oslo, Norway) adapted in our laboratories was tested using a multodisc. The medium used was Mueller Hinton agar. Two types of multodiscs were used: KQ 2/4 and KQ 3/4.

3.3. The effect of freeze-drying of eggs and of mixing tetracyclines and sulphonamides.

A suspension of sulfadimidine (Cosmos, Nairobi) of concentration 50 mg/ml was prepared. Oxytetracycline of concentration 5µg/ml yolk suspension was also prepared, a portion of which was freeze-dried to test the effect of freeze-drying on the sensitivity. Two ml of each of the above was mixed to form a third preparation.

After the incubation box had been prepared, 200 µl of each of the above preparations was pipetted into the wells made on the Mueller Hinton agar. The box was then incubated at 30^0C for 18 hours.

3.4. The effect of freeze-drying egg samples and differentiation of oxytetracycline zones of inhibition from those of sulphonamides.

Different concentrations of oxytetracycline in yolk suspension, 2.5 µg/ml, 5 µg/ml and 10 µg/ml, were prepared using analytical grade oxytetracycline hydrochloride (Sigma, USA).

Sulfadimidine (Cosmos, Nairobi) of concentration 0.125 g/ml was also prepared.

A portion of each of the three concentrations of oxytetracycline in yolk suspension was freeze-dried after which a portion was reconstituted with phosphate buffer, pH 4.5. Then 200 µl of each of the above preparations (oxytetracycline and sulfadimidine) was pipetted into wells dug on Mueller Hinton agar in the incubation box. The box was incubated at 30^0 C for 18 hours.

3.5 Results

3.5.1. Multodisc sensitivity

Tables 7 and 8 show the sensitivity of *B. cereus* to different drugs incorporated in the multodics.

Table 7: Sensitivity tests using multodisc KQ 2/4.

Antibiotics contained	Result
Tetracycline	+++++
Ampicillin	+
Sulfatriad	(+)
Gentamycin	+++
Streptomycin	+++
Kanamycin	++
Chloramphenicol	++
Cotrimoxazole	-

Table 8: Sensitivity tests using multodisc KQ 3/4.

Antibiotics contained	Result
Tetracycline	+++++
Nitrofurantoin	+++
Nalidixic acid	(+)
Streptomycin	+++
Sulfatriad	(+)
Cotrimoxazole	-
Gentamycin	++++
Ampicillin	++

Key:

+ = sensitive (number of +s depicts extent of sensitivity).

(+) =slightly resistant.

- = resistant.

The two drugs of interest were tetracycline and sulfonamides because they are some of the most widely used poultry antimicrobials in Kenya. From the results, it was concluded that the microorganism was most sensitive to tetracycline and slightly sensitive to sulfonamides. It was therefore easy to distinguish between inhibition zones due to tetracycline and those due to sulphonamide drugs by observing the zone outline.

3.5.2. Zones of inhibition following freeze-drying and mixing tetracyclines and sulphonamides

It was observed (fig. 1 and table 9) that when sulphonamides and oxytetracycline were mixed (C_1 and C_2), different zones made by each drug were identifiable. The inner zone was that of oxytetracycline and it has a clearly demarcated outline while the outer zone was that of sulphonamides and had a blurred outline. This result could only be achieved by using different concentrations of each drug.

The effect of freeze-drying is depicted by **B** and **D**. It was observed that the diameters of zones of inhibition of the freeze-dried samples (**D**) were wider than those of the none-freeze-dried ones (B). Freeze-drying, therefore, increased the drug concentration in the sample. **E** indicates that the outline of oxytetracycline zones are generally smooth.

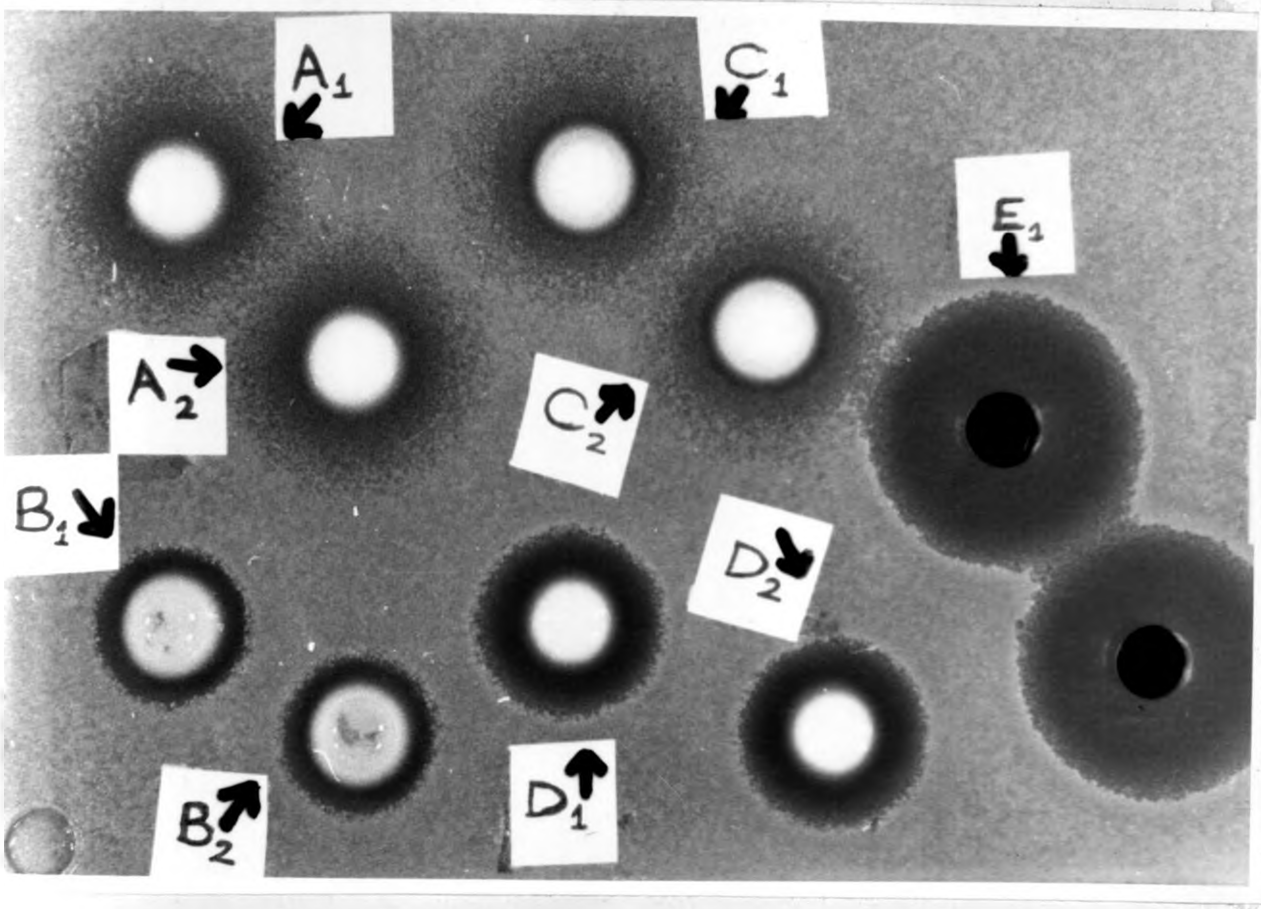


Figure 1: Zones of inhibition obtained following freeze-drying and mixing tetracyclines and sulphonamides.

Key:

A_1,A_2 - 50 mg/ml sulfur; **B_1, B_2** - 5 µg/ml Oxytetracycline; **C_1,C_2** - Sulfur and oxytetracycline mixture; **C_{11}, C_{21}**- Sulfur zones (Outer); **C_{12}, C_{22}**- Oxytetracycline zones (Inner); **D_1, D_2**- Freeze-dried oxytetracycline (portion of B); **E**- Control- 50 µg/ml oxytetracycline.

Table 9: The mean diameters of zones of inhibition obtained following freeze-drying and mixing tetracyclines and sulphonamides (figure 1).

	Zone diameter (mm)			
	1st measurement	2nd measurement	Mean (mm)	Overall mean
A_1	30	28	29	29*
A_2	29	29	29	
B_1	22	22	22	22*
B_2	22	21.5	21.75	
C_{11}	27	27	27	27
C_{12}	16	16	16	16
C_{21}	27	27	27	27
C_{22}	16	16	16	16
D_1	26	27	26.5	26*
D_2	26	26	26	

* = overall mean (mm) of 2 values.

Key:

A_1, A_2 - 50 mg/ml sulfadimidine; **B_1, B_2** - 5 µg/ml Oxytetracycline; **C_1, C_2** - Sulfur and oxytetracycline mixture; **C_{11}, C_{21}**- Sulfur zones (Outer); **C_{12}, C_{22}**- Oxytetracycline zones (Inner); **D_1, D_2**- Freeze-dried oxytetracycline (portion of B); **E**- Control- 50 µg/ml oxytetracycline.

3.5.3. Zones of inhibition for differentiation of oxytetracycline from sulphonamides and freeze-dried from non-freeze-dried samples.

Figures 2 and 3 show the effect of freeze-drying and also the difference between zones of inhibition of sulphonamides and those of oxytetracyclines. In both figures, **A** represents 2.5 µg/ml of oxytetracycline, **B** represents 5 µg/ml of oxytetracycline, and **C** represents 10 µg/ml of oxytetracycline. $\mathbf{A_1}$, $\mathbf{B_1}$, and $\mathbf{C_1}$ are the non-freeze-dried samples while $\mathbf{A_2}$, $\mathbf{B_2}$ and $\mathbf{C_2}$ are the freeze-dried samples respectively. The zones of inhibition of sulphonamides are depicted by the letter **S** in both plates.

From the two figures, it is observed that freeze-drying increases the sensitivity of detection because the zones of inhibition for freeze-dried samples are larger than those of the non-freeze-dried (see table 10). It is also observed that sulphonamide zones are easily distinguishable from those of oxytetracycline because the sulphonamide ones show incomplete zones of inhibition. The zones of inhibition of sulphonamides had a mean diameter of approximately 22.5 ± 0.71 mm.

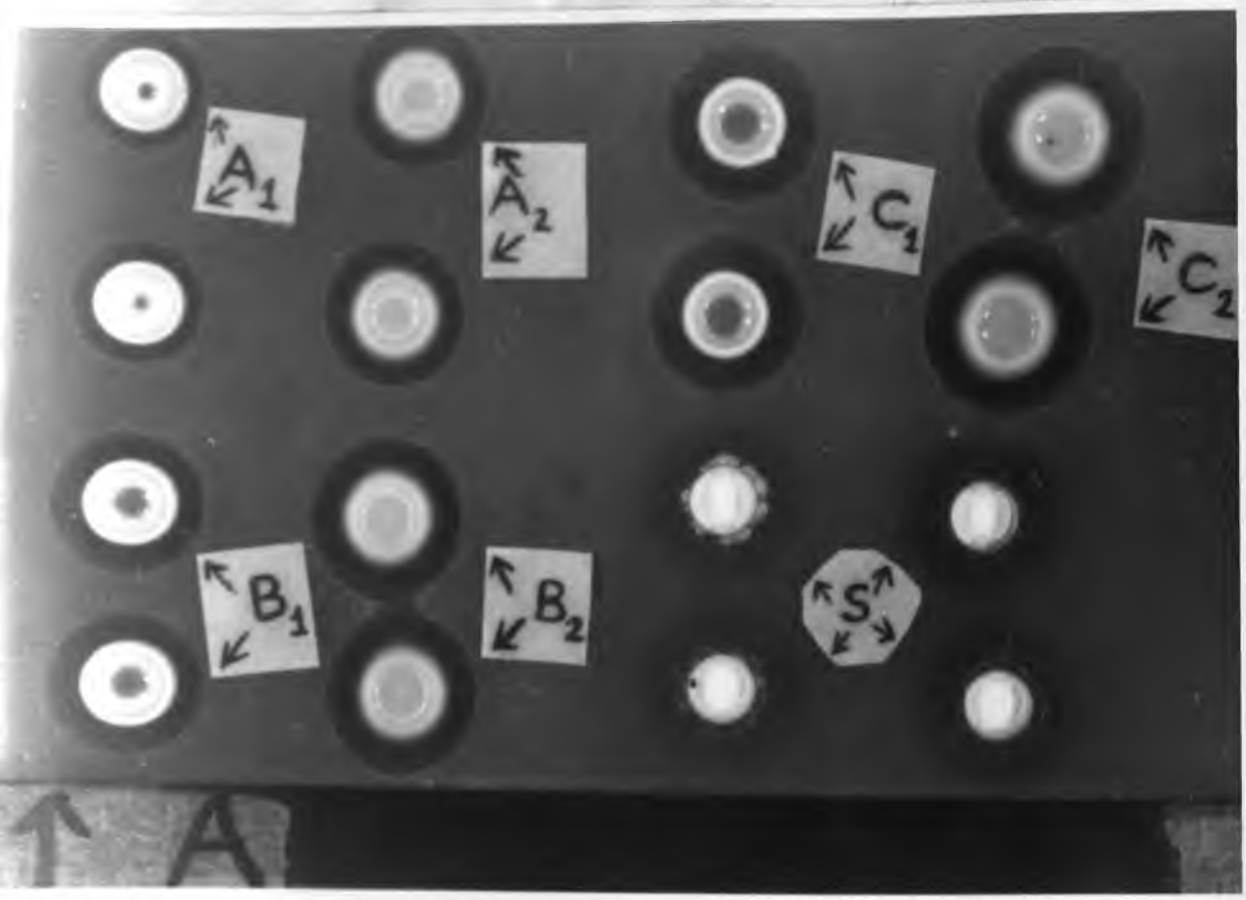


Figure 2: Effect of freeze-drying of egg samples and the differentiation of oxytetracycline from sulphonamides and freeze-dried from non-freeze-dried (A).

Key:

A1, **A2**- 2.5 µg/ml oxytetracycline; **B1**, **B2**- 5 µg/ml oxytetracycline; **C1**, **C2**- 10 µg/mloxytetracycline; **S**- sulfadimidine- 0.125 g/ml.

1- non-freeze dried; **2**- freeze dried.

NB. Diameter of all the wells = 10 mm. Inhibition zones include well diameter.

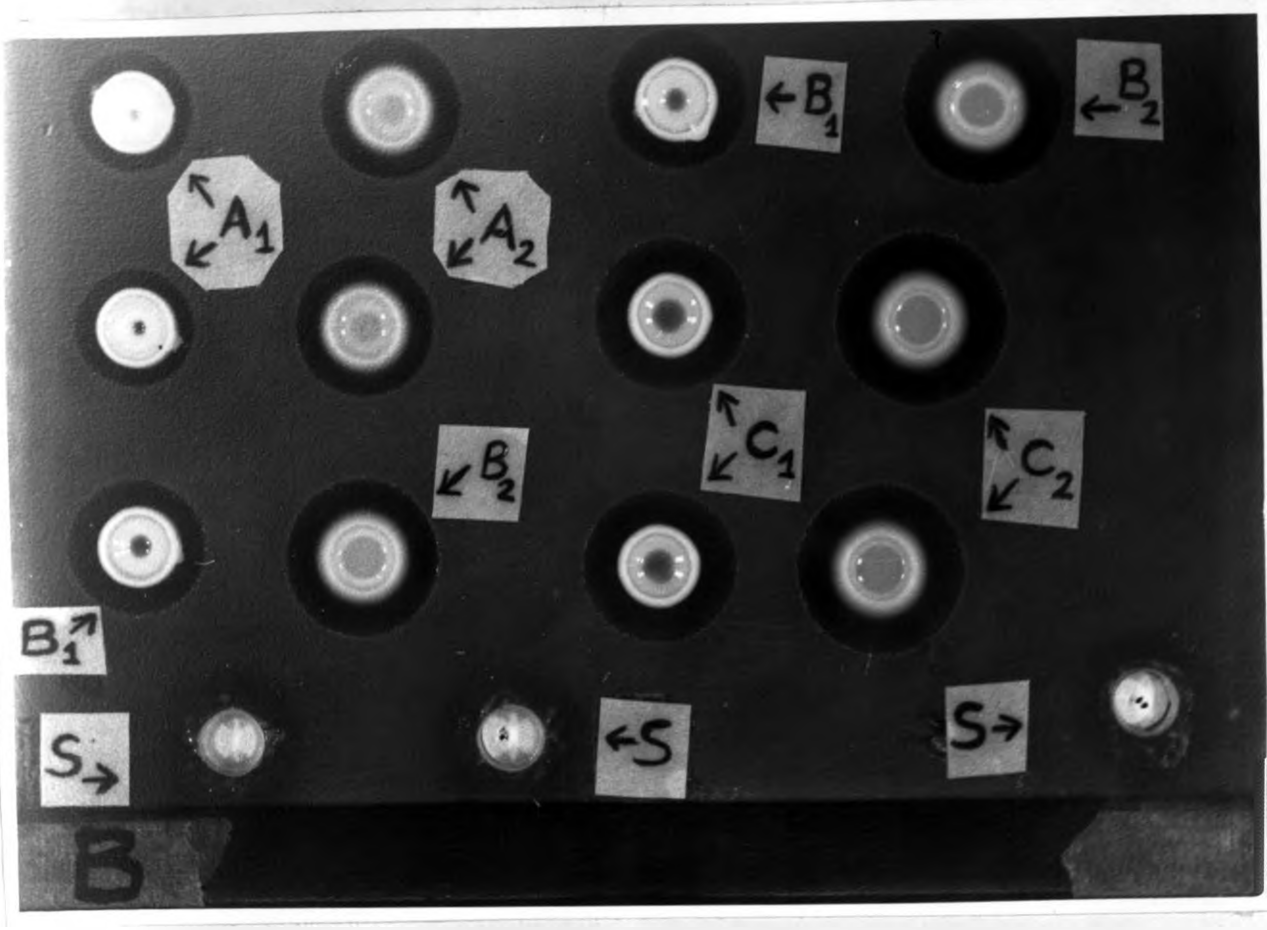


Figure 3: Effect of freeze-drying of egg samples and the differentiation of oxytetracycline from sulfur and freeze-dried from non-freeze-dried (B).

Key:

A_1, **A_2**- 2.5 µg/ml OTC; **B_1**, **B_2**- 5 µg/ml OTC; **C_1**, **C_2**- 10 µg/ml OTC; **S**- sulfadimidine/sulfur- 0.125 g/ml.

1- non-freeze dried; **2**- freeze dried.

NB. Diameter of all the wells = 10 mm. Inhibition zones include well diameter. OTC= Oxytetracycline.

Table 10: The mean diameters of zones of inhibition obtained following freeze-drying of egg samples.

	Mean zone diameter (mm ± S.D.)
A_1	17.50 ± 0.58
A_2	21.25 ± 0.29
B_1	20.25 ± 0.29
B_2	24.00 ± 0.00
C_1	23.00± 0.00
C_2	25.75 ± 0.29

Key:

A_1, **A_2**- 2.5 µg/ml OTC; **B_1**, **B_2**- 5 µg/ml OTC; **C_1**, **C_2**- 10 µg/ml OTC; **S**- sulfadimidine- 0.125 g/ml.

OTC = Oxytetracycline.

3.6. Standard Curve Preparation

3.6.1. Reagent solutions.

The following solutions were prepared, as described by Dornbush and Abbey (1972).

1. *0.1N Hydrochloric acid.*

2. *0.01N Hydrochloric acid.*

3. *4N Hydrochloric acid.*

4. *18N Phosphoric acid.*

5. *10N Potassium phosphate.*

6. *Acid acetone.* 1 volume 4N hydrochloric acid mixed with 13 volumes of reagent grade acetone, and 6 volumes of distilled water.

7. *0.1M phosphate buffer, pH 4.5..* 13.6 gm of potassium dihydrogen phosphate, 1000 ml of distilled water q.s. Either 18N phosphoric acid or 10N potassium phosphate was used to adjust the pH to 4.45-4.55 after sterilization.

8. *Methanolic hydrochloric acid.* 1 volume of undiluted hydrochloric acid was mixed with 50 volumes of reagent-grade anhydrous methanol.

9. *0.012M phosphate buffer, pH 7.* 790 mg of potassium dihydrogen phosphate and 1.0 gm of dipotassium phosphate, 1000 ml distilled water q.s. The pH was adjusted to 6.9-7.1.

10. *0.01N methanolic hydrochloric acid.* To 1.0 ml of 1N hydrochloric acid was added 100 ml of methanol q.s.

11. *1N sodium hydroxide.*

3.6.2. Oxytetracycline standard.

Oxytetracycline (Sigma Chemical Company, St. Louis. U.S.A.) was used as the standard. It was maintained in a tightly stoppered container, and kept in a refrigerator when not in use. Care was taken to allow the vessels to return to room temperature before the contents were removed.

Stock solution - 100 mg of the analytical oxytetracycline hydrochloride was weighed and dissolved in 100 ml of 0.01 N hydrochloric acid so that the resulting solution contained exactly 1000 µg of oxytetracycline hydrochloride activity per millilitre.

Working standard - One ml of the stock solution was added to 19 ml of 0.1 M phosphate buffer, pH 4.5, to give a concentration of 50 µg/ml (x 20 dilution). Using either antibiotic-free freeze-dried yolk or albumen, portions of this solution were further diluted to give concentrations of 0.075, 0.15, 0.3, 0.6, 1.2 and 2.4 µg/ml. Finally, 4 ml of each of these mixtures was diluted with 8 ml of the 0.1M phosphate buffer, so that the resulting antibiotic concentrations were 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 µg/ml (Dornbush and Abbey,1972).

A summary of the standard preparation is shown in figure 4.

OTC-HCl

↓ 100 mg into 100 ml 0.01N HCl

1000 µg/ml OTC Stored in a glass-stoppered vessel for < 4 days

↓ 1 ml + 19 ml of PB

50 µg/ml OTC

↓ 1 ml + 19.8 ml of A or Y suspension

2.4 µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.8 µg/ml OTC**

↓ 4 ml + 4 ml of A or Y suspension

1.2 µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.4 µg/ml OTC**

↓ 4 ml + 4 ml of A or Y suspension

0.6 µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.2 µg/ml OTC**

↓ 4 ml + 4 ml of A or Y suspension

0.3 µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.1 µg/ml OTC**

↓ 4 ml + 4 ml of A or Y suspension

0.15 µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.05 µg/ml OTC**

↓ 4 ml + 4 ml of A or Y suspension

0.075µg/ml OTC —— 4 ml + 8 ml of PB ——→ **0.025 µg/ml OTC**

Key:

OTC = Oxytetracycline.

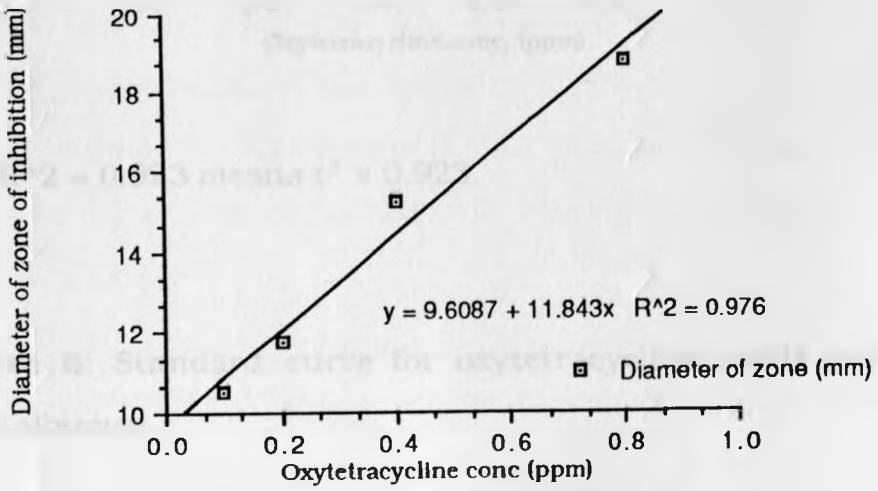
PB = 0.1 M phosphate buffer, pH 4.5.

A = Freeze-dried albumen.

Y = Freeze-dried yolk.

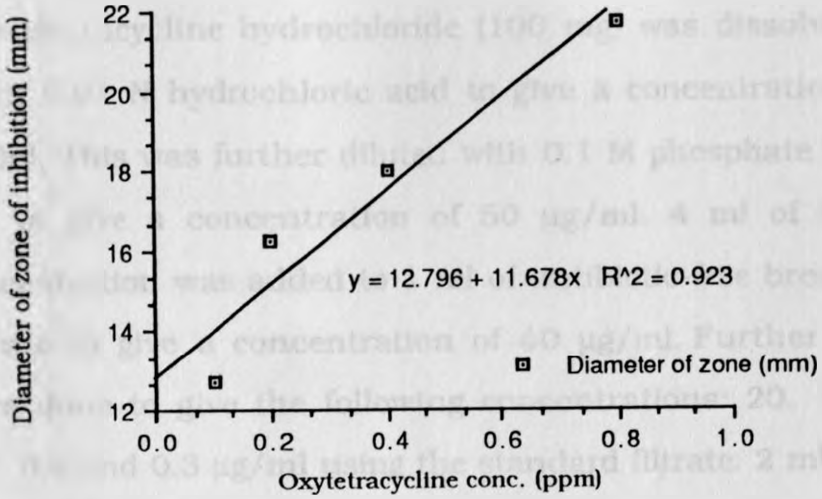
Figure 4: An outline of the standard curve preparation of oxytetracycline hydrochloride.

Four dilutions, i.e. 0.1, 0.2, 0.4 and 0.8 µg/ml (n = 6) were then used to prepare standard curves during the microbiological assay, by plotting concentration (µg/ml) versus diameter of zone of inhibition (mm). These curves are shown in figures 5 and 6 respectively.



NB. R^2 = 0.976 means $r^2 = 0.976$.

Figure 5: Standard curve for oxytetracycline levels prepared from yolk.



NB: R^2 = 0.923 means $r^2 = 0.923$.

Figure 6: Standard curve for oxytetracycline levels prepared from albumen.

3.6.3. Preparation of standards for meat samples

Oxytetracycline hydrochloride (100 mg) was dissolved in 100 ml of 0.01 N hydrochloric acid to give a concentration of 1000 µg/ml. This was further diluted with 0.1 M phosphate buffer, pH 4.5 to give a concentration of 50 µg/ml. 4 ml of this latter concentration was added to 1 ml of antibiotic-free breast muscle filtrate to give a concentration of 40 µg/ml. Further dilutions were done to give the following concentrations: 20, 10, 5, 2.4, 1.2, 0.6 and 0.3 µg/ml using the standard filtrate. 2 ml of each of the last four dilutions was added to 4 mls of phosphate buffer, pH 4.5 to give the following final dilutions, respectively: 0.8, 0.4, 0.2 and 0.1 µg/ml. A summary of the standard preparation is shown in figure 7.

1000 µg/ml of OTC HCl

↓ 1 ml + 19 ml of PB

50 µg/ml

↓ 4 ml + 1 ml of Std filtrate

40 µg/ml

↓ 2 ml + 2 ml of Std filtrate

20 µg/ml

↓ 2 ml + 2 ml of Std filtrate

10 µg/ml

↓ 2 ml + 2 ml of Std filtrate

5 µg/ml

↓ 2 ml + 2.2 ml of Std filtrate

2.4 µg/ml — 2 ml + 4 ml of PB → **0.8 µg/ml**

↓ 2 ml + 2 ml of Std filtrate

1.2 µg/ml — 2 ml + 4 ml of PB → **0.4 µg/ml**

↓ 2 ml + 2 ml of Std filtrate

0.6 µg/ml — 2 ml + 4 ml of PB → **0.2 µg/ml**

↓ 2 ml + 2 ml of Std filtrate

0.3 µg/ml — 2 ml + 4 ml of PB → **0.1 µg/ml**

Key:

OTC = oxytetracycline.

PB = 0.1 M phosphate buffer, pH 4.5.

Std. filtrate= antibiotic-free breast muscle filtrate.

Figure 7 : A schematic diagram showing how the breast muscle standards were prepared.

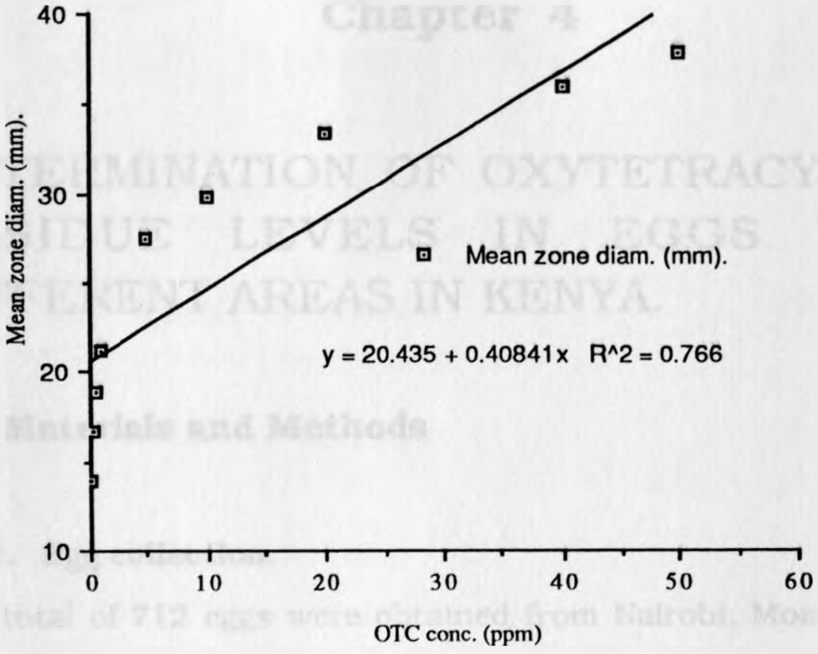
The sample as well as the standard filtrates were used for a microbiological assay as previously described (The standards were run in triplicate). The results are shown in table 11 and figure 8.

Table 11: Zones of inhibition for oxytetracycline levels prepared from breast muscle samples.

OTC conc. (ppm)	Mean zone diameter (mm ± S.D.)
50	38 ± 0.00
40	36 ± 0.00
20	33.5 ± 0.71
10	29.75 ± 1.06
5	27.5 ± 0.71
0.8	21.25 ± 0.35
0.4	18.83 ± 0.29
0.2	16.75 ± 0.35
0.1	14 ± 0.00

S.D. = standard deviation.

OTC = oxytetracycline.



NB: R^2 = 0.766 means $r^2 = 0.766$.

Figure 8: Standard curve for oxytetracycline levels in breast muscle.

Chapter 4

DETERMINATION OF OXYTETRACYCLINE RESIDUE LEVELS IN EGGS FROM DIFFERENT AREAS IN KENYA.

4.1 Materials and Methods

4.1.1. Egg collection.

A total of 712 eggs were obtained from Nairobi, Mombasa and Nakuru regions. A summary of the sources and number of eggs used in the study is shown in table 12.

Table 12: Source and number of eggs analysed.

Code	Area	No. of eggs
A	Thogoto	59
B	Kikuyu town	28
C	Uchumi, Kimathi St., Nairobi	59
D	Mustapha Farm, Mombasa	60
E	Jirani Traders, Mombasa	60

Table 12 (cont'd)

F	City Grocers, Mombasa	30
G	Coast Poultries, Mombasa	30
H	Farm Traders, Mombasa	30
J	Star of The Sea, Mombasa	30
K	Baobab Farm, Mombasa	30
L	Uhuru Farm, Mombasa	28
M	Uchumi, Aga Khan Walk, Nairobi	29
N	Uchumi, Muindi Mbingu St., Nairobi	30
P	Uchumi, Westlands, Nairobi	30
Q	Feedlot Meat Supply, Ronald Ngala St., Nairobi	30
R	Wangige market, Nairobi area	30
S	Muguku Farm, Kikuyu, Nairobi area	30
T	Kawangware market, Nairobi area	30

Table 12 (cont'd)

Y	Bahati, Nakuru	29
O	Bahati, Nakuru	30

Eggs were obtained from farms or from various markets/supermarkets. In the case of farms, the farmers had to indicate the type of drugs they may have added to either feed or water. The eggs were stored under refrigeration at +4°C in the laboratory up to 3 weeks pending analysis.

4.1.2. Microbiological assay.

4.1.2.1. Plate Preparation.

A rectangular framed glass plate of size 220 x 150 mm was used. The spacers were taped to the main glass plate to form a trough, after which it was wrapped in brown paper for sterilization in dry heat at 170°C.

4.1.2.2. Sample Preparation.

The yolk and albumen were separated aseptically and analysed separately. Each portion was diluted one in three (1:3) with 0.1M Monopotassium phosphate buffer, pH 4.5 ± 0.05. The samples were then homogenized with a Whirlimixer® (Fisons), and then stored in a freezer at -20°C until analysis was done.

The frozen yolk or albumen samples were thawed then dispensed into centrifuge tubes and centrifuged at 4000 g (5800

rpm) for 15 minutes, so that by using the supernatant, lipoproteins being partly discarded, the sensitivity increased.

4.1.2.3. Freeze-drying of the samples.

A portion of the sample was frozen in a universal bottle to form a slant so that there was increased surface area. The mouth of the bottle was then covered with a cheese cloth and the latter fastened with a rubber band. The sample was then freeze-dried using an Edwards High Vacuum® freeze-drier (Sussex) at a temperature of -40 to -60^{0}C and a vacuum pressure of 650 mm Hg. The sample was considered dry at 10^{-1} torr.

After freeze-drying, 100 mg of the sample was added to 0.5 ml of phosphate buffer pH 4.5, then thoroughly mixed with a Whirlimixer® (Fisons). 200 µl of the sample was dispensed into 10 mm diameter wells on Mueller Hinton molten agar and the experiment was done as described for the non-freeze-dried portion.

4.1.2.4. Performing the assay.

Mueller Hinton agar (3.8 gm) was weighed into a conical flask and 100 ml of distilled water added, as per the manufacturer's instructions. It was then sterilized in an autoclave at a pressure of 15 mm Hg and a temperature of 121^{0}C for 15 minutes, after which it was cooled to about 50^{0}C (the actual agar temperature was found to be 51.5^{0}C). The spore suspension, (1 ml), earlier incubated at 30^{0}C for 18 hours was then inoculated into the molten agar and mixed thoroughly to ensure uniform spore

distribution. The agar was poured into the rectangular glass plate and allowed to solidify. After the agar had hardened, wells of diameter 10 mm were dug out in the medium with a 10 mm diameter cork borer. The number and arrangement of the wells depended on the number of samples being run at a given time. One trough could accommodate up to 40 samples in duplicate, as well as the standards. The standards had to be run together with the samples each time to reduce the error of determination. Into each well, 200 µl of the sample or standard was dispensed, using a micropipette. After the lid was fastened on, a prediffusion time at room temperature of one hour was used as recommended by Fabiansson and Rutegaard (1979) before incubation at 30^{0}C for 18 hours.

4.1.2.5. Calibration of diameter of zones of inhibition.

The diameters of the inhibition zones of the standards and samples were measured with a vernier caliper to the nearest 0.5 mm. Each sample was run in duplicate and for each well, three measurements were made and the mean calculated.

4.2. Results.

Seven hundred and twelve (712) eggs were analysed, 355 from Nairobi area, 298 from Mombasa and 59 from Nakuru. A total of 6 eggs from Nairobi area had residue values which were above the detectable limit of 0.1 ppm. This was only detected from the freeze-dried samples and represented a percentage of 1.69%. Of these eggs, 5 were from Uchumi Supermarket, Kimathi Street, while one was from Wangige market (table 13). The mean concentration of residues in the eggs from Uchumi Supermarket was 0.475 ppm while the percentage of positive samples was 8.47%. All the positive results were obtained from the yolk following the freeze-drying procedure. None of the corresponding albumen samples gave values above 0.1 ppm. Out of the 30 eggs from Wangige market, only one egg had residue levels above the detection limit of 0.1 ppm, and unlike the samples from Uchumi Supermarket, both the yolk and albumen had residues above 0.1 ppm. Again, the residues were only detected in freeze-dried samples. The mean for the yolk was 0.492 ppm while that for albumen was 0.324 ppm. The percentage of positive samples was 3.33%.

Only one egg from Mombasa out of 298 eggs analysed gave zones of inhibition from the yolk. The zones of inhibition were, however, due to sulfonamides. No drug residue was detected from the albumen of the same egg. None of the 59 eggs from Nakuru gave zones of inhibition.

The mean concentration of positive yolk samples was 0.478 ppm which was 0.278 ppm above the acceptable limit, while the

mean concentration of positive albumen samples was 0.324 ppm which was 0.124 ppm above the acceptable limit. This shows that all the six yolk and one albumen samples had oxytetracycline concentrations above the acceptable limit of 0.2 ppm.

There were no positive samples with oxytetracycline concentrations below the acceptable limit.Tables 13, 14 and 15 show the analysis results from the respective areas.

In table 16, the oxytetracycline concentrations obtained in this survey is compared with the recommended maximum WHO/FAO standard of 0.2 ppm for oxytetracycline.

mean concentration of positive albumen samples was 0.324 ppm which was 0.124 ppm above the acceptable limit. This shows that all the six yolk and one albumen samples had oxytetracycline concentrations above the acceptable limit of 0.2 ppm.

There were no positive samples with oxytetracycline concentrations below the acceptable limit.Tables 13, 14 and 15 show the analysis results from the respective areas.

In table 16, the oxytetracycline concentrations obtained in this survey is compared with the recommended maximum WHO/FAO standard of 0.2 ppm for oxytetracycline.

Table 13: Oxytetracycline levels of eggs from Nairobi area.

Source of eggs	No.	No. of (+)ves	Mean (ppm)	% of (+)ves
Thogoto	59	0	-	-
Kikuyu town	28	0	-	-
Uchumi-Kimathi St.	59	5	0.475	8.47
Uchumi- Aga Khan Walk	29	0	-	-
Uchumi-Muindi Mbingu St.	30	0	-	-
Uchumi-Westlands	30	0	-	-
Feedlot Meat Supply	30	0	-	-
Wangige Market	30	1	Yolk- 0.492 Albumen-0.324	3.3%
Muguku Farm	30	0	-	-
Kawangware	30	0	-	-
TOTAL	355	6	0.456	1.69%

Key:

(+)ves means positives (drug residues detected).

- means not applicable.

Table 14: Oxytetracycline levels of eggs from Mombasa.

Source of eggs	No.	No. of (+)ves	Mean (ppm)	%of (+)ves
Mustapha Farm	60	1(sulphonamides)	-	1.67%
Jirani Traders	60	0	-	-
City Grocers	30	0	-	-
Coast Poultries	30	0	-	-
Farm Traders	30	0	-	-
Star of the Sea	30	0	-	-
Baobab Farm	30	0	-	-
Uhuru Farm	28	0	-	-
TOTAL	298	1 (sulphonamides)	Not measured	0.34%.

Key:

(+)ves means positives (drug residues detected).

- means not applicable.

• Although there were no oxytetracycline residues detected, one sample had sulphonamides.

Table 15: Oxytetracycline levels of eggs from Nakuru.

Source of eggs	No.	No. of (+)ves	Mean (ppm)	% of (+)ves
Bahati (young)	29	0	-	-
Bahati (old)	30	0	-	-
TOTAL	59	0	-	-

Key:

(+)ve means positives (drug residues detected).

- means not applicable.

Table 16: A comparison of the oxytetracycline concentrations obtained in the survey with the WHO/FAO acceptable standards.

	Nairobi area	Mombasa	Nakuru
No. of eggs analysed	355	298	59
No. of positives	6	0	0
No. of positive yolks	6	0	0
No. of positive albumens	1	0	0
% of positive yolks	1.69 %	-	-
% of positive albumens	0.28 %	-	-
No. of positive yolks above WHO/FAO Standard of 0.2 ppm	6	-	-
No. of positive albumens above WHO/FAO Standard of 0.2 ppm	1	-	-
Mean of positive yolks above WHO/FAO Standard (ppm)	0.478	-	-
Mean of positive albumens above WHO/FAO Standard (ppm)	0.324	-	-
No. of positive yolks below WHO/FAO Standard of 0.2 ppm	0	-	-
No. of positive albumens below WHO/FAO Standard of 0.2 ppm	0	-	-

N.B. Detection limit: 0.1 ppm; and - means not applicable.

4.3. Discussion.

It was observed from the results that the number of eggs with residue levels above the detectable limit of 0.1 ppm was very small- (6 out of 712 eggs). The oxytetracycline residues were only detected in freeze-dried samples because, from previous experiments, it was found that freeze-drying increases the sensitivity of detection. From this observation, it can be stated that commercial eggs collected from the areas of study are generally safe, but considering Uchumi Supermarket of Kimathi Street (table 13), it is noticed that 5 out of 59 eggs (i.e. 8.47%) had oxytetracycline residue levels above the acceptable limit of 0.2 ppm. In fact, 83.33% of all the positive yolk samples were from this supermarket. Although the eggs don't pose much danger to the consumer, one cannot rule out the fact that occasionally there could be a risk.

In all the albumen samples analysed, oxytetracycline residue levels above 0.1 ppm were only detected in one sample. This indicates that oxytetracyclines accumulate more in the yolk than in albumen. This is in agreement with work done by Siegmann (1982) who reported that the antibiotic accumulates in the pool of follicles of the yolk. Hence the elimination period from yolk is longer than that of albumen. This explains why hardly any oxytetracycline was detected in albumen.

The fact that all the positive samples were above the acceptable limit of 0.2 ppm is a coincidence. It could have been that these eggs were from birds that had been on oxytetracycline medication for a long time, because the residues can be detected

only when the concentration of the drug within the body is between 200-400 ppm. One other possibility for the oxytetracycline levels being above 0.2 ppm could be due to misuse of the drug by the farmer, either by adding too little water for reconstitution or adding too much of the drug into drinking water.

The low number of eggs positive for oxytetracycline may be due to either poor absorption of oxytetracycline from the gut because of the high calcium diet offered to layers or the deposition of oxytetracycline in the shell

The sensitivity threshold for the detection of oxytetracycline in albumen obtained in this experiment is different from that of other workers. Roudaut et al (1989) and Yoshimura et al (1991) reported a sensitivity threshold of 0.07 µg/g (ppm oxytetracycline). In this experiment, no zones of inhibition were obtained for albumen standards below 0.1 ppm. This detection limit of 0.1 ppm tallies with that of Katz et al (1972) but is slightly lower than that obtained by Yoshida et al (1973) who reported a limit of 0.27 ppm (µg/g). Roudaut et al (1987) reported a detection limit of 0.2 µg/g for yolk.

It may be concluded that freeze-drying increases the sensitivity of detection of oxytetracycline residues, since the positives were only obtained in freeze-dried samples. This was confirmed in sections 3.5.2 and 3.5.3.

No literature is available on a survey carried out in Kenya or elsewhere to determine the oxytetracycline residue levels in commercial eggs, although several workers have done feeding

studies whereby different concentrations of oxytetracycline are fed to chicken in drinking water.

The earliest recorded feeding trial was carried out by Durbin et al (1954) who could only detect antibiotic residues in eggs of hens fed 500 ppm of oxytetracycline and above. Katz et al (1972) fed chlortetracycline at levels of 0, 50, 100, 150 and 200 ppm but since they employed a chemical fluorimetric procedure as well as a microbiological method for analysis, they were able to detect residues in those chicken fed 200 ppm. Like Durbin et al (1954), Yoshida et al (1973) could detect oxytetracycline residues only when a minimum of 500 ppm of the antibiotic was fed. Feeding trials have also been done by Roudaut et al (1987 and 1989) to determine the kinetics of oxytetracycline elimination into eggs separately for albumen and yolk. They used both the oral and intramuscular (IM) routes.

All the authors mentioned used a microbiological method of analysis and the bacterium was *Bacillus cereus* var. *mycoides*, ATCC 11778. This bacterium is suitable for this method because it has been found to be resistant to the lysozyme of the albumen (Roudaut et al, 1987) and is also very susceptible to tetracyclines. The microbiological method is popular because it is cheap, easy to carry out and many samples can be run within a short time. The other methods available for analysis of tetracyclines are: fluorimetry (Kohn, 1961; Wilson, et al., 1972), chromatography (Wagman and Weinstein, 1973; Ryan and Dupont,1974; Bocker and Estler, 1979; Lin, 1985; Ashworth, 1985, Oka et al, 1985 and MacNeil et al., 1989) and

radioimmunoassay method (Faraj and Ali, 1981; Blomquist and Hanngren, 1966 and Ullberg, 1977). These methods are however expensive and have mainly been used for analysis of tetracyclines in other tissues such as muscle (Raynaud et al, 1976), liver (Meredith, 1965), bones (Jones et al, 1977) and kidney (Booth, 1988) and animal products such as milk (Maritim, 1985 and Booth, 1988).

An improved chemical method for analysis of tetracyclines has been demonstrated by Oka et al (1985). This method employs the chromatographic principle and it was used to analyse oxytetracycline, tetracycline, chlortetracycline and doxycycline from animal liver. Detection limits in the beef liver were 0.05 and 0.1 ppm for oxytetracycline and tetracycline and for chlortetracycline and doxycycline, respectively.

Chapter 5

DETERMINATION OF OXYTETRACYCLINE RESIDUE LEVELS IN EGGS AND MEAT FROM LAYERS ADMINISTERED DIFFERENT DOSAGES OF OXYTETRACYCLINE IN WATER.

5.1. Materials and Methods

5.1.1. Laying birds

Twenty Rhode's Island Red layers in their last stage of lay were obtained and after randomization, they were put in individual cages making a total of 4 groups. The environmental temperature in the cages was 25°C. The birds were fed on layers complete meal with Vitamealo® (Unga Feeds Ltd) and received water *ad libitum*. They were kept for one week to acclimatise to the surroundings and the study was only began when the egg production had stabilised.

5.1.2. Drug treatment

Group 1 (n = 5), which was the control, received antibiotic-free water, group 2 (n = 5) received water containing 400 ppm oxytetracycline, group 3 (n = 5) received water containing 600 ppm oxytetracycline and group 4 (n = 5) received water containing 800 ppm oxytetracycline. These concentrations were very much higher than the therapeutic dose of 50 ppm. The birds were offered medicated water *ad libitum* for 7 days. Eggs were collected on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16 and 17, after which they were marked and stored in a

refrigerator at +4^{0}C, pending analysis. Eggs obtained on day 0 were analysed to ensure that they did not have detectable levels of oxytetracycline, before oxytetracycline was administered.

5.1.3. Determination of oxytetracycline residues in breast muscle of the layers.

On day 17 (ten days after the cessation of drug administration), the birds which had been on oxytetracycline medication were sacrificed and the breast muscle removed. The muscle was stored at -20^{0}C until analysed. The breast muscle samples were thawed and cut into small pieces, after which 5 grams was weighed and blended twice with 20 ml 0.01N hydrochloric acid for 2 minutes and lastly, with 10 ml of the acid for 1 minute (modified from Oka et al, 1985), using a Mistral Scientific Equipment (M.S.E.) homogenizer. The samples were then centrifuged at 4000 g (5800 r.p.m.) for 10 minutes, the supernatant filtered and the filtrate used for a microbiological assay as described in section 4.1.2. Oxytetracycline levels were extrapolated from the standard curve as described in section 4.1.2.5.

5.1.4. Data analysis.

The data obtained from the study was analysed using a one-way analysis of variance (Snedecor and Cochran, 1982) on an IBM compatible computer, using panacea package, to determine whether there was any statistical difference between and among the groups fed different oxytetracycline levels at a probability level of 0.05.

5.2. Results

5.2.1. Oxytetracycline levels in eggs.

Freeze-dried samples exhibited higher concentration of oxytetracycline than the corresponding non-freeze-dried samples. Data was therefore taken from readings of freeze-dried samples.

5.2.1.1. *Yolk samples.*

In group 2 (400 ppm oxytetracycline), levels were detectable only from day 7, which was the last day of feeding, and only 6 out of 38 eggs (15.8%) gave positive results. All the positive eggs had oxytetracycline values above the WHO/FAO acceptable level of 0.2 ppm. The highest oxytetracycline level was 0.449 ppm, obtained on day 10 (Appendix 2), while the highest mean was 0.289 ppm, obtained on day 7. The mean oxytetracycline level (figure 9 and appendix 4) rose to a maximum on day 7 and then began to decline to undetectable levels on day 9. The levels then rose again on day 10 and by day 11, they were undetectable again.

In group 3 (600 ppm), the oxytetracycline residue levels were very erratic, being detectable on day 1 and day 2, only to become undetectable again until day 7. No egg was laid by any bird in this group on day 8. The oxytetracycline levels began to rise again on day 9, with the mean maximum concentration (0.526 ppm) occurring on the same day. Out of 27 eggs analysed, 11 eggs (40.7%) had detectable levels of oxytetracycline, and all of these had values above the acceptable level of 0.2 ppm. No oxytetracycline residues were detectable on day 13.

In group 4 (800 ppm), there were detectable oxytetracycline levels by day 1. The levels were undetectable on day 2, but began to rise from day 3 onwards. Out of 43 eggs analysed, 29 eggs (i.e. 67.4%) had detectable levels of oxytetracycline and all of these had values greater than the acceptable limit of 0.2 ppm. The latter were all obtained during the feeding exercise. The mean maximum concentration (0.583 ppm) was obtained on day 9, two days after the medication exercise was terminated. The oxytetracycline levels began declining after day 9, until none was detectable by day 13.

There was significant difference ($p<0.05$) among group 2 (400 ppm), group 3 (600 ppm) and group 4 (800 ppm). There was a significant difference ($p<0.05$) between groups 2 and 3 as well as between groups 3 and 4. There was also a significant difference ($p<0.05$) between groups 2 and 4.

5.2.1.2. *Albumen samples.*

None of the albumen samples in group 2 had detectable levels of oxytetracycline, while for group 3, only one egg out of the 27 eggs analysed (i.e.3.7%) had detectable levels (0.221 ppm) which was above the acceptable level of 0.2 ppm (Appendix 3). This egg was laid on day 7, which was the last day of drug administration.

In group 4 (fed 800 ppm), oxytetracycline levels were first detectable on day 2 of drug administration, although the mean levels (0.123 ppm) were below the acceptable level of 0.2 ppm (figure 10 and appendix 5). Out of 43 eggs analysed, 17 (i.e. 39.5%) had detectable levels of oxytetracycline. Thirteen of the

positive eggs had oxytetracycline levels above the acceptable level of 0.2 ppm, but when the mean values were considered, only four had values above 0.2 ppm. The mean oxytetracycline levels rose steadily with occasional drops, until a mean maximum oxytetracycline concentration of 0.280 ppm was achieved on day 7. The oxytetracycline levels became undetectable by day 10.

There was significant difference ($p<0.05$) among groups 2, 3 and 4. Although there was an apparent difference between groups 2 and 3, there was however no significant difference ($p>0.05$) between them. But there was a significant difference ($p<0.05$) between groups 3 and 4. There was also a significant difference ($p<0.05$) between groups 2 and 4.

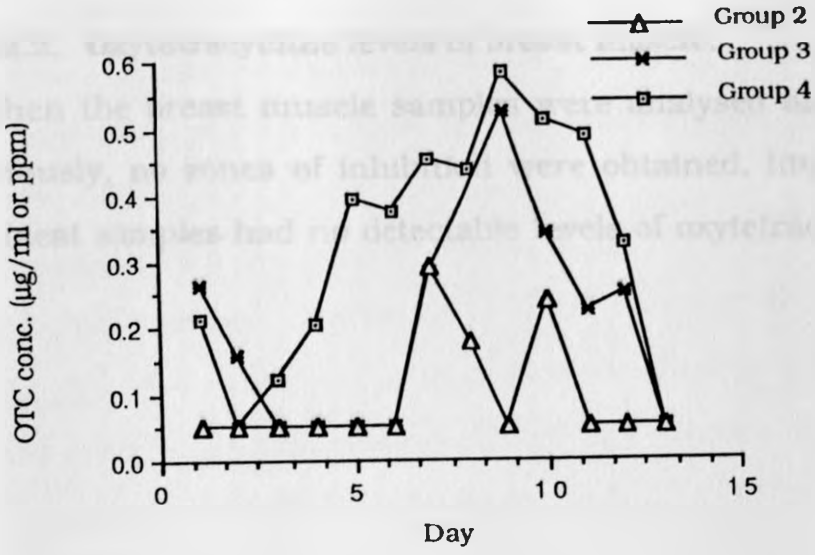


Figure 9: Mean concentration of oxytetracycline (ppm) in yolk for groups 2,3 and 4.

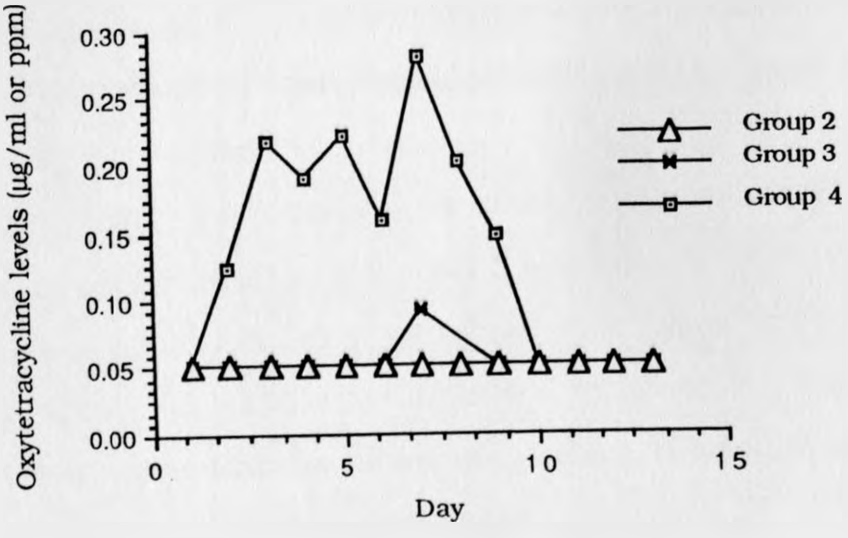


Figure 10: Mean concentration of oxytetracycline (ppm) in albumen for groups 2,3 and 4.

5.2.2. Oxytetracycline levels in breast muscle.

When the breast muscle samples were analysed as described previously, no zones of inhibition were obtained, implying that the meat samples had no detectable levels of oxytetracycline.

5.3. Discussion

An earlier pilot study indicated that no residues were detected when the birds were fed 20 ppm, 50 ppm (therapeutic level) or 200 ppm.

Residues were detected in the yolk from birds fed 400 ppm and above. However, Durbin et al (1954) couldn't detect residues when they fed 500 ppm in feed to birds. Roudaut et al (1987) could detect oxytetracycline residues in both yolk and albumen when the birds were fed 250 ppm, but in this case, while the residues could be detected in the yolk when the birds were fed 400 ppm, none was detected when the albumen was analysed. This could be because their experimental conditions and the sensitivity of the method used were different from those of this study. Katz et al (1972) obtained clear zones when they fed 200 g/ton (ppm) of chlortetracycline, while here, none was detected when the birds were fed 200 ppm of oxytetracycline. This shows that the detection limit for chlortetracycline is lower than that for oxytetracycline.

Since there were many eggs with oxytetracycline levels below the detectable limit of 0.1 ppm, this tended to affect the general trend of concentration of oxytetracycline, when the means were considered especially as observed in group 4. Since it was difficult to quantify water intake per bird, it was assumed that a bird, on average, drinks 200 ml/day.

Whereas the study first gave detectable residues in albumen on day 2, Yoshida et al (1973) could detect the residues as early as day 1. When Yoshimura et al (1991) fed 500 ppm of

oxytetracycline to layers, residue levels appeared in yolk 3 days after commencing medication.

The peak concentration of oxytetracycline in the yolk for groups 3 and 4 was obtained on day 9, two days after the last day of feeding of the compound. In group 2, the peak concentration was obtained on day 10, three days after discontinuation of feeding. In other words, the peak concentration of oxytetracycline in yolk was obtained 2-3 days after feeding was stopped. This is almost in agreement with the results reported by Yoshida et al (1973) who came to a conclusion of 1-2 days.

When Yoshida et al (1973) fed 4,000 ppm of oxytetracycline, they obtained a peak albumen concentration on day 4 while in this experiment, a peak was obtained on day 7. This could be explained by the big differences in the concentrations of oxytetracycline fed in each case.

When fed 400 ppm, no residues were detected in the yolk on day 11, which was four days after the last day of drug administration. This agrees with the withdrawal period of most commercial poultry products containing oxytetracycline, sold in Kenya. But it is one day more than that recommended by Raynaud et al (1976) in meat.

When the birds were fed 600 and 800 ppm of oxytetracycline, no residues were detectable in the yolk by day 13 which was six days after the discontinuation of medication and which is in agreement with the elimination period of tetracycline, as reported by Roudaut et al (1989) of between 6 and 11 days.

These workers also obtained an elimination period of 9 days in the case of chlortetracycline.

Oxytetracycline residues were not detectable in albumen after day 9 which was only two days after discontinuation of medication which tallies with the studies done by Roudaut et al (1989). But this is at variance with the work of Yoshimura et al (1991) who reported 6 days. So there was no consistency in the trend levels.

The peak concentration of oxytetracycline in yolk for group 3 occurred on day 10, for one bird in the group. However, when the mean concentrations were considered, the peak concentration fell on day 7, and this was by virtue of the fact that only one egg was laid on that day. This shows how the results are affected by considering the mean concentrations.

The results show that elimination lasts longer for yolk as compared to albumen. Siegmann (1982) ascribed this to the accumulation of antibiotic in the pool of follicles located in the yolk. The follicles are very lipophilic and are able to attract antimicrobial substances.

Although withdrawal periods are said to become unexpectedly longer when antibiotics are given via the drinking water in tropical countries (Yoshimura et al, 1991), this study has shown that when the birds were fed 400 ppm, the withdrawal period was 4 days, which is generally accepted. When fed 600 ppm or 800 ppm, the withdrawal period was 6 days. So it seems therefore, that the withdrawal period also depends on the concentration of the antibiotic fed to the birds.

The study has also confirmed the observation that oxytetracycline residues reach a peak level faster in albumen than in the yolk. Maximum yolk levels in this study were obtained after the discontinuation of feeding (this was 2-3 days after the last day of feeding).

Whereas other workers have reported peak oxytetracycline levels in albumen during feeding, this study gave peak albumen levels on the last day of feeding which was day 7.

5.3.1. Oxytetracycline levels in meat.

The tolerance levels of tetracyclines in various tissues in birds are: 0.6 mg/kg in kidney, 0.1 mg/kg in muscle and 0.3 mg/kg in liver.

Even though group 2 birds were fed 400 ppm of oxytetracycline, group 3 were fed 600 ppm and group 4 were fed 800 ppm of oxytetracycline, ten days after medication was stopped, no oxytetracycline residues could be detected in all the breast muscle samples. This means that even if there is overdosage of up to 800 ppm of oxytetracycline, after ten days, the meat will be safe enough for consumption. The results tally with that recommended by Raynaud et al (1976) who used broilers in their experiment.

Several questions were not answered by this study because of the design of the investigation. For instance, the concentration of oxytetracycline that distributes into the various tissues when either therapeutic dosages (50 ppm) or growth promotion dosages (20 ppm) is administered. These two questions could

only be answered if a more sensitive method of analysis was employed, such as High Performance Liquid Chromatography (HPLC). Also, it wouldn't be established how long it would take for the oxytetracycline in tissues to decrease to below the detectable limit of 0.1 ppm. This last question could not be answered by the experiment because the birds couldn't be sacrificed immediately after medication was terminated.

Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

The two studies utilised the microbiological method of analysis which is cheap, easy to carry out and convenient when the sample size is large. The appropriate test organism for this method is *Bacillus cereus* var *mycoides*, ATCC 11778 (Dornbush and Abbey, 1972).

In the first study, 712 eggs from Nairobi, Mombasa and Nakuru areas were analysed using the microbiological method. One portion of the yolk and the other of the albumen were freeze-dried to investigate the effect of freeze-drying on the samples. Out of 712 eggs analysed, only six had oxytetracycline residue levels above the WHO/FAO acceptable limit of 0.2 ppm and this was only observed after the samples were freeze-dried. From this study, it is concluded that freeze-drying increases the sensitivity of detection of tetracyclines.

The very low percentage of positive cases (0.84%) shows that commercial eggs from the areas studied are safe for human consumption, although isolated cases of high residue levels arise, such as the one observed in Uchumi supermarket of Kimathi Street in Nairobi, where 5 out of 59 eggs had residue concentrations of oxytetracycline above the acceptable limit of 0.2 ppm.

In the second study, laying hens were fed oxytetracycline in drinking water. The eggs were analysed and the results compared with those of other workers. It was only after 400 ppm of oxytetracycline was fed that residues could be detected, and yet the therapeutic dose is 50 ppm. This therefore means that for there to be a danger to human health, the chicken have to be fed more than ten times the therapeutic dose, hence the very low number of positive egg samples obtained in the survey. This observation indicates that oxytetracycline has a very high therapeutic index.

The study also showed when maximum oxytetracycline concentrations occur and the time taken for the levels to become undetectable. These were compared with those of other workers. The conclusion was that maximum oxytetracycline concentration in yolk occurs after medication has stopped, while that of albumen occurs during the medication period. The drug residues remain high for longer periods in yolk than in the albumen, and is attributed to the accumulation of the residues in the follicles in the yolk (Siegmann, 1982). This is evidenced by the result that only one of the six positive albumen samples had residue levels above the detectable limit of 0.1 ppm. Although workers such as Roudaut et al (1989) and Yoshimura et al (1991) reported a lower sensitivity threshold for albumen (0.07 μg/g) than for yolk (0.27 or 0.3 μg/g), this study resulted in a threshold of 0.1 ppm for both yolk and albumen. No residues were detected in the meat samples 10 days after the cessation of oxytetracycline administration in water.

More work needs to be done to determine the partitioning of oxytetracycline in the egg. The drug is deposited in the egg shell, egg yolk and albumen. While egg shell has a lot of calcium ions which attract the oxytetracycline residues, the egg yolk has follicles which also attract the oxytetracycline residues. What proportion goes to the shell, yolk and albumen needs to be investigated.

Usually, farmers need to feed a high calcium diet to their layers in order to reduce the incidence of egg abnormalities. But calcium ions are poorly absorbed in the gut, so when oxytetracycline is fed orally to the birds, the drug binds to the calcium ions in the gut and only a fraction of the total amount is absorbed.

Conclusions

The following conclusions were established from this study:

1. *Bacillus cereus* var. *mycoides* is not very sensitive to sulfur as compared to oxytetracycline. Therefore, it may be possible to distinguish the presence of oxytetracycline or sulfur in other poultry products, feeds or biological fluids using the microbiological method.
2. Freeze-drying increases the sensitivity of detection of tetracyclines in both yolk and albumen in the method used in the study.
3. Oxytetracycline distributes more in the yolk than in the albumen.
4. Oxytetracycline residues reach a maximum concentration faster in albumen than in yolk and the residue levels persist for a longer period in the yolk than in the albumen.
5. A withdrawal period of 10 days is adequate for elimination of oxytetracycline in meat to safe levels even when dosages as high as 800 ppm of oxytetracycline are fed to the birds.
6. Commercial eggs from the areas of study are still safe for human consumption, inspite of the observed widespread use of antibiotic feed additives by farmers and other food producers. However, periodic surveys should be carried out to monitor the antibiotic residue levels in eggs as well as in meat.

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Appendix 1:

OXYTETRACYCLINE RESIDUE LEVELS FROM EGGS FROM VARIOUS REGIONS

A-THOGOTO:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
A1	10	<0.1	<0.1	-
A2	10	<0.1	<0.1	-
A3	10	<0.1	<0.1	-
A4	10	<0.1	<0.1	-
A5	10	<0.1	<0.1	-
A6	10	<0.1	<0.1	-
A7	10	<0.1	<0.1	-
A8	10	<0.1	<0.1	-
A9	10	<0.1	<0.1	-
A10	10	<0.1	<0.1	-
A11	10	<0.1	<0.1	-
A12	10	<0.1	<0.1	-
A13	10	<0.1	<0.1	-
A14	10	<0.1	<0.1	-
A15	10	<0.1	<0.1	-
A16	10	<0.1	<0.1	-
A17	10	<0.1	<0.1	-
A18	10	<0.1	<0.1	-
A19	10	<0.1	<0.1	-
A20	10	<0.1	<0.1	-
A21	10	<0.1	<0.1	-
A22	10	<0.1	<0.1	-
A23	10	<0.1	<0.1	-
A24	10	<0.1	<0.1	-
A25	10	<0.1	<0.1	-
A26	10	<0.1	<0.1	-
A27	10	<0.1	<0.1	-
A28	10	<0.1	<0.1	-
A29	10	<0.1	<0.1	-
A30	10	<0.1	<0.1	-
A31	10	<0.1	<0.1	-
A32	10	<0.1	<0.1	-
A33	10	<0.1	<0.1	-
A34	10	<0.1	<0.1	-
A35	10	<0.1	<0.1	-
A36	10	<0.1	<0.1	-
A37- Egg broken				
A38	10	<0.1	<0.1	-
A39	10	<0.1	<0.1	-
A40	10	<0.1	<0.1	-
A41	10	<0.1	<0.1	-
A42	10	<0.1	<0.1	-
A43	10	<0.1	<0.1	-
A44	10	<0.1	<0.1	-
A45	10	<0.1	<0.1	-
A46	10	<0.1	<0.1	-
A47	10	<0.1	<0.1	-
A48	10	<0.1	<0.1	-
A49	10	<0.1	<0.1	-
A50	10	<0.1	<0.1	-

THOGOTO (cont'd)

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
A51	10	<0.1	<0.1	-
A52	10	<0.1	<0.1	-
A53	10	<0.1	<0.1	-
A54	10	<0.1	<0.1	-
A55	10	<0.1	<0.1	-
A56	10	<0.1	<0.1	-
A57	10	<0.1	<0.1	-
A58	10	<0.1	<0.1	-
A59	10	<0.1	<0.1	-
A60	10	<0.1	<0.1	-

B-KIKUYU TOWN:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
B1	10	<0.1	<0.1	-
B2	10	<0.1	<0.1	-
B3	10	<0.1	<0.1	-
B4	10	<0.1	<0.1	-
B5	10	<0.1	<0.1	-
B6	10	<0.1	<0.1	-
B7	10	<0.1	<0.1	-
B8	10	<0.1	<0.1	-
B9	10	<0.1	<0.1	-
B10	10	<0.1	<0.1	-
B11	10	<0.1	<0.1	-
B12	10	<0.1	<0.1	-
B13	10	<0.1	<0.1	-
B14	10	<0.1	<0.1	-
B15	10	<0.1	<0.1	-
B16	10	<0.1	<0.1	-
B17	10	<0.1	<0.1	-
B18- Egg broken				
B19	10	<0.1	<0.1	-
B20	10	<0.1	<0.1	-
B21	10	<0.1	<0.1	-
B22	10	<0.1	<0.1	-
B23- Egg broken				
B24	10	<0.1	<0.1	-
B25	10	<0.1	<0.1	-
B26	10	<0.1	<0.1	-
B27	10	<0.1	<0.1	-
B28	10	<0.1	<0.1	-
B29	10	<0.1	<0.1	-
B30	10	<0.1	<0.1	-

C-UCHUMI SUPERMARKET. KIMATHI ST.. NAIROBI.

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
C1	10	<0.1	<0.1	-
C2	10	<0.1	<0.1	-
C3	10	<0.1	<0.1	-
C4	10	<0.1	<0.1	-
C5	10	<0.1	<0.1	-
C6	10	<0.1	<0.1	-
C7	10	<0.1	<0.1	-
C8	10	<0.1	<0.1	-
C9	10	<0.1	<0.1	-
C10	10	<0.1	<0.1	-
C11	10	<0.1	<0.1	-
C12	10	<0.1	<0.1	-
C13	10	<0.1	<0.1	-
C14	10	<0.1	<0.1	-
C15	10	<0.1	<0.1	-
C16	10	<0.1	<0.1	-
C17	10	<0.1	<0.1	-
C18	10	<0.1	<0.1	-
C19	10	<0.1	<0.1	-
C20	10	<0.1	<0.1	-
C21	10	<0.1	<0.1	-
C22	10	<0.1	<0.1	-
C23	10	<0.1	<0.1	-
C24	10	<0.1	<0.1	-
C25	10	<0.1	<0.1	-
C26	10	<0.1	<0.1	-
C27-Egg broken				
C28	10	<0.1	<0.1	-
C29	10	<0.1	<0.1	-
C30	10	<0.1	<0.1	-
C31	10	<0.1	<0.1	-
C32	10	<0.1	<0.1	-
C33	10	<0.1	<0.1	-
C34	10	<0.1	<0.1	-
C35	10	<0.1	<0.1	-
C36	10	<0.1	<0.1	-
C37	10	<0.1	<0.1	-
C38	10	<0.1	<0.1	-
C39	10	<0.1	<0.1	-
C40	10	<0.1	<0.1	-
C41	10	<0.1	<0.1	-
C42	10	<0.1	<0.1	-
C43	10	<0.1	<0.1	-
C44	10	<0.1	<0.1	-
C45	13.8 (Yolk)	0.439	<0.1	+ for yolk
C46	10	<0.1	<0.1	-
C47	10	<0.1	<0.1	-
C48	10	<0.1	<0.1	-
C49	14.7 (Yolk)	0.487	<0.1	+ for yolk
C50	12.7 (Yolk)	0.380	<0.1	+ for yolk
C51	10	<0.1	<0.1	-
C52	16.5 (Yolk)	0.583	<0.1	+ for yolk
C53	14.7 (Yolk)	0.487	<0.1	+ for yolk
C54	10	<0.1	<0.1	-
C55	10	<0.1	<0.1	-

UCHUMI (cont'd)

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
C56	10	<0.1	<0.1	-
C57	10	<0.1	<0.1	-
C58	10	<0.1	<0.1	-
C59	10	<0.1	<0.1	-
C60	10	<0.1	<0.1	-

D- MUSTAPHA POULTRY FARM, MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
D1	10	<0.1	<0.1	±
D2	10	<0.1	<0.1	±
D3	10	<0.1	<0.1	-
D4	10	<0.1	<0.1	-
D5	10	<0.1	<0.1	-
D6	10	<0.1	<0.1	-
D7	10	<0.1	<0.1	-
D8	10	<0.1	<0.1	-
D9	10	<0.1	<0.1	-
D10	10	<0.1	<0.1	-
D11	10	<0.1	<0.1	-
D12	10	<0.1	<0.1	-
D13	10	<0.1	<0.1	-
D14	10	<0.1	<0.1	-
D15	10	<0.1	<0.1	-
D16	10	<0.1	<0.1	-
D17	10	<0.1	<0.1	-
D18	Zones identical to sulphonamides			-
D19	10	<0.1	<0.1	-
D20	10	<0.1	<0.1	-
D21	10	<0.1	<0.1	-
D22	10	<0.1	<0.1	-
D23	10	<0.1	<0.1	-
D24	10	<0.1	<0.1	-
D25	10	<0.1	<0.1	-
D26	10	<0.1	<0.1	-
D27	10	<0.1	<0.1	-
D28	10	<0.1	<0.1	-
D29	10	<0.1	<0.1	-
D30	10	<0.1	<0.1	-
D31	10	<0.1	<0.1	-
D32	10	<0.1	<0.1	-
D33	10	<0.1	<0.1	-
D34	10	<0.1	<0.1	-
D35	10	<0.1	<0.1	-
D36	10	<0.1	<0.1	-
D37	10	<0.1	<0.1	-
D38	10	<0.1	<0.1	-
D39	10	<0.1	<0.1	-
D40	10	<0.1	<0.1	-
D41	10	<0.1	<0.1	-
D42	10	<0.1	<0.1	-
D43	10	<0.1	<0.1	-
D44	10	<0.1	<0.1	-
D45	10	<0.1	<0.1	-
D46	10	<0.1	<0.1	-
D47	10	<0.1	<0.1	-
D48	10	<0.1	<0.1	-
D49	10	<0.1	<0.1	-
D50	10	<0.1	<0.1	-
D51	10	<0.1	<0.1	-
D52	10	<0.1	<0.1	-
D53	10	<0.1	<0.1	-

D54	10	<0.1	<0.1	-

MUSTAPHA POULTRY FARM (cont'd).

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
D55	10	<0.1	<0.1	-
D56	10	<0.1	<0.1	-
D57	10	<0.1	<0.1	-
D58	10	<0.1	<0.1	-
D59	10	<0.1	<0.1	-
D60	10	<0.1	<0.1	-

E- JIRANI TRADERS, MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
E1	10	<0.1	<0.1	-
E2	10	<0.1	<0.1	-
E3	10	<0.1	<0.1	-
E4	10	<0.1	<0.1	-
E5	10	<0.1	<0.1	-
E6	10	<0.1	<0.1	-
E7	10	<0.1	<0.1	-
E8	10	<0.1	<0.1	-
E9	10	<0.1	<0.1	-
E10	10	<0.1	<0.1	-
E11	10	<0.1	<0.1	-
E12	10	<0.1	<0.1	-
E13	10	<0.1	<0.1	-
E14	10	<0.1	<0.1	-
E15	10	<0.1	<0.1	-
E16	10	<0.1	<0.1	-
E17	10	<0.1	<0.1	-
E18	10	<0.1	<0.1	-
E19	10	<0.1	<0.1	-
E20	10	<0.1	<0.1	-
E21	10	<0.1	<0.1	-
E22	10	<0.1	<0.1	-
E23	10	<0.1	<0.1	-
E24	10	<0.1	<0.1	-
E25	10	<0.1	<0.1	-
E26	10	<0.1	<0.1	-
E27	10	<0.1	<0.1	-
E28	10	<0.1	<0.1	-
E29	10	<0.1	<0.1	-
E30	10	<0.1	<0.1	-
E31	10	<0.1	<0.1	-
E32	10	<0.1	<0.1	-
E33	10	<0.1	<0.1	-
E34	10	<0.1	<0.1	-
E35	10	<0.1	<0.1	-
E36	10	<0.1	<0.1	-
E37	10	<0.1	<0.1	-
E38	10	<0.1	<0.1	-
E39	10	<0.1	<0.1	-
E40	10	<0.1	<0.1	-
E41	10	<0.1	<0.1	-
E42	10	<0.1	<0.1	-
E43	10	<0.1	<0.1	-
E44	10	<0.1	<0.1	-
E45	10	<0.1	<0.1	-
E46	10	<0.1	<0.1	-
E47	10	<0.1	<0.1	-
E48	10	<0.1	<0.1	-
E49	10	<0.1	<0.1	-
E50	10	<0.1	<0.1	-
E51	10	<0.1	<0.1	-
E52	10	<0.1	<0.1	-
E53	10	<0.1	<0.1	-
E54	10	<0.1	<0.1	-
E55	10	<0.1	<0.1	-

JIRANI TRADERS (con't).

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
E56	10	<0.1	<0.1	-
E57	10	<0.1	<0.1	-
E58	10	<0.1	<0.1	-
E59	10	<0.1	<0.1	-
E60	10	<0.1	<0.1	-

F- CITY GROCERS, MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
F1	10	<0.1	<0.1	-
F2	10	<0.1	<0.1	-
F3	10	<0.1	<0.1	-
F4	10	<0.1	<0.1	-
F5	10	<0.1	<0.1	-
F6	10	<0.1	<0.1	-
F7	10	<0.1	<0.1	-
F8	10	<0.1	<0.1	-
F9	10	<0.1	<0.1	-
F10	10	<0.1	<0.1	-
F11	10	<0.1	<0.1	-
F12	10	<0.1	<0.1	-
F13	10	<0.1	<0.1	-
F14	10	<0.1	<0.1	-
F15	10	<0.1	<0.1	-
F16	10	<0.1	<0.1	-
F17	10	<0.1	<0.1	-
F18	10	<0.1	<0.1	-
F19	10	<0.1	<0.1	-
F20	10	<0.1	<0.1	-
F21	10	<0.1	<0.1	-
F22	10	<0.1	<0.1	-
F23	10	<0.1	<0.1	-
F24	10	<0.1	<0.1	-
F25	10	<0.1	<0.1	-
F26	10	<0.1	<0.1	-
F27	10	<0.1	<0.1	-
F28	10	<0.1	<0.1	-
F29	10	<0.1	<0.1	-
F30	10	<0.1	<0.1	-

G- COAST POULTRIES. MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
G1	10	<0.1	<0.1	-
G2	10	<0.1	<0.1	-
G3	10	<0.1	<0.1	-
G4	10	<0.1	<0.1	-
G5	10	<0.1	<0.1	-
G6	10	<0.1	<0.1	-
G7	10	<0.1	<0.1	-
G8	10	<0.1	<0.1	-
G9	10	<0.1	<0.1	-
G10	10	<0.1	<0.1	-
G11	10	<0.1	<0.1	-
G12	10	<0.1	<0.1	-
G13	10	<0.1	<0.1	-
G14	10	<0.1	<0.1	-
G15	10	<0.1	<0.1	-
G16	10	<0.1	<0.1	-
G17	10	<0.1	<0.1	-
G18	10	<0.1	<0.1	-
G19	10	<0.1	<0.1	-
G20	10	<0.1	<0.1	-
G21	10	<0.1	<0.1	-
G22	10	<0.1	<0.1	-
G23	10	<0.1	<0.1	-
G24	10	<0.1	<0.1	-
G25	10	<0.1	<0.1	-
G26	10	<0.1	<0.1	-
G27	10	<0.1	<0.1	-
G28	10	<0.1	<0.1	-
G29	10	<0.1	<0.1	-
G30	10	<0.1	<0.1	-

H- FARM TRADERS, MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
H1	10	<0.1	<0.1	-
H2	10	<0.1	<0.1	-
H3	10	<0.1	<0.1	-
H4	10	<0.1	<0.1	-
H5	10	<0.1	<0.1	-
H6	10	<0.1	<0.1	-
H7	10	<0.1	<0.1	-
H8	10	<0.1	<0.1	-
H9	10	<0.1	<0.1	-
H10	10	<0.1	<0.1	-
H11	10	<0.1	<0.1	-
H12	10	<0.1	<0.1	-
H13	10	<0.1	<0.1	-
H14	10	<0.1	<0.1	-
H15	10	<0.1	<0.1	-
H16	10	<0.1	<0.1	-
H17	10	<0.1	<0.1	-
H18	10	<0.1	<0.1	-
H19	10	<0.1	<0.1	-
H20	10	<0.1	<0.1	-
H21	10	<0.1	<0.1	-
H22	10	<0.1	<0.1	-
H23	10	<0.1	<0.1	-
H24	10	<0.1	<0.1	-
H25	10	<0.1	<0.1	-
H26	10	<0.1	<0.1	-
H27	10	<0.1	<0.1	-
H28	10	<0.1	<0.1	-
H29	10	<0.1	<0.1	-
H30	10	<0.1	<0.1	-

J- STAR OF THE SEA GIRLS, MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
J1	10	<0.1	<0.1	-
J2	10	<0.1	<0.1	-
J3	10	<0.1	<0.1	-
J4	10	<0.1	<0.1	-
J5	10	<0.1	<0.1	-
J6	10	<0.1	<0.1	-
J7	10	<0.1	<0.1	-
J8	10	<0.1	<0.1	-
J9	10	<0.1	<0.1	-
J10	10	<0.1	<0.1	-
J11	10	<0.1	<0.1	-
J12	10	<0.1	<0.1	-
J13	10	<0.1	<0.1	-
J14	10	<0.1	<0.1	-
J15	10	<0.1	<0.1	-
J16	10	<0.1	<0.1	-
J17	10	<0.1	<0.1	-
J18	10	<0.1	<0.1	-
J19	10	<0.1	<0.1	-
J20	10	<0.1	<0.1	-
J21	10	<0.1	<0.1	-
J22	10	<0.1	<0.1	-
J23	10	<0.1	<0.1	-
J24	10	<0.1	<0.1	-
J25	10	<0.1	<0.1	-
J26	10	<0.1	<0.1	-
J27	10	<0.1	<0.1	-
J28	10	<0.1	<0.1	-
J29	10	<0.1	<0.1	-
J30	10	<0.1	<0.1	-

K- BAOBAB FARM. MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
K1	10	<0.1	<0.1	-
K2	10	<0.1	<0.1	-
K3	10	<0.1	<0.1	-
K4	10	<0.1	<0.1	-
K5	10	<0.1	<0.1	-
K6	10	<0.1	<0.1	-
K7	10	<0.1	<0.1	-
K8	10	<0.1	<0.1	-
K9	10	<0.1	<0.1	-
K10	10	<0.1	<0.1	-
K11	10	<0.1	<0.1	-
K12	10	<0.1	<0.1	-
K13	10	<0.1	<0.1	-
K14	10	<0.1	<0.1	-
K15	10	<0.1	<0.1	-
K16	10	<0.1	<0.1	-
K17	10	<0.1	<0.1	-
K18	10	<0.1	<0.1	-
K19	10	<0.1	<0.1	-
K20	10	<0.1	<0.1	-
K21	10	<0.1	<0.1	-
K22	10	<0.1	<0.1	-
K23	10	<0.1	<0.1	-
K24	10	<0.1	<0.1	-
K25	10	<0.1	<0.1	-
K26	10	<0.1	<0.1	-
K27	10	<0.1	<0.1	-
K28	10	<0.1	<0.1	-
K29	10	<0.1	<0.1	-
K30	10	<0.1	<0.1	-

L- UHURU FARM. MOMBASA:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
L1	10	<0.1	<0.1	-
L2	10	<0.1	<0.1	-
L3	10	<0.1	<0.1	-
L4	10	<0.1	<0.1	-
L5	10	<0.1	<0.1	-
L6	10	<0.1	<0.1	-
L7	10	<0.1	<0.1	-
L8	10	<0.1	<0.1	-
L9	10	<0.1	<0.1	-
L10	10	<0.1	<0.1	-
L11	10	<0.1	<0.1	-
L12	10	<0.1	<0.1	-
L13	10	<0.1	<0.1	-
L14	10	<0.1	<0.1	-
L15	10	<0.1	<0.1	-
L16	10	<0.1	<0.1	-
L17	10	<0.1	<0.1	-
L18	10	<0.1	<0.1	-
L19	10	<0.1	<0.1	-
L20	10	<0.1	<0.1	-
L21	10	<0.1	<0.1	-
L22- Egg broken				
L23	10	<0.1	<0.1	-
L24	10	<0.1	<0.1	-
L25	10	<0.1	<0.1	-
L26- Egg broken				
L27	10	<0.1	<0.1	-
L28	10	<0.1	<0.1	-
L29	10	<0.1	<0.1	-
L30	10	<0.1	<0.1	-

M- UCHUMI SUPERMARKET, AGA KHAN WALK, NAIROBI.

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
M1	10	<0.1	<0.1	-
M2	10	<0.1	<0.1	-
M3	10	<0.1	<0.1	-
M4	10	<0.1	<0.1	-
M5	10	<0.1	<0.1	-
M6	10	<0.1	<0.1	-
M7	10	<0.1	<0.1	-
M8	10	<0.1	<0.1	-
M9	10	<0.1	<0.1	-
M10	10	<0.1	<0.1	-
M11	10	<0.1	<0.1	-
M12	10	<0.1	<0.1	-
M13	10	<0.1	<0.1	-
M14	10	<0.1	<0.1	-
M15- Egg broken				
M16	10	<0.1	<0.1	-
M17	10	<0.1	<0.1	-
M18	10	<0.1	<0.1	-
M19	10	<0.1	<0.1	-
M20	10	<0.1	<0.1	-
M21	10	<0.1	<0.1	-
M22	10	<0.1	<0.1	-
M23	10	<0.1	<0.1	-
M24	10	<0.1	<0.1	-
M25	10	<0.1	<0.1	-
M26	10	<0.1	<0.1	-
M27	10	<0.1	<0.1	-
M28	10	<0.1	<0.1	-
M29	10	<0.1	<0.1	-
M30	10	<0.1	<0.1	-

N- UCHUMI SUPERMARKET, MUINDI MBINGU ST., NAIROBI.

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
N1	10	<0.1	<0.1	-
N2	10	<0.1	<0.1	-
N3	10	<0.1	<0.1	-
N4	10	<0.1	<0.1	-
N5	10	<0.1	<0.1	-
N6	10	<0.1	<0.1	-
N7	10	<0.1	<0.1	-
N8	10	<0.1	<0.1	-
N9	10	<0.1	<0.1	-
N10	10	<0.1	<0.1	-
N11	10	<0.1	<0.1	-
N12	10	<0.1	<0.1	-
N13	10	<0.1	<0.1	-
N14	10	<0.1	<0.1	-
N15	10	<0.1	<0.1	-
N16	10	<0.1	<0.1	-
N17	10	<0.1	<0.1	-
N18	10	<0.1	<0.1	-
N19	10	<0.1	<0.1	-
N20	10	<0.1	<0.1	-
N21	10	<0.1	<0.1	-
N22	10	<0.1	<0.1	-
N23	10	<0.1	<0.1	-
N24	10	<0.1	<0.1	-
N25	10	<0.1	<0.1	-
N26	10	<0.1	<0.1	-
N27	10	<0.1	<0.1	-
N28	10	<0.1	<0.1	-
N29	10	<0.1	<0.1	-
N30	10	<0.1	<0.1	-

P- UCHUMI SUPERMARKET, WESTLANDS, NAIROBI:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
P1	10	<0.1	<0.1	-
P2	10	<0.1	<0.1	-
P3	10	<0.1	<0.1	-
P4	10	<0.1	<0.1	-
P5	10	<0.1	<0.1	-
P6	10	<0.1	<0.1	-
P7	10	<0.1	<0.1	-
P8	10	<0.1	<0.1	-
P9	10	<0.1	<0.1	-
P10	10	<0.1	<0.1	-
P11	10	<0.1	<0.1	-
P12	10	<0.1	<0.1	-
P13	10	<0.1	<0.1	-
P14	10	<0.1	<0.1	-
P15	10	<0.1	<0.1	-
P16	10	<0.1	<0.1	-
P17	10	<0.1	<0.1	-
P18	10	<0.1	<0.1	-
P19	10	<0.1	<0.1	-
P20	10	<0.1	<0.1	-
P21	10	<0.1	<0.1	-
P22	10	<0.1	<0.1	-
P23	10	<0.1	<0.1	-
P24	10	<0.1	<0.1	-
P25	10	<0.1	<0.1	-
P26	10	<0.1	<0.1	-
P27	10	<0.1	<0.1	-
P28	10	<0.1	<0.1	-
P29	10	<0.1	<0.1	-
P30	10	<0.1	<0.1	-

Q- FEEDLOT MEAT SUPPLY. NAIROBI:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
Q1	10	<0.1	<0.1	-
Q2	10	<0.1	<0.1	-
Q3	10	<0.1	<0.1	-
Q4	10	<0.1	<0.1	-
Q5	10	<0.1	<0.1	-
Q6	10	<0.1	<0.1	-
Q7	10	<0.1	<0.1	-
Q8	10	<0.1	<0.1	-
Q9	10	<0.1	<0.1	-
Q10	10	<0.1	<0.1	-
Q11	10	<0.1	<0.1	-
Q12	10	<0.1	<0.1	-
Q13	10	<0.1	<0.1	-
Q14	10	<0.1	<0.1	-
Q15	10	<0.1	<0.1	-
Q16	10	<0.1	<0.1	-
Q17	10	<0.1	<0.1	-
Q18	10	<0.1	<0.1	-
Q19	10	<0.1	<0.1	-
Q20	10	<0.1	<0.1	-
Q21	10	<0.1	<0.1	-
Q22	10	<0.1	<0.1	-
Q23	10	<0.1	<0.1	-
Q24	10	<0.1	<0.1	-
Q25	10	<0.1	<0.1	-
Q26	10	<0.1	<0.1	-
Q27	10	<0.1	<0.1	-
Q28	10	<0.1	<0.1	-
Q29	10	<0.1	<0.1	-
Q30	10	<0.1	<0.1	-

R- WANGIGE MARKET:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
R1	10	<0.1	<0.1	-
R2	10	<0.1	<0.1	-
R3 (a)	14.8 (Yolk)	0.492	-	+ for yolk & albumen
R3 (b)	14.2 (Albumen)	-	0.324	
R4	10	<0.1	<0.1	-
R5	10	<0.1	<0.1	-
R6	10	<0.1	<0.1	-
R7	10	<0.1	<0.1	-
R8	10	<0.1	<0.1	-
R9	10	<0.1	<0.1	-
R10	10	<0.1	<0.1	-
R11	10	<0.1	<0.1	-
R12	10	<0.1	<0.1	-
R13	10	<0.1	<0.1	-
R14	10	<0.1	<0.1	-
R15	10	<0.1	<0.1	-
R16	10	<0.1	<0.1	-
R17	10	<0.1	<0.1	-
R18	10	<0.1	<0.1	-
R19	10	<0.1	<0.1	-
R20	10	<0.1	<0.1	-
R21	10	<0.1	<0.1	-
R22	10	<0.1	<0.1	-
R23	10	<0.1	<0.1	-
R24	10	<0.1	<0.1	-
R25	10	<0.1	<0.1	-
R26	10	<0.1	<0.1	-
R27	10	<0.1	<0.1	-
R28	10	<0.1	<0.1	-
R29	10	<0.1	<0.1	-
R30	10	<0.1	<0.1	-

S- MUGUKU FARM. KIKUYU:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
S1	10	<0.1	<0.1	-
S2	10	<0.1	<0.1	-
S3	10	<0.1	<0.1	-
S4	10	<0.1	<0.1	-
S5	10	<0.1	<0.1	-
S6	10	<0.1	<0.1	-
S7	10	<0.1	<0.1	-
S8	10	<0.1	<0.1	-
S9	10	<0.1	<0.1	-
S10	10	<0.1	<0.1	-
S11	10	<0.1	<0.1	-
S12	10	<0.1	<0.1	-
S13	10	<0.1	<0.1	-
S14	10	<0.1	<0.1	-
S15	10	<0.1	<0.1	-
S16	10	<0.1	<0.1	-
S17	10	<0.1	<0.1	-
S18	10	<0.1	<0.1	-
S19	10	<0.1	<0.1	-
S20	10	<0.1	<0.1	-
S21	10	<0.1	<0.1	-
S22	10	<0.1	<0.1	-
S23	10	<0.1	<0.1	-
S24	10	<0.1	<0.1	-
S25	10	<0.1	<0.1	-
S26	10	<0.1	<0.1	-
S27	10	<0.1	<0.1	-
S28	10	<0.1	<0.1	-
S29	10	<0.1	<0.1	-
S30	10	<0.1	<0.1	-

T- KAWANGWARE MARKET:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
T1	10	<0.1	<0.1	-
T2	10	<0.1	<0.1	-
T3	10	<0.1	<0.1	-
T4	10	<0.1	<0.1	-
T5	10	<0.1	<0.1	-
T6	10	<0.1	<0.1	-
T7	10	<0.1	<0.1	-
T8	10	<0.1	<0.1	-
T9	10	<0.1	<0.1	-
T10	10	<0.1	<0.1	-
T11	10	<0.1	<0.1	-
T12	10	<0.1	<0.1	-
T13	10	<0.1	<0.1	-
T14	10	<0.1	<0.1	-
T15	10	<0.1	<0.1	-
T16	10	<0.1	<0.1	-
T17	10	<0.1	<0.1	-
T18	10	<0.1	<0.1	-
T19	10	<0.1	<0.1	-
T20	10	<0.1	<0.1	-
T21	10	<0.1	<0.1	-
T22	10	<0.1	<0.1	-
T23	10	<0.1	<0.1	-
T24	10	<0.1	<0.1	-
T25	10	<0.1	<0.1	-
T26	10	<0.1	<0.1	-
T27	10	<0.1	<0.1	-
T28	10	<0.1	<0.1	-
T29	10	<0.1	<0.1	-
T30	10	<0.1	<0.1	-

O- BAHATI. NAKURU:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
O1	10	<0.1	<0.1	-
O2	10	<0.1	<0.1	-
O3	10	<0.1	<0.1	-
O4	10	<0.1	<0.1	-
O5	10	<0.1	<0.1	-
O6	10	<0.1	<0.1	-
O7	10	<0.1	<0.1	-
O8	10	<0.1	<0.1	-
O9	10	<0.1	<0.1	-
O10	10	<0.1	<0.1	-
O11	10	<0.1	<0.1	-
O12	10	<0.1	<0.1	-
O13	10	<0.1	<0.1	-
O14	10	<0.1	<0.1	-
O15	10	<0.1	<0.1	-
O16	10	<0.1	<0.1	-
O17	10	<0.1	<0.1	-
O18	10	<0.1	<0.1	-
O19	10	<0.1	<0.1	-
O20	10	<0.1	<0.1	-
O21	10	<0.1	<0.1	-
O22	10	<0.1	<0.1	-
O23	10	<0.1	<0.1	-
O24	10	<0.1	<0.1	-
O25	10	<0.1	<0.1	-
O26	10	<0.1	<0.1	-
O27	10	<0.1	<0.1	-
O28	10	<0.1	<0.1	-
O29	10	<0.1	<0.1	-
O30	10	<0.1	<0.1	-

Y- BAHATI. NAKURU:

Egg No.	Mean zone of inhibition diameter (mm)	OTC conc. in yolk (ppm)	OTC conc. in albumen (ppm)	+ve or -ve for OTC
Y1	10	<0.1	<0.1	-
Y2	10	<0.1	<0.1	-
Y3	10	<0.1	<0.1	-
Y4	10	<0.1	<0.1	-
Y5	10	<0.1	<0.1	-
Y6	10	<0.1	<0.1	-
Y7	10	<0.1	<0.1	-
Y8	10	<0.1	<0.1	-
Y9	10	<0.1	<0.1	-
Y10	10	<0.1	<0.1	-
Y11	10	<0.1	<0.1	-
Y12	10	<0.1	<0.1	-
Y13	10	<0.1	<0.1	-
Y14	10	<0.1	<0.1	-
Y15	10	<0.1	<0.1	-
Y16	10	<0.1	<0.1	-
Y17- Egg broken				
Y18	10	<0.1	<0.1	-
Y19	10	<0.1	<0.1	-
Y20	10	<0.1	<0.1	-
Y21	10	<0.1	<0.1	-
Y22	10	<0.1	<0.1	-
Y23	10	<0.1	<0.1	-
Y24	10	<0.1	<0.1	-
Y25	10	<0.1	<0.1	-
Y26	10	<0.1	<0.1	-
Y27	10	<0.1	<0.1	-
Y28	10	<0.1	<0.1	-
Y29	10	<0.1	<0.1	-
Y30	10	<0.1	<0.1	-

Appendix 2:Oxytetracycline residue levels (ppm).in yolk.

Bird	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
4-1	0	-	0.263	0.503	-	0.503	0.503	-	0.663	0.565	-	0.529	0
4-2	0.263	0	-	0	0.396	-	0.423	0.423	-	0.512	0.476	-	0
4-3	0.263	-	0	0.369	-	0.396	0.503	0.529	-	0.574	0.503	0.396	0
4-4	0.263	0	0	0	-	0.343	0.396	-	-	0.405	-	-	0
4-5	-	0	-	0	-	0.263	-	0.369	0.503	0.503	-	0	-
Mean	0.210	0.050	0.121	0.204	0.396	0.376	0.456	0.440	0.583	0.512	0.490	0.325	0.050
3-1	-	-	-	0	-	0	-	-	-	-	-	0	-
3-2	0.263	-	0	0	-	0	0.343	-	0.529	-	0	-	0
3-3	-	-	-	-	-	-	0	-	-	-	-	-	-
3-4	-	0.263	0	0	-	0	0.396	-	0.458	0.343	0	-	0
3-5	-	0	-	-	0	-	0.345	-	0.592	-	0.565	0.458	-
Mean	0.263	0.157	0.050	0.050	0.050	0.050	0.283	-	0.526	0.343	0.222	0.252	0.050
2-1	0	-	0	-	0	-	-	0.263	-	0	-	0	0
2-2	0	0	-	0	0	-	0.289	0.263	-	0.405	-	-	-
2-3	0	0	-	0	0	0	-	0.263	-	0.449	-	0	0
2-4	0	0	0	-	0	0	-	0	0	-	-	0	-
2-5	0	-	0	0	-	0	-	0	-	0	0	-	0
Mean	0.050	0.050	0.050	0.050	0.050	0.050	0.289	0.178	0.050	0.239	0.050	0.050	0.050

N.B: "0" represents <0.100 ppm of oxytetracycline and is taken to be 0.05 ppm.

Appendix 3: Oxytetracycline residue levels (ppm).in albumen.

Bird	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
4-1	0	-	0.314	0.314	-	0.314	0.361	-	0.245	0	-	0	0
4-2	0	0.268	-	0.175	0.221	-	0.175	0	-	0	0	-	0
4-3	0	-	0.291	0.198	-	0.221	0.361	0.361	-	0	0	0	0
4-4	0	0	0	0.221	-	0	0.221	-	-	0	-	-	0
4-5	-	0	-	0	-	0	-	0.198	0	0	-	0	-
Mean	0.050	0.123	0.218	0.191	0.221	0.159	0.280	0.201	0.148	0.050	0.050	0.050	0.050
3-1	-	-	-	0	-	0	-	-	-	-	-	0	-
3-2	0	-	0	0	-	0	0	-	0	-	0	-	0
3-3	-	-	-	-	-	-	0	-	-	-	-	-	-
3-4	-	0	0	0	-	0	0	-	0	0	0	-	0
3-5	-	0	-	-	0	-	0.221	-	0	-	0	0	-
Mean	0.050	0.050	0.050	0.050	0.050	0.050	0.093	-	0.050	0.050	0.050	0.050	0.050
2-1	0	-	0	-	0	-	-	0	-	0	-	0	0
2-2	0	0	-	0	0	-	0	0	-	0	-	-	-
2-3	0	0	-	0	0	0	-	0	-	0	-	0	0
2-4	0	0	0	-	0	0	-	0	0	-	-	0	-
2-5	0	-	0	0	-	0	-	0		0	0	-	0
Mean	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050

N.B: "0" represents <0.100 ppm of oxytetracycline and is taken to be 0.05 ppm.

Appendix 4.

Mean oxytetracycline residue levels (ppm) in yolk.

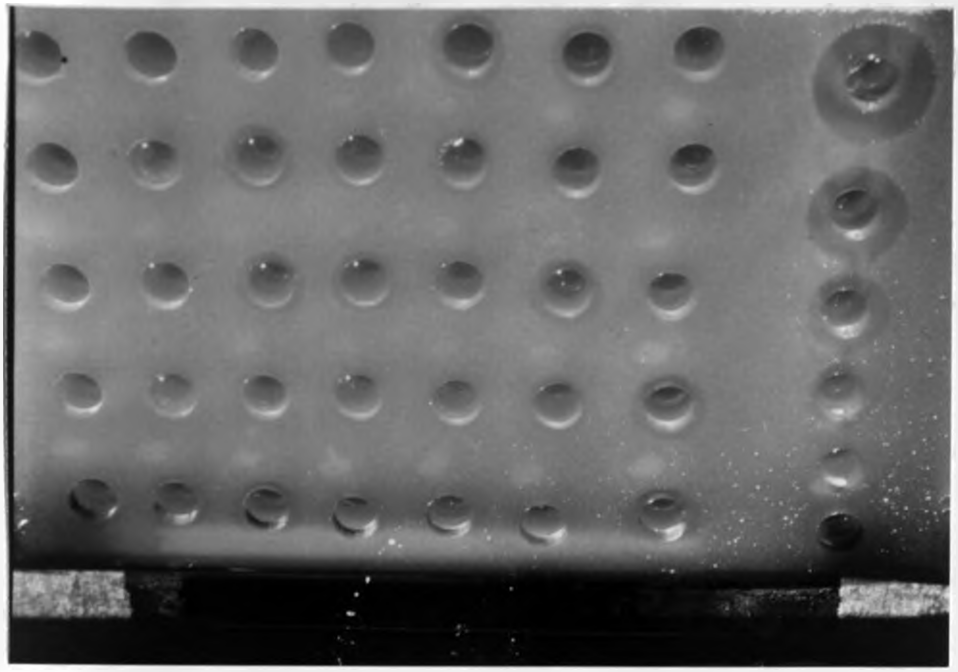
	Day of study												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Grp.2	0.050	0.050	0.050	0.050	0.050	0.050	0.289	0.178	0.050	0.239	0.050	0.050	0.050
Grp 3	0.263	0.157	0.050	0.050	0.050	0.050	0.283	-	0.526	0.343	0.222	0.252	0.050
Grp 4	0.210	0.050	0.121	0.204	0.396	0.376	0.456	0.440	0.583	0.512	0.490	0.325	0.050

Appendix 5.

Mean oxytetracycline residue levels (ppm) in albumen.

	Day of study												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Grp.2	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Grp.3	0.050	0.050	0.050	0.050	0.050	0.050	0.093	-	0.050	0.050	0.050	0.050	0.050
Grp.4	0.050	0.123	0.218	0.191	0.221	0.159	0.280	0.201	0.148	0.050	0.050	0.050	0.050

N.B: "0" represents <0.100 ppm of oxytetracycline and is taken to be 0.05 ppm.



Appendix 6