

AN ABSTRACT OF THE THESIS OF

Ali A. Youssef Hakimi for the degree of Master of Science  
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Title: Sulfa Residue Studies with Market Turkeys: Repletion,  
Depletion and Recycling Effect from Reused Litter

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Abstract Approved: \_\_\_\_\_

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A series of four experiments were conducted to study sulfa residues with market turkeys and to observe the repletion, depletion and possible recycling effect from reused litter.

In the first experiment, sulfaquinoxaline (S.Q.) was fed at 0.015% level from 8 to 16 weeks of age. No significant effects were observed among the treatments in body weights, feed consumption, and mortality. The level of S.Q. remained slightly above 0.1 ppm after 12 days of withdrawal. Litter and the feed of the sulfa treated birds were 80, and 137 ppm.

The second and third experiments were conducted to observe the depletion of sulfadimethoxine (SDM) from kidney tissue and to observe the carry-over effect from reused litter (1 and 2 times) to the edible tissue in market turkeys fed SDM at 0.00625% level from day old to 17 weeks of age. Body weights, feed consumption and mortality were not significantly different among the treatments. The level

of SDM in the litter indicated an accumulation effect. No direct transfer of SDM was observed from the litter to the tissue. However, SDM levels in the litter did affect the depletion of the drug from the kidney tissue resulting in concentrations slightly above tolerance levels of 0.1 ppm 7 days after the initiation of the withdrawal period. High correlation coefficients were obtained between kidney, liver and breast tissues with the levels of SDM at the start of the withdrawal period.

A fourth experiment was conducted to determine the repletion rate and plateau levels of SDM in the whole blood of market turkeys fed either a prophylactic level (0.00625%) or a therapeutic level (0.03125%) for 24 days. In the prophylactic treatment, SDM reached the plateau of 1 ppm after 15 days with the highest levels around 2 ppm at 14 days. In the therapeutic treatment, the level of SDM leveled at 24 hours with 4 ppm and the highest levels were obtained at 40 ppm at 11 days.

SULFA RESIDUE STUDIES WITH MARKET TURKEYS:  
REPLETION, DEPLETION AND RECYCLING  
EFFECT FROM REUSED LITTER

by

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## CONTRIBUTIONS OF THE AUTHORS

Dr. Harry S. Nakaue is an Associate Professor at Oregon State University Poultry Science Department and one of the project leaders for the Residue Avoidance Program (RAP), a grant received by the Department of Poultry Science from the United States Department of Agriculture Extension Service. Dr. Nakaue has directed all the research involved with the demonstration of sulfa drug recycling through built up litter of market turkeys. Dr. Nakaue has also acted as the major advisor for the senior author.

Professor Charles M. Fischer, the O.S.U. Poultry Extension Specialist, is also one of the project leaders with the RAP program and has directed the field monitoring of pesticides and sulfonamide residues in market turkeys.

Molly L. Murphy is a graduate student involved with the monitoring phase and the development of the qualitative sulfa blood analytical test as a partial fulfillment of her Master of Agriculture thesis.

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# SULFA RESIDUE STUDIES WITH MARKET TURKEYS: REPLETION, DEPLETION AND RECYCLING EFFECT FROM REUSED LITTER

## CHAPTER I

### INTRODUCTION

Uses of drugs in livestock and poultry production for the purpose of disease treatment is one of the oldest practices in the field of Veterinary Medicine. The major risk involved with some of these drugs is their transfer to meat, eggs and milk from the treated animals. This concern is of a greater importance since the use of these compounds has been expanding rapidly during the past three decades.

One of the classes of drugs used extensively in poultry production is sulfonamides. These drugs are used for their coccidiostatic, bacteriostatic, viricidal and growth promoting properties. Because of the extensive uses of these compounds, efforts have been made to monitor any drug residue problems which have been most significant in swine and turkey industries.

Many reasons were suggested for the high incidence of drug residues seen in animals. These include failure to comply with suggested drug withdrawal periods indicated on the label, mismanagement, cross contamination of feed, and recycling of the drug through litter material.

The commercial turkey producer uses sulfa drugs much more than the broiler grower or the egg producer. For this reason, there are more sulfa residue problems confronting the turkey industry even though good management practices may be used. Therefore, the purposes of the following experiments were to study the drug recycling from the litter material to the edible tissue, and to study the repletion and depletion of sulfadimethoxine in market turkeys.

## CHAPTER II

## REVIEW OF LITERATURE

The sulfonamides have found varied uses as clinically effective drugs since their discovery. Prior to the discovery of penicillin and other antibiotics, sulfa drugs were the mainstay of bacterial chemotherapy. Applegate (1983) states that sulfonamides are the most widely used antimicrobials in the world today. Their success has been due mostly to their low cost and high effectiveness against a wide range of antimicrobial activity.

History of the Sulfonamides:

Sulfanilamide was first synthesized in 1908 by Gelmo (Gelmo, 1908). The antibacterial activity of Prontosil, an azo dye containing p- aminobenzene sulfonamide group was demonstrated in 1935 (Domagk, 1935a, 1935b). As a result, Domagk received the Nobel prize in medicine in 1938. The therapeutic significance of the drug was not established until 1937 with the synthesis of sulfapyridine. Fuller (1937) confirmed that Prontosil was broken down in vivo to sulfanilamide. In later years, it was learned that Prontosil was excreted as a colorless product in the urine of the animals receiving the dye. Due to the fact that aromatic amines were known to be excreted as acetyl derivatives following administration, it was shown subsequently that acetyl sulfonamides were a major excretory product of the dye. Trefouels et al. (1935), made the assumption that

sulfonamide was the antibacterially active compound present in Prontosil. In 1936, Fournau et al. showed that pure sulfonamide was highly effective when administered to mice which were inoculated with lethal doses of bacteria.

According to Woods (1962) prior to World War II, sulfonamides were the only successful agents for chemotherapy of bacterial infections. Sulfonamides were the first anti-coccidial used successfully in the treatment of coccidiosis from 1940 to 1948 (Grumbles et al., 1947, 1948a, 1948b). During following years, over five thousand derivatives of sulfonamides were synthesized and tested for their antibacterial activities. Less than thirty of these drugs are presently used as clinically effective drugs in different domestic animals and approved by the Federal government (Table 1).

#### Chemistry of Sulfonamides:

Sulfanilamide and other amide of sulfonic acid (para amino benzene sulfonic acid) and its derivatives are commonly known as sulfonamides or sulfa drugs (Figure 1). They generally contain a benzene or other aromatic nucleus, an amino group and a weakly acidic group, possibly a carboxylic group. The nitrogen molecules in the compound are designated as N1 and N4 (amino nitrogen). According to Bevill (1982), most antibacterial sulfonamides have been synthesized by chemical substitution at the N1 position. Substitution of the compound at the N4 position greatly reduces the antibacterial activity when compared to their unsubstituted

counterparts. However, certain N4 substituted compounds provide some antibacterial action. The most important chemical feature of sulfonamides is the direct linkage of the sulfur to the benzene ring without which the drug is inactivated.

As a chemical class, sulfonamides are white crystalline powders that are relatively insoluble in water, exhibit amphoteric behavior and form salts in both strongly acidic and strongly basic solutions. The antibacterial efficiency has been shown to be dependent upon pH, and maximum competition with PABA is shown at higher pH (Schmelkes et al., 1942). Generally, sulfonamides behave as weak organic acids. Schmelkes et al. (1942) also stated that the active agent in a sulfonamide solution is an anionic specie of the drug. Sodium salts of the sulfonamides have greater solubility than the parent compounds. A major development has been the synthesis of highly soluble derivatives in the urine to reduce the incidences of renal toxicity. Lehr (1945) demonstrated that solubility of a sulfonamide is not influenced by the presence of other sulfonamides in the solution thus obeying the "Law of Independent Solubility". Some relevant structures are shown in Figure 1.

#### Mode of Action of Sulfonamides:

Sulfonamides are bacteriostatic agents which inhibit the multiplication of bacteria. Sulfonamide activity is best described by Woods and Fildes (1940), who were further

supported by Wyss (1941) explain it as follows. Competition of the drug with para amino benzoic acid (PABA), its most prominent antagonist which is necessary for the biosynthesis of bacterial folic acid, inhibits formation of tetrahydro-folic acid (THFA), a vital cofactor for amino acid metabolism relative to purine synthesis hence the formation of RNA. Reduced RNA synthesis leads to the inhibition of bacterial protein production and multiplication of the bacteria is arrested. This inhibition process does not interfere with the protein synthesis scheme in the animal's body. The animal supplies its cells with folic acid from the diet. Sulfonamides stop the rapid growth of bacteria population in a disease, but the phagocytosis of the bacteria by the defense mechanism has to take place for the full effectiveness against the disorder. Bevill (1982) states that the best therapeutic efficacy of the drug is achieved in the early stages of the bacterial infection because of the following reasons. First, due to the high metabolic rate of the bacteria, it causes them to pick up sulfonamides by mistake into the cellular biosynthesis scheme. Second, the animal possesses a high capability for phagocytosis at an early stage of the disease which causes the process to take place at a higher rate. The diffusion of the drug into the infectious sites takes place faster because the tissue barriers from inflammatory reactions have not yet been produced to obstruct the diffusion of the drug. One last reason is because at an early



stage of bacterial infection, there is no cellular debris to limit drug action.

#### Bacterial Resistance of Sulfonamides:

Bacterial resistance will be exhibited very rapidly, both in vitro and in vivo. This resistance is irreversible and can remain for many generations. Selser et al. (1944) has shown that some strains of pneumococcus which have acquired a high degree of resistance to the sulfonamide retain that resistance for an indefinite period of time, while strains which have acquired only a moderate degree of resistance may lose this characteristic after removal of contact with the drug. Acquired resistance can also limit the therapeutic efficacy of the drug. The resistance will not be limited to one sulfonamide. It develops based upon changes for the requirement of the PABA by the bacterial cell which can, with time, be circumvented by the use of sulfonamide due to the similarity of the two compounds. At this stage, some bacteria may even become dependant on a sulfonamide for their growth. Woods (1940) was the first to suggest that resistance may be based upon the ability of the bacteria to synthesize enough PABA to antagonize the drug.

#### Metabolism of Sulfonamides:

Much of the data pertaining to sulfonamide metabolism remains unpublished, and available literature is very limited on the subject. Mandell and Sande (1981) stated that the primary metabolic alterations with sulfonamides take place

in the liver. Oshima et al. (1964) has shown that sulfadimethoxine administered to poultry orally was absorbed rapidly from the gastrointestinal tract with the small intestine being the major site. Little absorption also takes place in the stomach and hardly any from the crop. However, the presence of the crop did prove to be an important factor in absorption due to variation in the plasma concentration after oral administration when compared to intravascular administration. Mandell and Sande (1981) mentioned that 70 to 100 percent of the oral dose of the drug is absorbed, and the drug can be found in the urine within 30 minutes of administration. The degree of absorption depends mostly on the specie of the animal and the drug administered. The absorption mechanism is not affected by the water solubility of the drug which is evenly distributed throughout most tissues except the brain. It readily passes the placental barrier to the limit where the fetal blood level may approach that of the maternal levels. The binding of the drug takes place primarily with plasma protein, albumin (Bevill, 1982), and appears to decrease with the age of the plasma sample employed in the chicken (Bankowski and Johnson, 1948).

Sulfonamides may undergo many different transformations before excretion. The most common reaction is acetylation, which in turn inactivates the drug while retaining the toxic potentialities of the parent substance. The degree of acety-

lation varies with the drug and the animal; it is considered moderate in most domestic animals. The drug also undergoes glucuronic acid conjugation and sulfate conjugation to a much smaller extent. Excretion of sulfonamides and their metabolic products is almost entirely by the kidney, resulting in high urinary excretions from body tissues. This is confirmed by high levels of residues present in the kidney of the contaminated animals. The excretion takes place in the form of free drug, acetylated and conjugated form. Small amounts are also excreted in bile, pancreatic and intestinal juices, saliva and milk. With poultry, Mercer (1975) mentioned that the subcutaneous glandular systems may play an active role in the excretion of the drug because of the levels of residues found in skin tissues. Problems can arise when dehydration occurs, reducing the excretion of sulfonamides and prolonging blood levels which can lead to residue problems. On the other hand, excessive excretion of the drug can occur with increased defecation of water. Excretion of the drug may also vary with the degree of solubility of the sulfonamide. The greater the solubility, the more easily excreted. If the concentration of the sulfonamide exceeds that of saturation limits, crystals are formed and deposited in the excretory system of the animal causing damage to the tissues involved. High enough levels of crystal formation can also affect the nervous system. Chronic toxicity can cause neuritis and suppression of egg

production in chickens. Morrison, et al. (1954) reported that in diets low in vitamin K, graded levels of vitamin K were needed to correct the prolonged clotting time induced by addition of sulfaquinoxaline and states the reason to be the influence of sulfa drugs in general on prothrombin formation and inhibition of intestinal synthesis of vitamin K. This finding was also supported by Frost and Spruth (1955). Other side effects have been also observed in humans, such as hypersensitivity along with headache, nausea and vomiting and possible hepatitis (Dujovne et al., 1967).

#### Blood Concentrations of Sulfonamides:

Concentration of a sulfonamide in the blood is a net result of absorption, metabolism, distribution and excretion of the drug from the body. Any small variation in any of these mechanisms can change the overall blood concentration and the depletion scheme over a period of time. The efficacy of a sulfonamide can be correlated to some extent with the concentration of the drug in the blood. According to Bevill, (1982) blood concentrations between 5 and 15 mg/100 ml are recognized as safe and efficacious. High concentrations may lead to an unsatisfactory response. Weinstein et al. (1960) have demonstrated a crude correlation between sulfonamide blood concentrations and therapeutic response. Correlation can vary with bacterial activity, extent of metabolism of the drug, status of the defense mechanism and other factors, thus correlation coefficients cannot be consistent and dependable.

Regulation of Sulfonamides:

According to Mercer (1975), the regulatory control over new animal drugs and medicated feed originated in 1938 under the New Drug and Cosmetic Act. Approval of all medicated feeds and animal drugs was required by the Food and Drug Administration under these provisions. During the following years, until 1962, the law was strengthened and broadened to ensure the effectiveness of the drugs and to avoid residual characteristics of the new drugs. One of these amendments was the 1958 Food Additive Amendment which allowed the establishment of a tolerance limitation of other than zero for a "safe" food additive in human food, as the number of products increased steadily. Antibiotic production doubled from 1960 to 1965 and there was a sixfold increase in feed additive usage from 1960 to 1970. Today, the FDA, through Food Safety and Inspection Service (FSIS), is responsible for assuring the absence of any illegal residues. The meats from all livestock and poultry are sampled and inspected continuously for any possible residue in the edible tissues. These studies mostly apply to food animal drugs which were approved since 1962. Many of the sulfonamides were approved prior to this amendment, and drug manufacturers were not required to submit data on tissue residues following their use in food producing animals. In 1973, the FDA ruled that all sulfonamides containing drugs for oral, injectable, intramammary and intrauterine use in food producing animals

would be considered new animal drugs. The meaning of this ruling was that a "no effect" level had to be determined with target animals in the laboratory. Tissue residue data have to be submitted also for the establishment of safe withdrawal periods to ensure the absence of any drug residues in the meat or eggs of the treated animals.

Federal Drug Administration Drug Withdrawal Requirement:

Drug withdrawal requirements set by FDA are based upon complete information from the supplier on identity of the drug along with all the physical and chemical properties, metabolism, methodology for the determination and experimental data on all species for which the drug has been developed. This information is then printed on the label of the drug under withdrawal days once the product is approved. Based on the same information, drugs are then classified as to their tolerance for residues. Sulfonamides can be categorized as "negligible". This group embodies compounds for which the toxic and non-toxic levels have been determined and the best sensitivity achieved with analytical assay and methodology for those levels. The analytical sensitivity and/or tolerance levels for all sulfonamides is set at 0.1 ppm in muscle, kidney and eggs and 0.01 ppm in milk.

Sulfonamide Residues:

Miller (1983) states the FDA definition for residues as follows: "An illegal residue is a drug substance in edible tissues of animals at concentrations in excess of the

tolerance established by Food and Drug." Data from FSIS (Table 2) shows consistency of residue violation with sulfonamides in livestock and poultry. The greatest portion has been seen with swine and turkeys. These values are all in excess of the tolerance level for the drug which has been set at 0.1 ppm in the tissue. In poultry, the greatest violation occurred in the third and fourth quarter of 1974 in North and South Carolina. Some areas in the two states showed a high incidence of sulfa residues in turkeys. As a result, several thousand pounds of processed meat were destroyed. Studies conducted by governmental agencies during this period have indicated 4.7% incidence. Most similar surveys with different species have indicated that sulfa residues are a frequent problem in the livestock and poultry industries. As a result, disruptions occur in the marketing of the products causing heavy financial losses to the producer. The most significant loss incurred by the producer is the loss encountered when the drug is removed from the feed in order to avoid residues, according to Van Houweling (1981). Cromwell (1983) blames the occurrence of the residues on cross contamination of sulfa feed and clean feed due to the electrostatic properties of the drug and concluded that the granulated form of the drug should help in reducing residue violations in pork. Pneumarthu et al. (1975) saw improper observation of withdrawal period as the main reason for violation. Possibility of drug recycling

through litter material has been studied by Whipple et al. (1980) where accumulation of the drug did occur in the kidney and liver of untreated pigs in 5 to 14 days without consumption of any sulfamethazine. No such data has been conducted with market turkeys to study recycling and possible carry-over of sulfa drugs from litter to the edible tissue.

Sulfaquinoxaline:

Sulfaquinoxaline (S.Q.), chemically known as 2- sulfanilamido quinoxaline, first reported by Weilljard et al. (1944) is one of a series of sulfonamide drugs which proved to be superior to the parent drug, sulfanilamide. Smith and Robinson (1944) showed S.Q. to be four times as effective as sulfonilamide in vitro. Delaplane (1945) was the first to report its successful use against upper respiratory infection due to Pasteurella avicida at levels of 0.05 and 0.01 percent in a mash diet. In a following report, Delaplane et al. (1947) showed that the same concentrations were both prophylactically and therapeutically effective against cecal coccidiosis and fowl cholera in chickens (Delaplane and Higgins, 1948). Soon there were reports on possible toxic effects with this drug, and extensive studies were conducted to study possible toxic effects of S.Q. Delaplane and Milliff (1948), showed that 0.05 percent S.Q. in the diet was toxic to laying hens and observed whitish foci in spleen, liver, kidney, lungs, and heart tissue of the animals 8 days after medication. Davies and Kendall (1953)



reported hemorrhage, pale bone marrow and some mortality in poultry. Yacowitz et al. (1955) showed also some hemorrhage and mortality, and Sadeck et al. (1955) suggested an anemic condition associated with S.Q. toxicity. Faddoul et al. (1967) observed increased mortality, decreased body weight along with focal necrosis in the liver, and gross pathological changes in kidney and spleen from levels of 0.0125 percent S.Q. with chickens up to 15 weeks of age. S.Q. was reported to interfere with vitamin K synthesis in the gut, according to Morrison et al. (1954) and Frost and Spruth (1955). More recently, Mian (1980) reported effects of S.Q. on the maturation of bone marrow cells in chickens. On the other hand, Cuckler and Ott (1955) reported S.Q. to be extremely well tolerated by chickens up to 64 times the recommended levels of use without any gross pathological effects found under the conditions studied. They failed to observe the accumulation of the drug in the blood after long continuous administration. Despite the problems, S.Q. has been popular over the years. Singen et al. (1948) reported the tendency of S.Q. to slightly increase feed efficiency in poultry. Atkinson et al. (1971), in studies with S.Q. in laying turkeys, observed highest level of production, fertility and hatchability with the group of birds receiving S.Q. at 0.024 percent in the diet and showed no detrimental effects on reproductive performance when S.Q. was fed continuously up to 0.036 percent in the diet. Schleckner

and Simmons (1950) stated the preference of S.Q. over the more common sulfonamides is mostly due to its antibacterial effect against a wide range of bacteria and its greater accumulation and retention time in the blood. The binding of the drug to plasma has been said to be in direct proportion with the blood concentration of the drug as well as its ready penetration into the egg and its accumulation in the tissue (Davis, et al., 1942).

Sulfadimethoxine:

Sulfadimethoxine (SDM), chemically known as 2, 4 dimethoxy 6 sulfonilamido 1, 3 diazine was first synthesized by Bretschneider and Kloetzer in 1955. Its antibacterial activity was first reported by Schnitzer et al. (1955). It is a white, odorless and almost tasteless crystalline powder, slightly soluble in water. Its solubility increases with increases in the pH. It has shown to have remarkable activity in a wide range of experiments with gram positive and gram negative bacteria. Its activity is reversed by PABA, like other sulfa drugs. It is a strong bacteriostatic agent with high chemotherapeutic potency exerted in vivo. Schnitzer et al. (1955) speculated the possibility that the presence of the methoxy group could be correlated to the toxicological and chemotherapeutic characteristics of the compound. It is very well absorbed in the blood and maintained well at high concentrations, in addition to its slow excretion rate in a highly soluble form. This charac-

teristic of SDM will permit great flexibility in the administration of the drug and offers the convenience of infrequent administrations. Studies conducted on the toxicity of the drug do not agree, and the results differ widely depending on dosages and the species used. Randall et al. (1959) reported hypertrophy and hyperemia of the thyroid glands with mice along with kidney damage. In humans, Weinstein et al. (1960) mentioned abdominal pains, fever and rash in a review of literature with sulfonamide toxicity. In poultry, Mitrovic (1968) and Mitrovic and Bauernfeind (1967) showed SDM to be effective against coccidiosis in turkeys. Bajwa and Singh (1977) reported that SDM at 0.05 percent in water affected growth rate after 21 days and caused premature development of combs and wattles in cockerels. A great deal of research has been conducted on the use of Rofenaid, a potentiated mixture of SDM and ormetoprim in poultry. Mitrovic et al. (1969a, 1969b) showed the effectiveness of Rofenaid as an anticoccidial and an antibacterial agent in chickens. Subsequent studies by Mitrovic et al. (1971a, 1971b) have shown the mixture to be equally as effective in turkeys. Mitrovic and Bauernfeind (1971) also showed that SDM at 0.0125% is effective against all pathogenic species of coccidia and against fowl cholera and infectious coryza (Mitrovic, 1967) in both chickens and turkeys. The safety and compatability of the drug was also studied by the same researchers and established to be 0.02% in broiler

rations and 0.01% in replacement pullet rations on a continuous basis (Marusich et al., 1969) and turkeys (Marusich et al., 1971). In the same studies, he also reported no adverse effects on broilers based on feed conversion, mortality, hematology and gross pathology with 0.08%, 4 times the proposed use level, when fed continuously for 8 weeks. Mitrovic et al. (1971a,b) stated also that the interference of the drug with folic acid, dihydroxy folic acid and its beneficial therapeutic response enhance the activity based on lower drug concentrations and decreased toxicity and mild drug resistance. The toxicity of SDM has also been reported to be lower than S.Q. and sulfadimidine due mostly to its higher solubility (Bevill, 1982).

#### Depletion and Residue Studies:

Many researchers have dedicated their efforts in studying depletion with sulfonamides with different species such as swine and poultry (Righter et al., 1970, 1973). The initial withdrawal period set for S.Q. was to be at 5 days prior to slaughter in meat animals. Different studies have shown a need for longer than 5 days withdrawal period for the complete disappearance of the drug from the tissue. In subsequent studies, it was suggested that the rate of depletion is highly proportional to tissue concentrations. Blom (1975) stated the reason for longer persistence of the drug in the egg as compared to the blood is due to the fact that water soluble proteins which are bound to the drug are in

reserve in the oviduct in sufficient amounts for 2 eggs. Righter et al. (1970) showed persistence of the drug in the renal tissue and suggested a withdrawal period in excess of 7 days was required for S.Q. in laying hens and broilers in order to reduce residues in kidney and eggs to or below 0.1 ppm. Righter et al. (1973) stated that a withdrawal period in excess of 10 days was needed with turkey poults given S.Q. at both prophylactic (0.0175%) and therapeutic (0.01%) levels in the water for 7 days. The new withdrawal period set for S.Q. is now 10 days prior to slaughter. S.Q. can be fed both continuously and intermittently to poultry. Grumbles et al. (1948a) suggested a feeding schedule of 0.05% for intermittent feeding and 0.033% for continuous feeding. Later however, the continuous level was reduced to 0.0125% of the diet. After studies conducted at Merck Laboratories with 12 week old turkey, Miller (1982), reported a withdrawal period of 10 days prior to slaughter with S.Q. This was long enough to reduce residue levels below the recommended levels of 0.1 ppm in all tissues when administered at 0.0175% in feed and 0.01% in drinking water. SDM has been reported to require 4, 6, and 10 days to disappear from plasma, albumin, and yolk, respectively, (Onodera et al., 1970). Fellig et al. (1971) suggested a period of 2 days for complete disappearance of the drug from the tissue. Randall et al. (1959) had shown that 4 day persistence in the tissue was seen with rats and a plateau was reached at 2-3 days with

levels of 25 mg/kg of body weight. Rofinaid has a 5 day withdrawal period associated with it. Laurencot et al. (1972) suggested 9 days for SDM and 7 days for ormetoprim from the renal tissue and 14 days for SDM and 12 days for ormetoprim to disappear from the egg.

## Chapter II.

Table 1. Sulfonamide drugs used for livestock raised for meat and poultry production\*

Livestock and Poultry	Drug	Use level	Withd.	Tol. in edible tissue
			period (days)	(ppm)
Cattle & Calves	Sulfaethoxypyridine	25 mg/lb BW/day	16	0.1
	Sulfamethazine	350 mg/hd/day	7	0.1
	+ Chlortetracycline	350 mg/hd/day for 28 days		
Swine	Sulfaethoxypyridine	100 g/ton 4-10 days	10	0
	Sulfamethazine + Tylosin	100 g/ton 100 g/ton	5	0
	Sulfathiozole + Chlortetracycline + Procaine penicillin	100 g/ton 50 g/ton 100 g/ton	7	0
Chickens	Sulfaquinoxaline	.015 to .025 %	10	0.1
	Sulfadimethoxine + Ormetoprim (Rofenaid)	0.0125% 0.0075%	5	0.1
Turkeys	Sulfaquinoxaline	0.0175%	10	0.1
	Sulfadimethoxine + Ormetoprim (Rofenaid)	0.00625% 0.00375%	5	0.1

\*Pneumathy et al. (1975)

## Chapter II.

Table 2. Percent Sulfa violations in livestock and poultry (1978 to 1982)\*

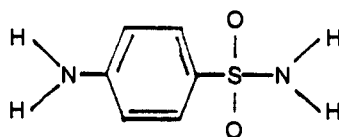
Animal Species	Percent Violation in Year				
	1978	1979	1980	1981	1982
Livestock					
Rabbits	0	2.2	2.7	0	0
Cattle & Calves	3.6	3.9	2.5	3.4	2.8
Swine	9.7	6.5	8.5	6.0	4.2
Poultry					
Chickens	0.9	0	0	0.9	0
Turkeys					
Fryer/ Roaster	9.0	0.7	1.5	0	0
Young	2.6	4.4	4.7	1.9	1.3
Mature	5.9	6.4	0	8.6	3.3
Ducks & Geese	0	4.8	0	0.4	0

\*All data from USDA and FSIS, average level of violative drug residue in all samples from FSQS (Food Safety and Quality Service) is 1 to 2%.

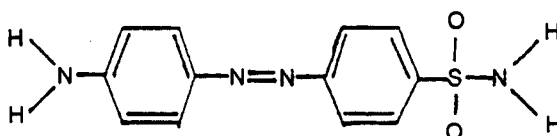


## Chapter II.

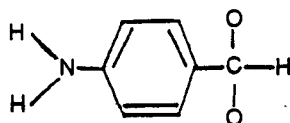
Figure 1. Some relevant structures



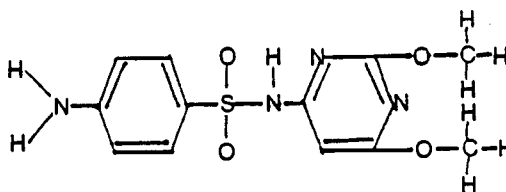
a. Sulfanilamide



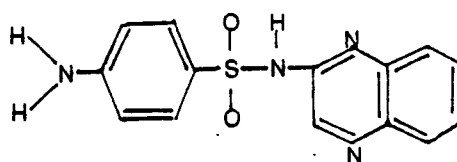
b. Prontosil



c. Para-aminobenzoic Acid



d. Sulfadimethoxine



e. Sulfiquinoxaline

## CHAPTER III

DEPLETION OF TISSUE RESIDUE AND LITTER SCCUMULATION OF  
SULFAQUINOXALINE FED AT 0.015% TO MARKET TURKEYS<sup>1</sup>

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## ABSTRACT

A study was conducted to observe the effect of the accumulation of sulfaquinoxaline (S.Q.) in the litter and to study the depletion scheme of S.Q. from kidney tissues of market turkeys. One hundred twenty, straight run, Medium White turkey poults were placed equally into 4 pens. Two pens each were fed (S.Q.) at 0.015% level starting from 8 to 16 weeks of age and two pens were fed non-medicated feed. All poults were raised on clean litter.

Body weights, feed conversion and mortality were not significantly different among the treatments at 8, 12, and 16 weeks of age.

The birds were sacrificed from 16 weeks of age after 0, 3, 6, and 12 days of drug withdrawal. The levels of S.Q. in the kidneys were 4.75, 1.5, 0.33, and 0.12 ppm for 0, 3, 6, and 12 days of withdrawal, respectively.

Sulfa levels in the litter were less than 0.05 ppm and 80 ppm from the non-medicated and the medicated pens, respectively. The levels in the non-medicated feed were less than 0.05 ppm and 137 ppm for the medicated feed. Ten day withdrawal period did not prove sufficient for the disappearance of S.Q. from the kidney tissue. There is a possibility of carry-over from the litter to the tissue due to the persistence of kidney tissue levels after 12 days of withdrawal.

## INTRODUCTION

Sulfaquinoxaline (S.Q.) was first synthesized by Weijlard et al in 1944. It was first used successfully in the prevention of upper respiratory tract infections (Delaplane, 1945). Later, it was used also in the control of coccidiosis infections in chickens (Delaplane et al., 1947; Grumbles et al., 1948). S.Q. is one of the sulfonamides used exclusively for poultry and preferred over the more common sulfonamides because of its greater accumulation and longer retention time in the blood (Schlenker and Simmons, 1950). It is also preferred for turkeys due to its greater palatability (Brander and Pugh, 1977).

Schlenker and Simmons (1950) monitored the behavior and excretion of S.Q. in poultry and observed greater accumulation of the drug in the blood and its diffusion into the egg. Righter et al. (1970) determined residue levels in tissue and eggs of chickens administered S.Q. at a prophylactic dose (0.025%) in the feed and therapeutic dose (0.05%) in water of laying hens and cockerels. They concluded that residues persisted longest in the renal tissue and withdrawal longer than 7 days was necessary in order to reduce drug residues in kidney and eggs to 0.1 ppm. In a subsequent report, Righter et al. (1973), in studying S.Q. depletion in turkey poults at prophylactic (0.0175%) and therapeutic (0.1%) levels for 7 days, concluded a need for greater than 10 days for total depletion of S.Q. How-

ever, Miller (1982), did not detect any residues above 0.1 ppm in kidney, fat, liver, blood, or muscle in either of the treatments, when S.Q. was fed a prophylactic (0.0175%) or provided therapeutically (0.1%) in the water for growing turkeys. He concluded that a 10 day withdrawal was adequate for total depletion of S.Q. The withdrawal period is now set at 10 days in poultry (Feed Additive Compendium, 1983).

No study has yet been conducted to monitor the levels of S.Q. in poultry litter. Thus, this study was conducted to observe the possibility of drug accumulation and carry-over, if any, into the tissue and to study kidney depletion of S.Q., when market turkeys were fed S.Q. at the 0.015% level from 8 weeks to 16 weeks of age.

## MATERIALS AND METHODS

One hundred twenty straight run Medium White turkey poults were randomly placed equally into four pens. All poults were wing banded at day-old to monitor individual birds during the experimental period. The poults were fed a corn-soy diet as listed in Table 1. Each pen (3.3 m X 4.6 m) contained 30 poults, and each bird was provided with an area of 0.51 meter square. The floor was covered with wood shavings litter (approximately 10 cm deep). One 40 watt light bulb provided light in each pen. Continuous lighting was provided for the first week, gradually decreasing to ten hours per day using natural light when available. Room temperature was set initially at 32.2C using a Merco brooder, then manually reduced as the birds grew older. Natural ventilation was provided by windows on the East-West walls, controlling the air flow accordingly. Feed and water were provided ad libitum throughout the period. Trough feeders (1.5 m) were used for the first four weeks of the test, changing to adjustable hanging tube feeders (40 cm in diameter). One Little Giant automatic waterer was used in each pen. At 8 weeks of age, S.Q. was mixed in the feed at 0.015% and fed to the poults in 2 pens. In order to avoid cross contamination of the litter, different footwear was worn for each treatment. Litter was stirred when there was any sign of caking. Body weight and feed

consumption were recorded for each pen at 8, 12, and 16 weeks of age. Mortality was noted daily. Dead birds were replaced up to one week of age with another poult. Litter and feed samples were collected at the end of the experiment for the analyses of S.Q. levels. Birds were sacrificed starting at 16 weeks of age, after 0, 3, 6, 12 days of S.Q. withdrawal. The levels of S.Q. were determined in the kidney according to the method described by Fellig and Westheimer (1968). Feed and litter samples were analyzed by the thin layer chromatography method proposed by U.S.D.A. (1982) in the determination of sulfa levels.

One way analyses of variance for all parameters measured were carried out by the method outlined by Snedecor and Cochran, (1980).

## RESULTS AND DISCUSSION

Table 2 shows the performance data for the birds throughout the S.Q. feeding period. Body weights were numerically higher, on the average, for the S.Q.-fed birds with higher feed efficiency as the period progressed, but these data were not significantly different between the two treatments. The mortality rate was higher in birds fed S.Q. The main cause for the high mortality was cannibalism because the birds were not beak trimmed and due to abnormally high temperatures during the experimental period.

The depletion curve of S.Q. from kidney tissue is presented in Figure 1. S.Q. levels at day 0 were 4.75 ppm, and the residue level decreased to one third (1.5 ppm) by the third day, 0.33 ppm by the sixth day, and at 0.12 ppm by twelve days post withdrawal, slightly above the tolerance level set by the FDA (i.e., 0.1 ppm). These results are in agreement with Righter et al. (1970, 1973) in that the tissue levels remained higher than the tolerance level after suggested withdrawal procedures were followed. Further, persistence of residues longer than 12 days may suggest possible contamination from the bedding material once the feed was withdrawn 12 hours prior to each slaughtering day.

The levels of S.Q. in the feed and litter are presented in Table 3. The levels of S.Q. in the litter are significantly greater in S.Q.-fed pens than the non-medicated fed pens. The level of S.Q. in the feed while lower



approximates with quantity added to in the feed (150 ppm). This speculation can be further tested by having a group of medicated turkeys maintained on the same used litter while having another medicated group moved on to clean litter during the withdrawal period. Both groups can then be monitored for drug residue at the same sampling period. Under the conditions of this study, 10 days withdrawal period did not suffice to eliminate S.Q. levels below 0.1 ppm in the kidney tissue.

## Chapter III.

Table 1. Composition of turkey diets from day old up to the market age

Ingredients	Starter	Grower	Grower	Grower
	(0-4 wks)	I (4-8 wks)	II (8-12 wks)	III (12-16 wks)
	%	%	%	%
Corn	41.86	42.75	55.0	64.97
Fat, animal	1.24	3.01	-	2.32
Soybean ml, 47.5% CP	48.87	47.43	40.60	28.40
Herring meal, 70% CP	2.0	1.0	-	-
Dehy. alfalfa ml, 17%	2.0	2.0	1.0	1.0
Defluo. phosphate	2.65	2.7	1.97	1.95
Limestone flour	0.53	0.34	0.59	0.51
Salt (iodized)	0.3	0.3	0.3	0.3
Trace min. premix <sup>1</sup>	0.05	0.05	0.1	0.1
Vitamin premix <sup>2</sup>	0.4	0.4	0.4	0.4
d, l methionine	0.1	0.02	-	0.012
S.Q. 40 <sup>3,4</sup>	-	-	0.0375	0.0375

## Calculated analyses:

Crude protein, %	28.73	27.4	24.35	19.40
Met. energy,				
Kcal/kg	2816	2904	2873	3102
Calcium, %	1.28	1.2	1.02	0.95
Avail. phos., %	0.65	0.65	0.49	0.47
Lysine, %	1.70	1.60	1.35	1.0
Methionine, %	0.57	0.45	0.38	0.33
Metho. + cyst., %	1.05	0.90	0.76	0.67

<sup>1</sup> Supplied per kilogram of ration: calcium, 97.5 mg; manganese, 60 mg; iron, 20 mg; copper, 2 mg; iodine, 1.2 mg; zinc, 27.5 mg.

<sup>2</sup> Supplied per kilogram of ration: vit A, 3304 I.U.; vit. D<sub>3</sub>, 1111 I.C.U.; riboflavin, 3.3 mg; d-pantothenic acid, 5.51 mg.; niacin, 22.01 mg.; choline, 191 mg.; vit B<sub>12</sub>, 5.51 mcg; vit E, 1.1 I.U.; vit K, .55 mg.; folacin, .22 mg.

<sup>3</sup> Graciously provided by Merck & Co., Rahway, NJ.

<sup>4</sup> All non-medicated birds were fed Amprolium (Merck & Co.) as coccidiostat at 0.0125% level.

Chapter III.

Table 2. Performance data for Medium White market turkeys fed Sulfaquinoxaline (S.Q.) at 0.015% from 8 weeks to 17 weeks of age<sup>1</sup>

S.Q. in feed	Age (Weeks)								
	8			12			17		
	Mean B.W.	Feed conv.	Mort. cum.	Mean B.W.	Feed conv.	Mort. cum.	Mean B.W.	Feed conv.	Mort. cum.
%	(kg)		%	(kg)		%	(kg)		%
0	1.82	1.96	3.3	3.52	2.33	6.7	5.53	2.87	11.7
0.015	1.91	1.97	1.6	3.69	2.4	8.3	5.67	2.96	15.0

<sup>1</sup>No significant differences in each column were noted at  $P \leq 0.05$ .

## Chapter III.

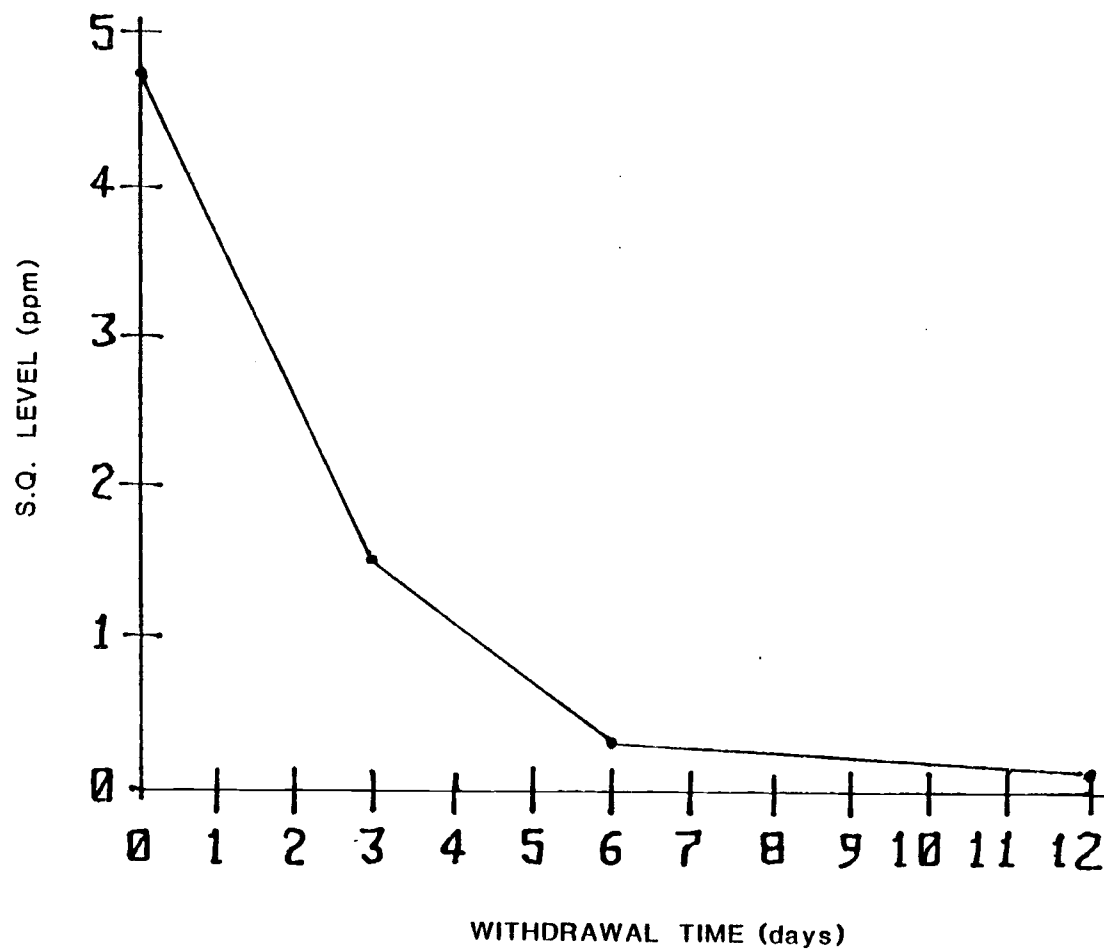
Table 3. Levels of S.Q. in litter and feed at 16 weeks of age

Treatment		Level of S.Q. <sup>1</sup>	
S.Q. in feed	Litter	Litter	Feed
%		ppm	ppm
0	Unused	< 0.05 <sup>a</sup>	< 0.05 <sup>a</sup>
0.015	Unused	80 <sup>b</sup>	137 <sup>b</sup>

<sup>1</sup> Different superscripts in each column indicate significance at  $P \leq 0.05$ .

Chapter III.

Fig. 1: Depletion of S.Q. from kidney tissue with Medium White turkeys fed from 8 to 16 weeks at 0.015% level.



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## CHAPTER IV

DEPLETION FROM TISSUE AND CARRY-OVER EFFECT OF SULFADIMETHOXINE  
FROM REUSED LITTER IN MARKET TURKEYS<sup>1</sup>

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## ABSTRACT

Two experiments were conducted to study the depletion from the tissue and the carry-over effect of sulfadimethoxine (SDM) from the use of built-up litter in market turkeys.

Four hundred-twenty turkey poults were raised on new litter, litter used once, and litter used twice. All but 120 were fed SDM at 0.00625% level from day old to market age (17 weeks of age).

Body weight, feed consumption and mortality were not significantly different among the treatments at 4, 8, and 16 weeks of age for both experiments.

The level of SDM in the litter indicated accumulation of the drug as it was building up levels, at 64, and 35 ppm at 8 weeks, 12 and 0.76 ppm at 17 weeks for the used litter and the new litter in the first experiment, respectively. In the second experiment, SDM levels dropped to 0.32, 0.23 and 0.17 ppm at 8 weeks for the unused, used once litter and used twice litter, respectively. The levels for 17 weeks litter samples were 0.73 and 0.94 ppm for the litter used once and twice. The lower levels were obtained due to dilutions with clean litter and to the binding of the SDM with the shaving. Litter levels in the non-medicated pens were below 0.05 ppm.

Levels of SDM in the kidney, liver and breast tissues at 0 day withdrawal period did not indicate the presence of any direct built-up effect from the litter. The depletion

in the kidney was also independent of the litter. However, the litter had an effect on withdrawal period lasting more than 5 days. A high correlation between the tissues was indicated with the values averaging at 4.26, 1.15, and 0.85 ppm in the kidney, liver and breast, respectively.

Despite the absence of any carry-over effect, sulfa levels in the litter can alter the depletion scheme in the tissue thus delaying the disappearance of the drug past the recommended withdrawal period.

## INTRODUCTION

Sulfadimethoxine (SDM), one of the newer sulfa drugs, was first synthesized by Bretschneider and Kloetzer in 1955. Its antibacterial activity has been described in detail by Schnitzer et al. (1955). It is characterized by low toxicity, long lasting concentrations in the blood, and high activity resulting in an optimal chemotherapeutic ratio (Delorenzo and Schnitzer, 1959). Randall et al. (1959), in studying the toxicologic and metabolic aspects of SDM, reported levels in blood, kidney, liver, muscle and brain tissues in descending order at 24 hours after administration and a four day persistence in the blood when SDM was ingested by mice at the level of 25 mg. Tissue plateau levels were reached at 2-3 days. The report failed to mention any damage to tissues other than the kidneys. Rofenaïd which is an agent containing SDM and ormetoprim in a 5:3 ratio, has been shown to have anticoccidial and antibacterial activities as well as safety and compatibility with chickens (Mitrovic et al., 1969a, 1969b; Marusich et al., 1969). The same properties hold true for turkeys (Mitrovic et al., 1971a, 1971b; Marusich et al., 1971).

In poultry, SDM fed as Rofenaïd has proven to be very effective against coccidiosis and fowl cholera at levels from 0.0075 to 0.025% (prophylactic) in the feed, and 0.00625 to 0.05% (therapeutic) in the water in turkeys. The optimum levels were determined to be 0.0125% in the feed and

0.025% in the water (Mitrovic and Bauernfeind, 1971).

SDM at levels of 0.05% in the drinking water of chicks reduced growth rate significantly after 21 days and caused premature development of combs and wattles (Bajwa and Singh, 1977). In the same study, minimal and maximal blood levels of SDM were seen at 0 and 18 days, respectively. Considerable work has been conducted on the clearance of SDM and Rofenaid from eggs, edible tissue and blood of poultry (Onodera et al., 1970; Fellig et al., 1971; Laurencot et al., 1972; Yamamoto et al., 1979).

Onodera et al. (1970), observed 4, 6, and 10 days disappearance of SDM from plasma, yolk and albumin, respectively, after feeding 0.02% SDM to chickens for 30 days. He found the highest levels in the gall bladder, plasma and kidneys. Fellig et al. (1971) administered Rofenaid to broiler chickens for a period of 8 weeks at the recommended use level of 0.02% in the feed and found all tissues to be free of SDM and ormetoprim 2 days after cessation of the treatment. When administered at 0.01% in the feed of turkeys for 13 weeks, all tissues were cleared 2 days after withdrawal. Laurencot et al. (1972), noted 9 and 14 days clearance for SDM from chicken and turkey eggs, respectively, when they were fed 0.02% to chickens and 0.01% to turkeys. Yamamoto and Kohenawa (1979) obtained the following results: disappearance rates were noted at 3 days for the liver; 7 days for breast and thigh muscles, and plasma; and 10

days for skin with fat along with the kidneys. The highest levels of SDM were noted in the plasma and lowest in the fat at 0 days when given laying hens SDM as sodium salt in drinking water at two times the therapeutic dose for 4 days. The withdrawal period set by the FDA for Rofenaid is 5 days (Feed Additive Compendium, 1983).

One of the reasons associated with persistence of sulfa drugs longer than the prescribed withdrawal period in the tissue is the recycling of such compounds through bedding material. In experiments conducted with swine, Samuelson et al. (1979) reported that untreated pigs, that were placed on bedding in pens formerly occupied by the treated group (sulfamethazine at 550g/1000 lb), developed tissue residues at or above 0.1 ppm. Similar results were also obtained by Whipple et al. (1980). No such data are available on poultry.

The following experiments were undertaken to determine the clearance pattern of SDM from kidney, liver, and muscle tissues as influenced by built-up litter; to observe possible carry-over effect to the tissue from the litter, and to attempt to establish a correlation coefficient between the tissues investigated.

## MATERIALS AND METHODS

Experiment 1. One hundred eighty straight run, Medium White turkey poults were divided equally into six pens. Four pens were fed SDM at the prophylactic level (0.00625%) starting at day-old. Of these pens, two were raised on clean wood shaving litter (approximately 10 cm deep), and two were raised on litter which had been used by one brood of turkeys. Two other pens serving as control, were fed Amprolium as coccidiostat and were raised on used litter.

Experiment 2. Two hundred-forty, sexed, Large White turkey poults were equally distributed into eight pens. Six pens were fed SDM at the prophylactic level (0.00625%) starting at day-old. Poults in two pens were raised on clean litter, two pens on litter used once, and two pens on litter used twice used for two broods of turkeys raised to market age. Two pens which served as control were fed Amprolium as coccidiostat and were raised on litter used twice.

Management conditions and procedures were common to both experiments. All poults were wing banded for individual monitoring and beak trimmed to avoid injuries. All poults were fed standard corn-soy diets as listed in Table 1. SDM was added as Rofenaid and substituted pound per pound with corn. Each pen (3.3 m X 4.6 m) contained 30 poults, giving each poult an area of 0.51 meter square. In the pens where litter was used for one or two broods, new shavings were spread around the brooding area and all caked materials

were removed. One 40 watt light bulb provided light in each pen and an infrared light was hung about 30 cm above the litter. Lighting was provided continuously for the first week, gradually decreasing to eight hours per day using natural light when available. The heater lamps were removed when the poults reached two weeks of age. Natural ventilation was provided by windows located on the East-West walls controlling the flow accordingly. Feed and water were provided ad libitum throughout the experiments. Trough feeders (1.5 m long) were used for two weeks and then replaced by adjustable hanging feeders (40 cm in diameter) which were raised as the birds grew. Little Giant automatic waterers were used, one in each pen. In order to avoid cross contamination between used and clean litter pens, different footwear was worn for each treatment. Litter was also stirred when there was any sign of caking and new litter was added due to the great degree of moisture in some pens.

Body weight, and feed consumption were recorded for each pen at 4, 8, and 16 weeks of the experiment. Mortality was noted daily, and dead birds were replaced up to one week of age. Litter samples were collected at 8, and 16 weeks for the analyses of SDM levels. Samples of the feed were also analyzed for each experiment. Birds were sacrificed at 17 weeks of age after 0, 1, 3, 5, and 7 days of SDM withdrawal. Levels of SDM were determined in kidney, liver, and breast tissue using thin layer chromatography as approved

by USDA (1982).

The data were analyzed by one way analysis of variance and significant treatment means were separated by Least Significant Difference (LSD) test (Snedecor and Cochran, 1980).



## RESULTS AND DISCUSSION

Tables 2 and 3 show the performance data of the birds for Experiments 1 and 2. There were no significant differences among the treatment means at 4, 8, and 16 weeks of age in mean body weight, feed conversion and mortality for both experiments. The cause for mortality of some of the birds were as follows: enteritis in two birds, intestinal coccidiosis in one, ruptured aorta in one and were all independent of the treatments or the feeding regime.

Table 4 shows the tissue and litter residue levels of SDM in the first experiment. Litter levels were significantly higher from pens in which one brood of turkeys had been grown out. The reason for the decline in the levels from 8 to 17 weeks may be due to dilutions made with the new litter due to the high degree of moisture caused by watery droppings in the used litter.

Tissue levels were not significantly different among the sulfa-fed birds but are significant when compared to the non-medicated treatment. There is no indication of the direct carry-over effect from built-up litter to the tissue (Table 4). The level of SDM in the feed was analyzed at about 37 ppm.

Table 5 shows the tissue and litter residue of SDM in Experiment 2. The litter levels indicate accumulation of the drug despite the dilutions which were made. The low levels are also indicative of the high degree of binding of

the sulfa drugs with the wood shavings suspected during the analysis of the litter samples. However, the litter levels do not appear to have a direct effect on the tissue levels prior to withdrawal. The differences among the treatments were non-significant. The levels of SDM in the feed for this experiment were established at 37 ppm, on the average.

The depletion curve of SDM from the kidney tissue from the first experiment is presented in Figure 1. At 0 day withdrawal the levels were 5.0 and 5.2 ppm for the birds grown on used and new litter, respectively. The levels were less than 0.05 ppm in the non-medicated pens. Kidney residues dropped to 0.05 ppm by the fifth day and were elevated to .32 and .78 ppm by 7 days for the used and clean litter, respectively. The main reason for these high increases could be associated with the consumption of the litter material. This could also be associated with the individual birds and not representative of the population.

The depletion of SDM from the kidney tissue in Experiment 2 is presented in Figure 2. The levels at 0 day withdrawal were highest in the pens with litter used once (7.95 ppm) and were 2.85 and 2.36 ppm for the new and twice used litter, respectively. The greatest drops of SDM residues were from 0 to 1 day and withdrawal levels dropped to 1.85, 0.56, and 0.23 ppm in pens with the litter used once, twice, and new litter, respectively. The fifth day, which is the

recommended withdrawal period for SDM levels were at 0.08 in the treatment with twice used litter, and slightly above 0.1 ppm in the other two. When kidney tissues were analyzed for SDM levels from the birds raised on litter used twice at 7 days withdrawal, the level was back up to 0.2 ppm.

Data from 16 samples (32 birds) shows strong evidence of a high degree of correlation among kidney, liver and breast tissue levels of SDM. The following coefficients were obtained: 0.83 between kidney and liver; 0.92 between liver and breast; 0.91 between kidney and breast with the values averaging at 4.26, 1.15, and 0.85 ppm in kidney, liver, and breast, respectively, at 0 day withdrawal.

Under the conditions of these experiments, the five day withdrawal period was not sufficient to lower the SDM levels below the tolerance level of 0.1 ppm in the kidney tissue. This finding is not in agreement with Fellig et al. (1971), who noted two days disappearance of SDM from all tissues when turkeys were fed 0.00625% SDM for 13 weeks. This difference could be attributed mainly to the consumption of litter material, as we have seen in the crop of the slaughtered turkeys. This has also been observed by Samuelson et al. (1979), and Whipple et al. (1980), who found levels of sulfamethazine in the tissue of swine fed non-medicated feed raised on sulfa contaminated litter. In our results, however, levels of sulfa from the built-up litter did not appear to have a direct effect on the tissue levels of SDM and only

delayed the depletion scheme in the kidney tissue. The primary reason is that once a saturation level is reached within the tissue small variations from the sulfa levels in the litter become negligible.

## Chapter IV.

Table 1. Composition of turkey diets from day old up to the market age

Ingredients	Starter	Grower	Grower	Grower
	(0-4 wks)	I (4-8 wks)	II (8-12 wks)	III (12-16 wks)
	%	%	%	%
Corn	41.86	42.75	55.00	64.97
Fat, animal	1.24	3.01	-	2.32
Soybean meal, 47.5% CP	48.87	47.43	40.60	28.40
Herring meal, 70% CP	2.00	1.00	-	-
Dehy. alfalfa meal, 17% CP	2.00	2.00	1.00	1.00
Defluo. phosphate	2.65	2.70	1.97	1.95
Limestone flour	0.53	0.34	0.59	0.51
Salt (iodized)	0.30	0.30	0.30	0.30
Trace min. premix <sup>1</sup>	0.05	0.05	0.10	0.10
Vitamin premix <sup>2</sup>	0.40	0.40	0.40	0.40
d, l methionine	0.10	0.02	-	0.012
Rofenaid 40 <sup>3,4</sup>	0.01	0.01	0.01	0.01
Calculated analyses:				
Crude protein, %	28.73	27.40	24.35	19.40
Met. energy, kcal/kg	2816	2904	2873	3102
Calcium, %	1.28	1.20	1.02	0.95
Avail. phos, %	0.65	0.65	0.49	0.47
Lysine, %	1.70	1.60	1.35	1.00
Methionine, %	0.57	0.45	0.38	0.33
Meth. + Cyst., %	1.05	0.90	0.76	0.67

<sup>1</sup>Supplied per kilogram of ration: calcium, 97.5 mg; manganese, 60 mg; iron, 20 mg; copper, 2 mg; iodine, 1.2 mg; zinc, 27.5 mg.

<sup>2</sup>Supplied per kilogram of ration: vit A, 3304 I.U.; vit D<sub>3</sub>, 1111 I.C.U.; riboflavin, 3.3 mg; d-pantothenic acid, 5.51 mcg; vit.E, 1.1 I.U.; vit.K, .55 mg; folacin, .22 mg.

<sup>3</sup>Gratuitously provided by Hoffmann La Roche Inc., Nutley, NJ.

<sup>4</sup>All non-medicated birds were fed Amprolium (Merck & Co) as coccidiostat at 0.0125% level.

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Table 2. Performance data<sup>1</sup> for Medium White market turkeys fed sulfadimethoxine (SDM) at 0.00625% from day-old to 16 weeks of age and raised on clean and reused litter (Experiment 1)

SDM in feed	Litter type	Age (weeks)								
		4			8			16		
		Mean B.W.	Mean feed conv	Cum. mort	Mean B.W.	Mean feed conv	Cum. mort	Mean B.W.	Mean feed conv	Cum. mort
%		(kg)		(%)	(kg)		(%)	(kg)		(%)
0	Used, 1X*	.80	1.71	7.8	2.51	3.10	9.3	8.25	2.81	9.3
0.00625	Used, 1X*	.69	1.56	6.7	2.49	2.23	6.7	7.76	3.8	11.6
0.00625	Unused	.75	1.58	6.7	2.57	2.72	8.2	7.85	3.24	9.6

<sup>1</sup>All values are average of all males and females in each treatment.

\*Grown on litter used for one brood with Sulfa-Q (Hakimi et al., 1984).

There was no significant differences among the treatments at  $P \leq 0.05$  for each treatment.

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Table 3. Performance data<sup>1</sup> for Large White market turkeys fed sulfadimethoxine (SDM) at 0.00625% day-old to 16 weeks of age and raised on clean and reused litter (Experiment 2)

SDM in feed	Litter type	Age (weeks)								
		4			8			16		
		Mean B.W.	Mean feed conv	Cum. mort	Mean B.W.	Mean feed conv	Cum. mort	Mean B.W.	Mean feed conv	Cum. mort
%		(kg)		(%)	(kg)		(%)	(kg)		(%)
0.00625	New	1.05	1.61	0	3.08	2.24	0	8.15	2.95	1.7
0.00625	Used,1X*	1.01	1.63	0	3.12	2.12	0	8.44	2.82	0
0.00625	Used,2X**	1.00	1.75	0	3.03	2.17	1.7	7.74	2.91	3.3
0	Used,2X**	1.03	1.77	0	2.97	2.23	3.2	8.29	2.89	4.8

<sup>1</sup>All values are average of all males and females in each treatment.

\*Grown on litter used for one brood with Sulfa-Q. (Hakimi *et al.*, 1984).

\*\*Grown on litter used for two broods one with Sulfa-Q and the second with SDM.

No significant differences in each column were noted at  $P \leq 0.05$ .

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Table 4. Litter and tissue levels of SDM from Medium White turkeys fed SDM at 0.00625% from day-old up to 17 weeks (Experiment 1)

Treatment		Litter Levels of SDM		Tissue level of SDM (0 day withdrawal)**		
SDM in feed	Litter type	8 wks	17 wks	Kidney	Liver	Breast
%		ppm		ppm		
0	Used, 1X*	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>
0.00625	Unused	35 <sup>b</sup>	0.76 <sup>a</sup>	5.2 <sup>a</sup>	1.9 <sup>a</sup>	0.73 <sup>a</sup>
0.00625	Used, 1X	64 <sup>b</sup>	12 <sup>b</sup>	5.0 <sup>b</sup>	7.2 <sup>b</sup>	0.65 <sup>a</sup>

Different superscripts indicate significance at  $P < 0.05$  in each column.

\*Grown on litter used once for a brood of turkeys fed S.Q. (Hakimi *et al.*, 1984)

\*\*Tissue levels are on average of 6 samples (2 birds each), half male and half female.



## Chapter IV.

Table 5. Litter and tissue levels of SDM from Large White turkeys fed SDM at 0.00625% from day-old up to 17 weeks (Experiment 2)

Treatment		Litter levels of SDM		Tissue level of SDM (0 day withdrawal)***		
SDM in feed	Litter type	8 wks	17 wks	Kidney	Liver	Breast
%		ppm		ppm		
0	Used, 2X**	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>
0.00625	Unused	0.32 <sup>a</sup>	-	2.85 <sup>a</sup>	1.06 <sup>a</sup>	0.88 <sup>a</sup>
0.00625	Used, 1X*	0.23 <sup>a</sup>	0.73 <sup>a</sup>	7.94 <sup>b</sup>	1.18 <sup>a</sup>	1.11 <sup>a</sup>
0.00625	Used, 2X**	0.17 <sup>a</sup>	0.94 <sup>a</sup>	2.36 <sup>a</sup>	1.20 <sup>a</sup>	0.64 <sup>a</sup>

Different superscripts in each column indicate significance at  $P < 0.05$ .

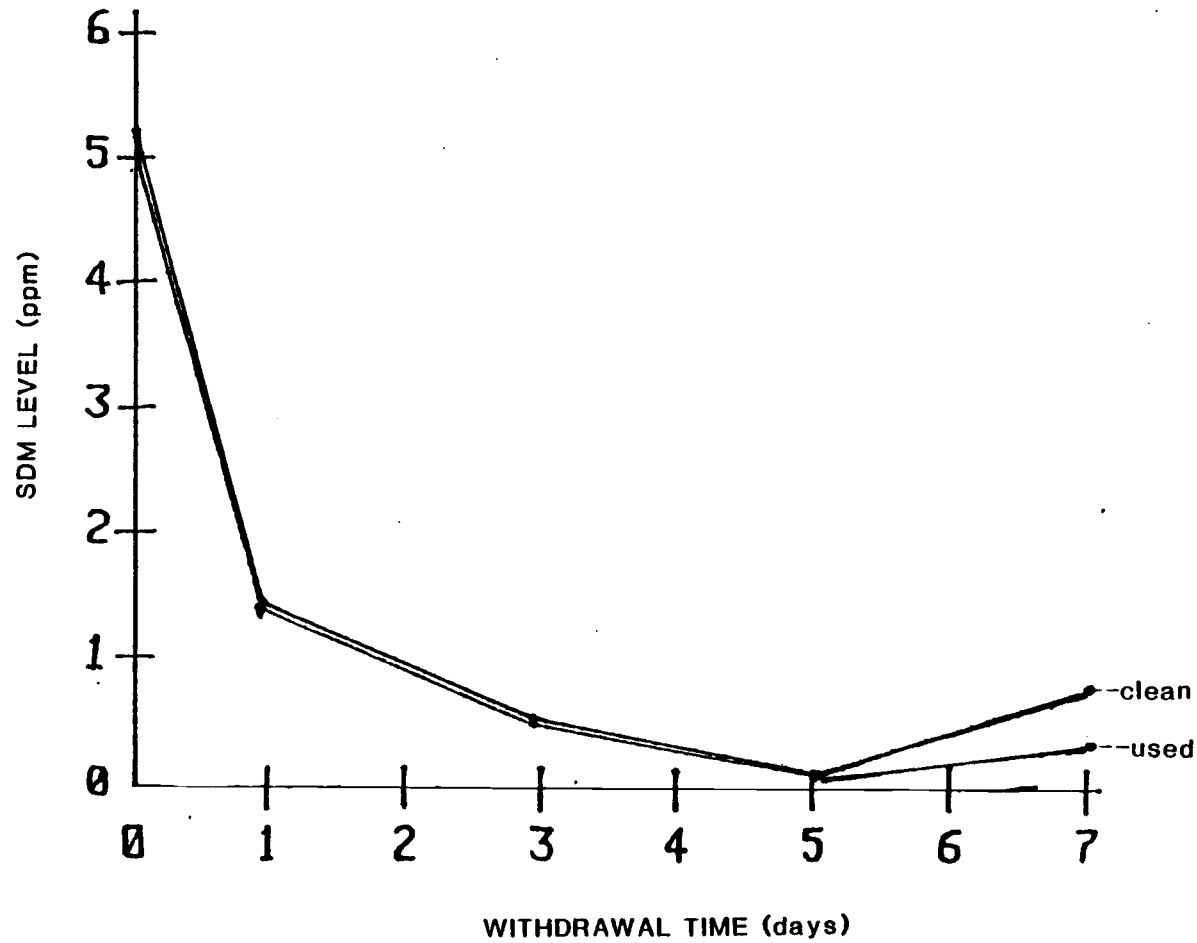
\*Grown on litter used once for growing one brood of turkeys (Hakimi *et al.*, 1984)

\*\*Grown on litter used twice for growing two broods of turkeys.

\*\*\*Tissue levels are an average of 6 samples (2 birds each) half male and half female.

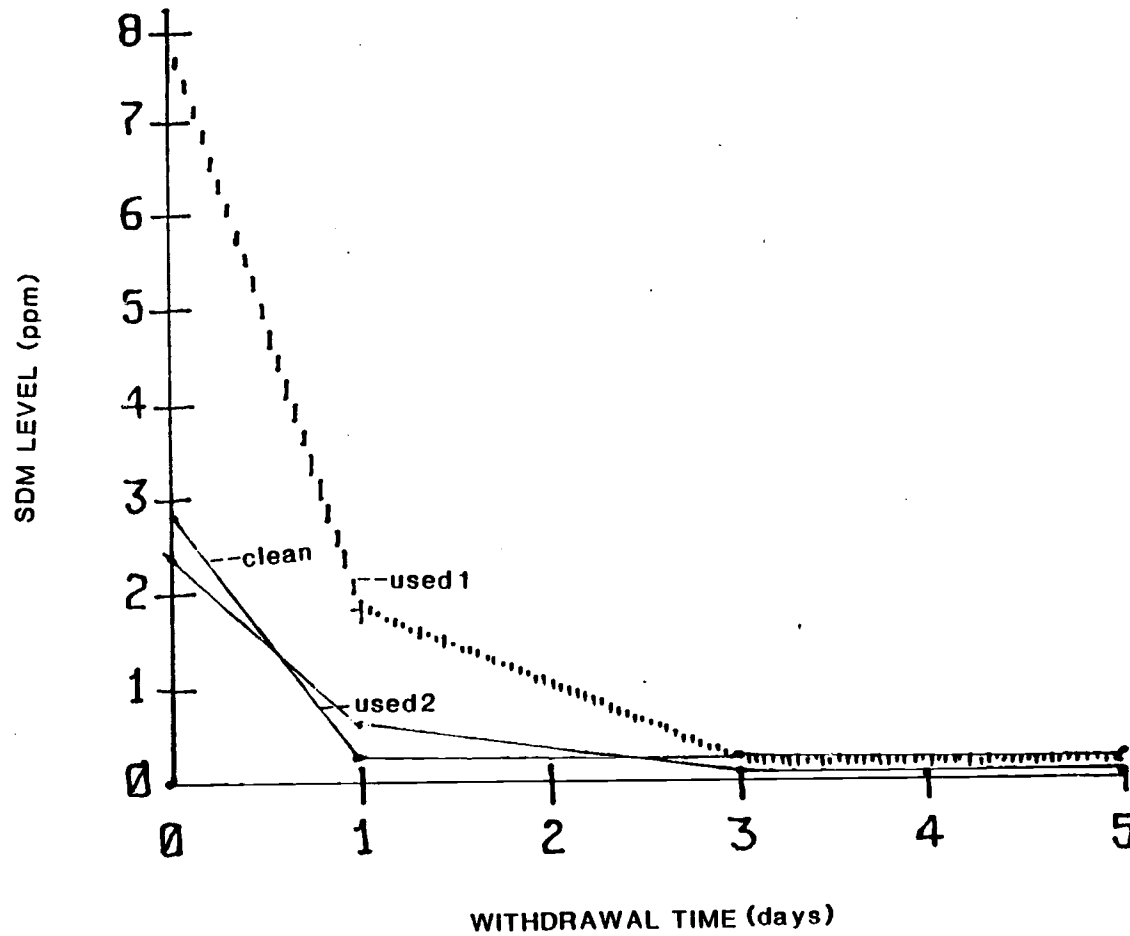
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Figure 1. Depletion of SDM from kidney tissue from Medium White turkeys fed SDM at 0.00625% level from day-old up to market age raised on clean and used litter (Exp. 1).



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Figure 2. Depletion of SDM from kidney tissue from Large White turkeys fed SDM at 0.00625% level from day-old up to market age raised on clean and used litter (Exp. 2).



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## CHAPTER V

REPLETION RATE AND PLATEAU LEVELS OF SULFADIMETHOXINE IN  
THE WHOLE BLOOD OF MARKET TURKEYS<sup>1</sup>

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## ABSTRACT

A study was conducted to determine the repletion rate and the plateau level of sulfadimethoxine (SDM) in the blood of market turkeys. Fifty-two (12 weeks old) female turkeys were fed SDM at either prophylactic (0.00625%) or therapeutic levels (0.03125%) for 24 days.

A semi-qualitative test, the whole blood sulfa test (WBST), was used to determine the sulfa levels in the whole blood.

Blood samples were obtained at 0, 3, 6, 9, 12, and 24 hours for the first day for both drug levels. Blood sampling was continued at 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 days in the prophylactic, and at 2, 3, 12, and 24 days in the therapeutic treatments. Six birds were chosen at random for each drug level for the sampling period.

The level of SDM reached its plateau of about 0.9 ppm after 15 days of feeding with the highest level around 1.2 ppm at 14 days in the prophylactic treatment. In the therapeutic treatment, the level of SDM in the whole blood leveled at 24 hours with approximately 3.5 ppm and the highest levels were attained at 30 ppm at 11 days on the drug.

## INTRODUCTION

Sulfadimethoxine (SDM), one of the newer sulfa drugs, was first synthesized by Bretschneider and Kloetzer in 1955. Its antibacterial activity has been described in detail by Schnitzer et al. (1955). SDM is characterized by low toxicity, long lasting concentrations in the blood, and high activity resulting in an optimal chemotherapeutic ratio (Delorenzo and Schnitzer, 1959). In poultry, Rofenaid, which is a product containing SDM and ormetoprim in a 5:3 ratio, has proven to be an effective coccidiostat and bacteriostat for both chickens and turkey (Mitrovic et al. 1969a, 1969b, 1971a, 1971b). Its safety and compatability has also been reported in both species (Marusich et al., 1969, 1971). Most of the studies conducted with SDM deal with its depletion scheme from the tissue and the blood.

Onodera et al. (1970), noted peak levels of SDM in plasma between 2 to 4 days when feeding 0.02% SDM to chickens for 30 days. Fellig et al. (1971) found SDM levels from 0.9 to 1.3 ppm at 0 day withdrawal when SDM was administered at the prophylactic level (0.00625%) to turkeys for 13 days. Bajwa and Singh (1977) fed 0.05% SDM in drinking water of chickens for 21 days, and observed maximal levels of sulfa in the plasma at 3 days after administration. Atef et al. (1978) noted that the concentration of the sulfa in the plasma reached its highest level during the first six hours before declining rapidly, when sulfamerazine was administered



at 200 and 500 mg/kg of body weight by intramuscular and oral routes. No study has been reported on the repletion rate of sulfa drugs in the blood of growing turkeys. There is a need for the user of any animal drug to know the time it takes for the drug to reach the desired level for more efficacious therapeutic response.

The following experiment was conducted to study the repletion rate of SDM in the blood as well as the time it takes for it to reach plateau levels in the blood of market turkeys when fed at prophylactic (0.00625%) and therapeutic (0.03125%) levels for 24 days.

## MATERIALS AND METHODS

Fifty two female Medium White twelve-week-old-turkeys were placed randomly in 2 pens. One pen was fed SDM at a prophylactic (0.00625%) and the other at a therapeutic (0.03125%) level in a corn-soy holding diet (Table 1). Rofenaid was used as the source for SDM and the weight was adjusted by replacement of corn. Feed and water were provided ad libitum. Continuous lighting was available in order to give full access to the feed at all times. Six birds were chosen randomly from each pen, and blood samples were obtained 0, 3, 6, 12, 24 hours for both levels during the first day and at 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 days for the prophylactic pen, and 2, 3, 12, 24 days for the therapeutic pen thereafter. The levels of SDM were measured in the blood by a semi-quantitative plating method, the whole blood sulfa test (WBST), as developed by Murphy et al. (1984).

For both drug levels, the time intervals were divided in two groups. The first time group indicated a sharp rise in SDM blood levels, up to 10 days for the prophylactic and up to 1 day for the therapeutic level. The second group showed a more leveled line. Least square regression lines were plotted for each group as described by Snedecor and Cochran (1981). The intercept of the two lines for each drug level signified the appropriate time and the level of SDM had reached its plateau.

## RESULTS AND DISCUSSION

The repletion of SDM in whole blood in the prophylactic treatment is shown in Figure 1. The levels prior to 2 days were below 0.9 ppm and were noted as negligible. A rapid rise was observed up to 10 days into the experiment with an average of about 0.9 ppm. From 12 to 24 days, the levels increased slightly and leveled off at .95 ppm. The highest level noted for the whole period was about 1.2 ppm on the average at 14 days. The plateau (1 ppm) of the drug in the whole blood was estimated at 15 days.

The repletion of SDM in whole blood in the therapeutic treatment is shown in Figure 2. Detectable levels (0.85 ppm) were observed starting at 3 hours after the start of the experiment. A sharp rise was noted up to and the end of the first day with the levels of 4.2 ppm. From one day up to 24 days the rise was not as sharp and little variation was seen with levels averaging around 9.7 ppm. Highest levels in this treatment were attained at 11 days (30 ppm). The plateau level (4 ppm) was estimated at 24 hours.

The results from the prophylactic level are in agreement with Fellig et al. (1971), who noted levels of 0.9 to 1.3 ppm after 13 days of feeding SDM at 0.00625%. The results in the therapeutic treatment support the idea of fast absorption of sulfa drugs and is in agreement with the results of Atef et al. (1978) who noted highest plasma levels of sulfamerazine after 6 hours of administration, but not in

agreement with Onodera et al. (1970) who noted peak levels of SDM in the blood after 2 to 4 days of feeding 0.2% SDM to poultry. One important aspect seen in this experiment was the absence of any tissue damage to the birds fed the therapeutic treatment, emphasizing the low toxicity of SDM, since the turkeys were fed for 24 days at the therapeutic levels. SDM is generally fed or administered in the drinking water from 3 to 5 days during a disease condition. The data from this experiment indicate that 3 to 5 days of the therapeutic dose is sufficient for maximum efficacy of the drug.

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Table 1. Composition of holding diet for twelve week old female turkeys

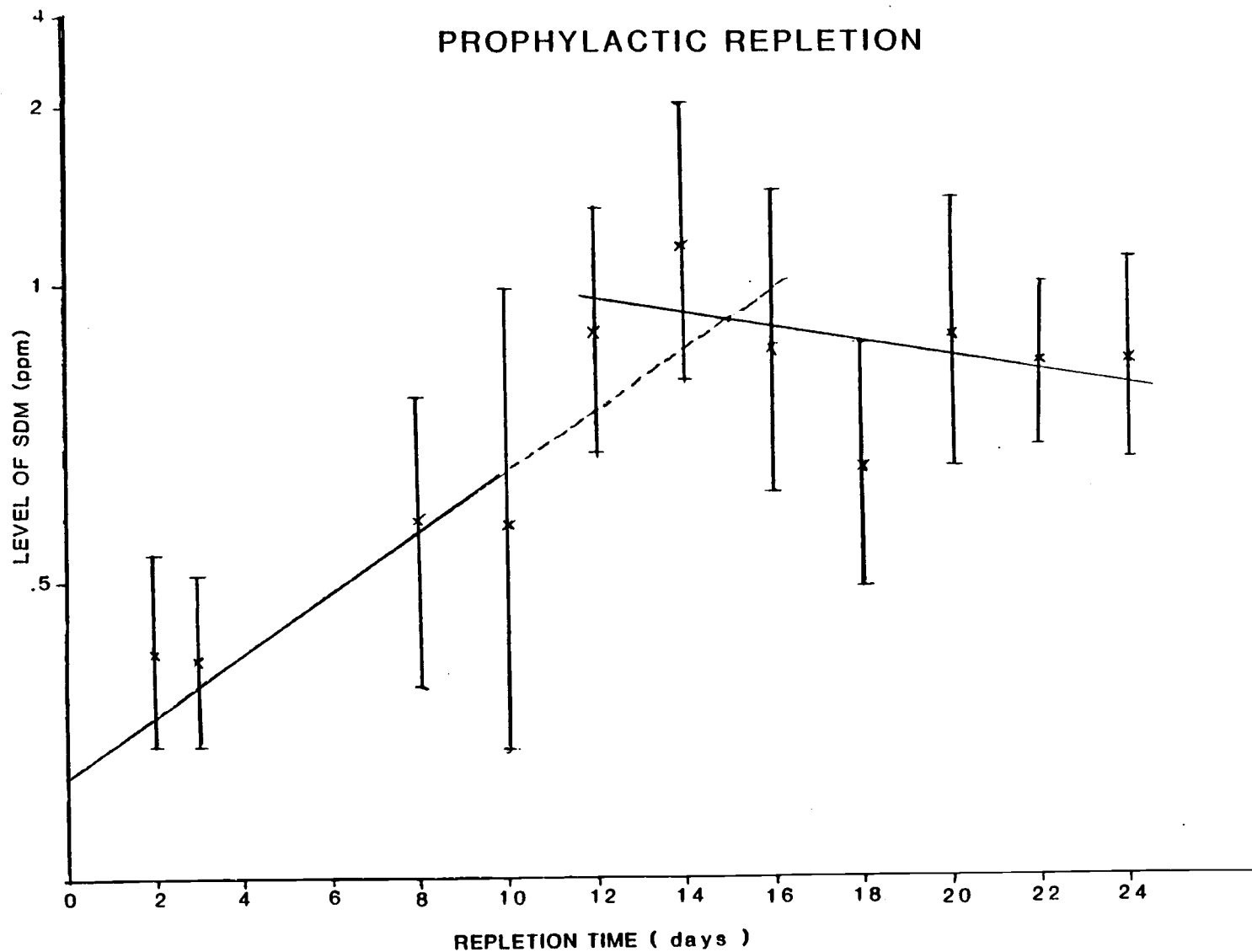
Ingredients <sup>1</sup>	Holding ration (12-15 wks)
	%
Corn	81.30
Soybean ml, 47.5% CP	8.75
Meat meal with bone, 50% CP	5.00
Whey, dried	1.25
Dehy. alfalfa ml, 17% CP	2.50
Limestone flour	0.50
Dical. phosphate	0.25
Salt (iodized)	0.25
Trace min. premix <sup>2</sup>	0.03
Vitamin premix <sup>3</sup>	0.15
d, l, methionine	0.03
Calculated analyses:	
Crude protein, %	14.50
Met. energy, kcal/kg	3150
Calcium, %	0.91
Avail. phos., %	0.45
Lysine, %	0.63
Methionine, %	0.27
Meth. + Cyst., %	0.55

<sup>1</sup>SDM was added as Rofenaid at prophylactic (0.01%) and therapeutic (0.05%) levels by substitution with corn. Rofenaid was provided gratuitously by Hoffmann La Roche Inc., Nutley, NJ.

<sup>2</sup>Supplied per kilogram of ration: calcium, 97.5 mg; manganese, 60 mg; iron, 20 mg; copper, 2 mg; iodine, 1.2 mg; zinc, 27.5 mg.

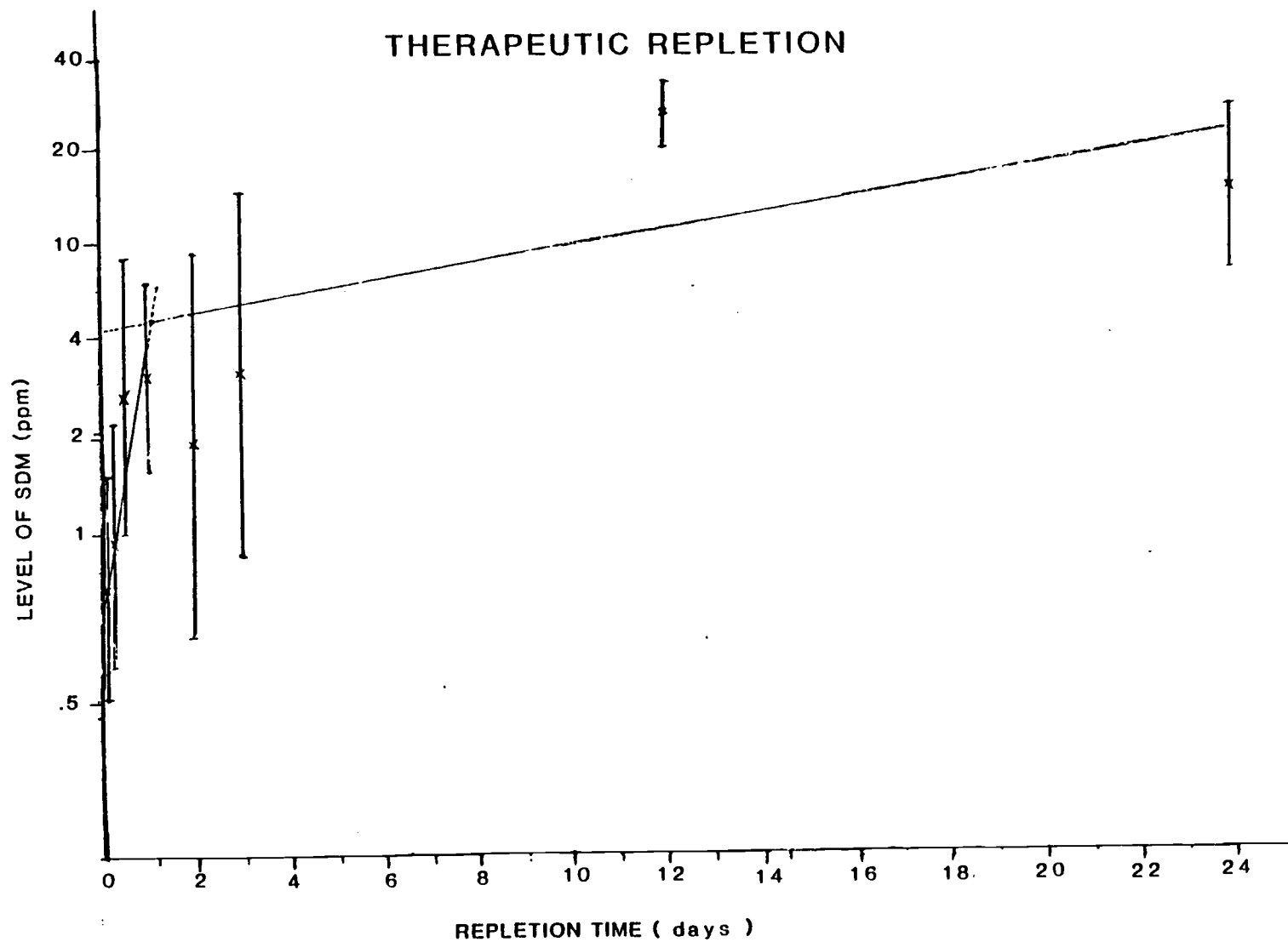
<sup>3</sup>Supplied per kilogram of ration: vit A, 3304 I.U.; vit. D<sub>3</sub>, 1111 I.C.U.; riboflavin, 3.3 mg; d-pantothenic acid, 5.51 mg; niacin, 22.01 mg; choline, 191 mg; vit.B<sub>12</sub>, 5.51 mcg; vit.E, 1.1 I.U.; vit K, .55 mg; folacin, .22 mg.

Fig. 1: Semi log graph of SDM levels in the whole blood of female market turkeys when fed prophylactic level (0.00625%) of SDM for 24 days.



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Fig. 2: Semi log graph of SDM levels in the whole blood of female market turkeys when fed therapeutic level (0.03125%) for 24 days.



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## CHAPTER VI

## CONCLUSION

Sulfa drugs have been used successfully for the treatment of infectious diseases in man, livestock, and poultry for many years. One of the main problems associated with these drugs is their transfer to meat, eggs, and milk of the treated animals. Over the years, consistent violations have been observed in particular with swine and market turkeys and efforts to detect unsafe residues after the animals have been slaughtered have proven expensive. Drug recycling may account for some of the observed residue violations. Due to a lack of data, experiments were undertaken to observe the possibility of drug recycling from used litter material containing sulfa drugs to the edible tissue in market turkeys.

Under the conditions of these studies, there was accumulation of sulfa drug in the litter material. However, the level of sulfa in the litter did not appear to effect tissue levels directly.

When sulfaquinoxaline or sulfadimethoxine was fed on a continuous basis, maximum levels were reached in the tissue within two weeks after administration at the prophylactic, and within 24 hours at the therapeutic level. Once the withdrawal period started the excretion was altered and levels did not drop as rapidly. This hypothesis is coupled with the absorption of the drug from the bedding material

consumed by the animal resulting in persistence of the compound in the tissue, at very low levels, past the recommended withdrawal period. This can result in violations particularly in the industry where large flocks of birds are raised in confinement and where the possibility of litter consumption is greater.

Further research is needed to observe tissue levels of sulfa drugs in the tissue well past recommended withdrawal periods to investigate the possibility of fluctuations in the tissue levels when birds are fed on a continuous basis.

## CHAPTER VII

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