This is to certify that the

thesis entitled

Studies in Drug Interactions: Fenbendazole, Tylosin and Lincomycin in the Growing Pig.

presented by

Ann Ruth Donoghue

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Dept. of Large Animal ~~Clinical~~ Sciences

J. C. Lucas & Rees

Major professor

Date 24 Sept. 1990

STUDIES IN DRUG INTERACTIONS: FENBENDAZOLE, TYLOSIN AND LINCOMYCIN IN THE GROWING PIG

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

1990

ABSTRACT

STUDIES IN DRUG INTERACTION:
FENBENDAZOLE, TYLOSIN AND LINCOMYCIN
IN THE GROWING PIG

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Ann Ruth Donoghue

The effects of drugs when given concurrently are not necessarily predictable based on the actions of the individual drugs.

Tylosin (TY) and lincomycin (LI) are antibiotics commonly used at low doses in the feed of pigs to promote growth and reduce disease. Fenbendazole (FBZ) is a broad spectrum anthelmintic administered in the feed to eliminate internal parasites. The effect of feeding fenbendazole concurrently with tylosin or lincomycin is not known. We conducted three trials to determine if drug interactions occurred between FBZ and TY or LI.

At 200 g/T LI or 100 g/T TY, efficacy of FBZ (9 mg/kg total dose divided over 3, 6 or 12 days) against *Ascaris suum* and *Trichuris suis* in the pig was not diminished. Feeding 1, 3 or 5 times the recommended dose of FBZ (3 mg/kg/day, 3 days) with the same levels of TY (100 g/T) or (LI 200 g/T) did not produce any adverse health affects in growing pigs as measured by complete blood count and serum chemistry

profile. When FBZ was fed at 3 mg/kg/day for 3 days or .75 mg/kg/day for 12 days with TY (100 g/T, 12 days) or LI (100 or 200 g/T, 12 days) residues in the kidney and liver 12, 24, 72 or 144 hours after withdrawal of the drugs was within expected ranges.

We conclude that based on efficacy, health and residue patterns in young pigs, there was no interaction between FBZ and TY or LI.

This thesis is dedicated to five especially important people;
I hope they know how much I love and appreciate them.

TO:

My Parents, Marlene and Wally Donoghue,
who, as always, made it all possible;
My friend, Alice Murphy,
who brightens my day, every day;
My mentor, Tjaart Schillhorn van Veen,
who truly inspires me to be my best,
and who is the very finest role model;
My husband and lover, Steve Radecki,
who is the best friend I could ever want.

ACKNOWLEDGMENTS

In this space I acknowledge and thank the important people who helped me throughout my program.

The Clinical Parasitology Laboratory student workers, they include:
Carrie Kane, Julie Gauthier, Beth Burmeister, John Calhoun, Barb Porter, Kevin Todd, and Linda Hagerman. Thank you for helping with all the dirty work! I hope you learned as much as I did.

Nariaki Nonaka, my office-mate, my close friend. Thanks for sharing your philosophy, brain-power, and strong arms. You'll never be forgotten.

Maggie Hofmann, your friendship and generosity are greatly appreciated; also your slide making capabilities!!

Susan Ewart, Julie Gauthier and Mary Ellen Shea, all I can say is: Thanks, without you, the final form of this thesis would never have been produced.

The Barn Guys: Dale Pollock, Mike Stevens and Dennis Frei; thanks for all the help with my animals. I learned a lot from you all.

Mom and Dad Donoghue, Mom and Dad Radecki: it sure helps to have your love to support me.

Steve Radecki, the statistics wizard, the husband extraordinaire. We made it!!!

Alice Murphy, the dearest friend a person could want, a terrific parasitologist.

Tjaart Schillhorn van Veen, the best of the best. Thanks for pushing so hard, and having confidence in my abilities.

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Chapter 1:

The History of Pharmacology and an Introduction to Pharmacokinetics and the Mechanisms of Drug Interactions

A. History of Pharmacology

The early uses of chemotherapy in human history was based on extracts of plant and animal materials mainly used to alleviate pain and rid the body of illness. Empirical discovery of new treatments occurred over generations of time, as healers experimented with new mixtures of old drugs and new applications of previously unused plant and animal products. Great reference books (*Pharmacopeia* and *Materia medica* (Levine, 1978)) evolved describing hundreds of plants, herbs and animal parts for the cure of disease. In many cases several drugs were given at the same time or in sequence. Mixing of drugs to produce an effect was epitomized in "mithridatium", a mixture of 54 substances used as a universal antidote against poisoning, concocted by Mithridates VI Eupator and expanded by Galen who called it theriac (containing 64 ingredients) (Watson, 1966; Mez-Mangold, 1971). This antidote application of one drug to alleviate the action of another provides the earliest evidence for interactions of drugs. Eventually, as the basic techniques of chemical analysis developed, they were applied to the budding discipline of pharmacology. These techniques expanded considerably in the 19th century.

In 1806, Friedrich Serturner isolated morphine from opium. This discovery led to investigations of many other crude drugs and soon several active ingredients (mainly alkaloids) were isolated from plants, among these were strychnine from *nux vomica* in 1818, caffeine from coffee beans in 1821 and quinine from cinchona bark in 1820. The expansion of the still empirical drug discovery and production led some to an enlargement in scale, from apothecary shops into supply houses for the compounds; George Merck's discovery of papaverine from opium led ultimately to the creation of Merck and Co., Rahway, NJ. (Leake, 1971).

The ability to use purified compounds allowed scientists to study the mechanisms of drug action and also to determine normal physiologic processes. Francois Magendie isolated emetine from ipecac in 1822 and this in turn led to his critical study of the physiology of vomiting and swallowing. Magendie and his pupils, Claude Bernard and James Blake at the College de France, laid the early foundations for the science of pharmacology by describing the specific problems not found in any other branch of science: dose effect relationships, factors involved in absorption, distribution, biotransformation and excretion, localization of the site of action, specific mechanism of action, and the relation between structure and biological activity.

At the same time toxicology was given a firm scientific base, particularly through Mathien Orfila, a Spanish chemist who published a two volume book *Traité des poisons* in 1814-1815. In this work the toxicity of arsenical salts (often used as

poisons) in dogs was quantitatively described. Bernard (1857) also critically described toxic substances in *Leçons sur les effets des substances toxiques et médicamentouses.*

Both pharmacology and toxicology are linked with the profession of pharmacy, a unique branch of pharmacology and medicine, originated by the Arabs, among whom pharmacists were regulated, inspected and held up to quality standards of drug preparation. The formation of pharmacies as separate establishments did not occur in Europe until the thirteenth century (Levine, 1978) In North America this occurred in the mid 1700's. Chemists, pharmacists and physicians developed specialized roles in the study and use of drugs.

B. Introduction to Pharmacokinetics

The early developments of chemistry and pharmacy were an introduction to the development of pharmacology, a discipline dealing with the nature, chemistry, effects and uses of drugs. Pharmacology contains several sub-disciplines including pharmacokinetics, pharmacodynamics, pharmacotherapeutics and toxicology. The study of pharmacokinetics is based on the principles outlined by Magendie, Bernard and Blake, and can be divided into four basic aspects:

1. drug entry and absorption
2. distribution
3. biotransformation
4. excretion.

1. Absorption

Absorption is essentially the transfer of the drug across membranes into the bloodstream. This transfer is accomplished via two mechanisms, passive transport and carrier mediated transport.

The flow of a drug down a concentration gradient (diffusion) is passive transport. This may occur by simple filtration based on water and lipid solubility. Drugs which are water soluble and small enough (4 Å or less; molecular weight of <200) will pass through the aqueous channels of cell membranes.

Drugs which are too large to move through the aqueous channels may pass through by diffusion through the lipid portion of the membrane. The nonionized drug is usually lipid soluble and can diffuse through the membrane. Therefore the lipid solubility is highly influenced by the pH. This in turn is based on the Henderson-Hasselbach equation, which for an acid is $pH=pK_a$ + log Ionized/Non-ionized (Sato et al., 1984). The lipid:water partition coefficient is the second important aspect of this process. A drug that alters the pH of the system will alter the passive diffusion of other drugs dependent on passive transport (Mayer et al., 1980; Sato et al., 1984).

Carrier mediated transport is accomplished via two mechanisms; active transport or facilitated diffusion. Active transport is an energy requiring process that is selective and competitive, i.e., a carrier with a receptor is used to carry the drug, and will move a drug against a concentration gradient by using energy.

Facilitated diffusion however does not require an energy input and therefore cannot go against the concentration, yet it can move a drug across the membrane when the lipid solubility or size precludes the drug's movement alone.

Both active forms of transport use receptors that have some affinity for certain compounds. The competition between drugs for a carrier receptor determines which drug, if more than one is present at one time, will be carried across the membrane (Mayer et al., 1980; Sato et al., 1984).

Absorption is also influenced by other factors, including the drug vehicle, local blood flow at the site, drug composition and area of absorption which is often dependent on the route of administration.

Enteral administration via the oral route provides a large area of absorption, but the absorption may be affected by several processes. These processes include presence of food to which drugs may adsorb, enzymes which may alter the drug structure, transit time of digesta (and drugs), concentration of drug in the bloodstream which is important for establishing a concentration gradient across the gut wall, and presence of other drugs which may chemically complex with the drug of interest. Additionally, the formulation of the drug is important when oral administration is used. Drugs must be able to maintain integrity in the low pH (1-2) of the stomach and the drug must be able to dissolve in the digesta for absorption across the gut wall. Rectal or sublingual administration bypasses some of these actions but the surface area for absorption is reduced.

Administration by any other route is parenteral and includes topical, subcutaneous, intramuscular, inhalation, intraperitoneal and intravenous. Each of these modes of administration may lead to differences in absorption depending on the membranes which have to be crossed to reach the bloodstream, and the surface area of application (Sato et al., 1984).

2. Distribution

Once the drug has entered the bloodstream it passes through several phases of distribution. Initially, the highly perfused organs receive most of the dose, i.e. the heart, liver, kidney and central nervous system. In the second phase, it reaches less perfused tissues such as muscle, skin, viscera and fat. The diffusion rates in each of these tissues is dependent on the lipid solubility of the drug, drug binding to receptors, concentration gradients, intracellular binding and fat partitioning. Lastly, there may be accumulation in particular tissues. For example, for a drug which is highly lipid soluble, when first administered, the concentration gradient between the bloodstream and fat is high, favoring movement of the drug into fat. Over time however, the drug will accumulate in the fat due to it's high lipid solubility, and once in the fat, the concentration gradient must be high before the drug will move out of the fat and back into blood (Sato et al., 1984).

At each of these sites the drug needs to cross several barriers to reach the site of action. These barriers include the capillary walls and cell membranes which divide

total body water into two compartments, intracellular and extracellular fluids. Extracellular is made up of the plasma and interstitial fluids, comprising approximately 17% of body weight. Intracellular fluid makes up 43% of the body weight for a total of 60%. This percentage is influenced by maturity and obesity. The rest of total body weight is made up of carbon, nitrogen, sulfur, sodium, potassium, calcium, phosphorus and other elements. These elements may have an effect on the total distribution of a drug in the body (Sato et al., 1984).

Distribution in these compartments is not only affected by the barriers presented but also by interactions between the drug and various tissues. Plasma proteins, particularly albumin may bind the drug and reduce drug availability at sites of action. This mechanism is saturable and competitive between drugs. Certain tissue sites may become depots; fat is often a depot when the drug is highly lipid soluble (Mayer et al., 1980).

3. Biotransformation

The liver is the primary site for biotransformation with the kidney, gastrointestinal endothelium, plasma and lung playing a less important role for the majority of drugs. The four basic mechanisms for drug metabolism consist of oxidation, hydrolysis, reduction and conjugation.

A majority of drugs are biotransformed via oxidation. The major reactions involved in oxidation are: aliphatic side-chain oxidation, aromatic hydroxylation, N-,

O- and S-dealkylation, oxidative deamination, sulfoxide formation, desulfuration, and N-oxidation. These occur primarily in the microsomal mixed function oxidase (MFO) system located on the smooth endoplasmic reticulum in the liver. The terminal oxidase of the MFO system is a hemoprotein designated cytochrome P-450 because it absorbs light at 450nm in the presence of carbon monoxide (which is also used for analytical determination). The primary electron donor is NADPH, and electron transfer involves a flavoprotein, cytochrome P-450 reductase. The overall substrate specificity of the enzyme system is low, allowing for a wide variety of drugs to be metabolized (Figure 1). An additional cytochrome, P-448 has been identified which has limited substrate specificity, oxidizing primarily polycyclic hydrocarbons.

The MFO system can be actively inhibited by drugs. The best example of this is SKF 525A (beta-diethylaminoethyl-2,2-diphenylpentanoate; proadifen). The mechanism of action is either simple competition or consists of multiple mechanisms involving competitive and non-competitive interference with binding of substrates to cytochrome P-450 and with the reduction of cytochrome P-450 which may bind irreversibly with the enzyme (Mayer et al., 1980).

In contrast to this, the activity of the microsomal enzymes can also be increased. There are several hundred compounds known to induce activity. These compounds are loosely classified into two categories: those that resemble phenobarbital and those similar to the carcinogenic polycyclic hydrocarbons. Phenobarbital induces biotransformation by increasing synthesis of cytochrome P-450, cytochrome P-450

reductase and other enzymes involved in biotransformation. This induction cannot be reproduced *in vitro*, implying that the process is mediated by several factors not yet attainable in an *in vitro* setting (Mayer et al., 1980).

Reduction and hydrolysis are performed by microsomal and nonmicrosomal enzymes. These enzyme-catalyzed biotransformations occur in liver and other tissues such as plasma and the gastrointestinal tract. The process involves mainly the reduction of azo- and nitro- groups to amines. The insertion of water and subsequent inactivation of the compound is the main function of hydrolysis (Sato et al., 1984).

Oxidation, reduction and hydrolysis are called nonsynthetic reactions, whereas the last category of biotransformation to be discussed, conjugation, is termed synthetic. In this mechanism, additive reactions take place. Glucuronic acid, sulfate, acetyl group, amino acids, glutathione, inorganic sulfate, etc. are added to the compound where high energy (conjugable) bonds of -OH, $-NH_2$, -COOH or -SH exist. Many of the enzymes for these reactions are found in the liver, however, some metabolism also occurs in the kidney and other tissues. The enzymes are different from the mixed function oxidases, and may be microsomal or nonmicrosomal. Most of these reactions render the drug inactive and more water soluble. Nonmicrosomal enzymes are generally not capable of being induced or inhibited (Levine, 1978; Mayer et al., 1980; Anadon, 1983; Sato et al., 1984).

Differences in biotransformation ability between different species of animals and between newborn and adult animals are evident from studies utilizing the S9 fraction

of the liver. In these studies, different substrates (often radiolabelled) are added to an *in vitro* culture system and the resulting metabolites are quantified. The results of these types of studies are frequently used to make comparisons and predictions of the pharmacokinetics or toxicity of a drug in one specie and the potential of that specie to serve as a model for others (particularly man) (Chhabra et al., 1974; Caldwell, 1981; Parke, 1983; Smith, 1983; Weber, 1983; Smith et al., 1984). Studies in neonates are used to predict potential toxicities in newborns which may not be able to biotransform (and inactivate) drugs (Short and Davis, 1970; Burrows et al., 1983). Additionally, these types of studies may clarify the role of tissues other than the liver in biotransformation. The rumen and intestinal epithelium have some potential for biotransformation which in some cases may also be inducible (Chhabra et al., 1974; Smith et al., 1984).

4. Excretion

Drug excretion may occur in the urine, bile/feces, sweat, tears, saliva, and expired air. Urine is the primary source for excreted drugs, the kidney serving as a filtering, secreting and reabsorbing device. Any molecule smaller than albumin (MW=69,000, 31Å) may pass through the glomerular pores of the kidney. At the tubular level the drug may be actively or passively secreted or reabsorbed. Two active, carrier mediated, competitive processes for secretion take place in the proximal tubule; one for organic acids, another for organic bases. These processes are somewhat

bidirectional, in that some compounds are reabsorbed. However, most exogenous compounds are predominately secreted. In the proximal and distal tubule, passive reabsorption can occur. This is dependent on a concentration gradient created by passive reabsorption of water during active transport of sodium and other inorganic ions. Nonionic substances are more able to permeate through the membranes than ionic substances, therefore pH plays a primary role in the process. Drugs that change the pH of the urine will alter the reabsorption of other drugs. For example, tylosin is a weak base with a pK_a of 7.1. In urine with a pH of 6.1, 90% of tylosin will be ionized and unable to be reabsorbed (Rasmussen, 1983). Urine acidifying drugs, therefore, may decrease the reabsorption (and increase excretion) of tylosin.

Some drugs are secreted into the bile by one of three carrier mediated systems, one each for organic bases, organic acids and steroid compounds. Again, this process is nonselective and competitive. Many of the drugs are reabsorbed in the gastrointestinal tract. This cycling between the liver and gastrointestinal tract is called enterohepatic circulation (Levine, 1978; Mayer et al., 1980; Sato, 1984).

C. Drug Interactions

The four principles of pharmacokinetics: absorption, distribution, biotransformation and excretion, can be used to predict and understand the potential for drug interactions. Drug interaction or drug interference is a phenomenon which occurs when the effects of one drug are modified by the prior or concurrent administration

of another (or the same) drug(s) (Cadwallader,1983). This phenomenon has been recognized for many years, yet the mechanisms for many interactions is often not known. As mentioned previously, mithridatium and theriac were probably the earliest use of a drug (and drug combination) in a formalized way to counteract the affects of another drug. A drug used for such a purpose is known as an antidote. The use of one drug to counteract the effects of another was entirely empirical until the mechanisms for individual drug action were understood. Ruckebusch (1983) reported on a survey of adverse drug reactions seen in veterinary practices in France. All were clinical reports, without indication of mechanism of action. Ruckebusch concluded that adverse drug reactions occur frequently and are often due to ignorance of the pharmacology of the drugs used, and irrational use of multiple drugs in an emergency.

1. Interactions associated with absorption

For orally administered drugs, interactions may occur prior to absorption in the gastrointestinal tract. Chemical complexation of two or more drugs or adsorption to digesta may occur. For example tetracycline is bound and made inactive by calcium and magnesium ions present in antacid products and lincomycin is known to bind to kaolin. Ion-exchange resins such as cholestyramine and cholestipol hydrochloride intended for complexation with bile acids will complex with other drugs (Cadwallader, 1983). Alterations in the pH of gastric and intestinal contents will alter the state of

ionization, with the result that drugs such as quinine may no longer be absorbed when in an ionized state (Levine, 1978). Changes in the rate of gastric emptying and intestinal motility will alter the amount of time available for drug absorption. Atropine decreases gastric emptying, delaying transit from stomach to intestine. Irritant laxatives increase gastric emptying and may speed transit through the gastrointestinal tract, decreasing the time available for absorption (Cadwallader, 1983).

Absorption in the ruminant animal is complex. A variety of events may take place in the rumen which affect drug dissolution, absorption and distribution. Microbial action, rumen pH, volume turnover and chemical reactions (including adsorption to feed) in the rumen make comparisons between monogastric and ruminant animals impossible. Additionally, these various factors require that each drug with potential oral use be studied with respect to rumenal influences on the drug (Dunlop, 1983; Koritz, 1983).

At the site of absorption, interactions may be complex and indirect. Decreases of intestinal bacterial populations following antibiotic treatment may lead to a loss of bacteria that conjugate drugs. Digoxin metabolism by bacteria may occur in the gut of humans; if the bacterial population is reduced, the patient may have a higher and potentially dangerous level of digitalization. Erythromycin and tetracycline are reported to have this action (Cadwallader, 1983). A more indirect interaction may be the decreased production of Vitamin K, a necessary component for blood

coagulation, by intestinal bacteria. Decreased serum levels of Vitamin K will ultimately increase the effectiveness of anticoagulant therapy. (Melmon and Gilman, 1980). Absorption itself may be altered by changes in the enzyme transport systems. Allopurinol, for instance, may block the system that inhibits iron absorption. This in turn leads to high iron levels in the body (Cadwallader, 1983).

2. Interactions associated with distribution

Plasma protein binding plays an important role in the transport of many drugs in the bloodstream. Acid drugs bind strongly to proteins, displacing less acidic (less strongly binding) drugs. Therefore, an equilibrium, in the bloodstream and at the site of action, can develop based on the strength of the bond. Phenylbutazone strongly binds plasma proteins and will displace others with moderate binding such as warfarin. Displacement of warfarin, an anticoagulant may lead to hemorrhage (Cadwallader, 1983, Melmon and Gilman, 1980). In neonates, sulfonamides and salicylates may displace bilirubin which in it's unconjugated free form will cross the blood brain barrier, damaging the central nervous system and causing death (kernicterus)(Cadwallader, 1983).

3. Interactions associated with biotransformation

More than 200 drugs have been shown to increase their own biotransformation or the biotransformation of other drugs (Mayer et al., 1980; Anadon, 1983).

Examples of these include barbiturates, phenytoin and probenecid. These drugs may induce the enzymes necessary for their biotransformation or the biotransformation of other drugs. Alcohol is known to decrease the half-life of phenytoin, tolbutamide and warfarin (Cadwallader, 1983). Generally, this change in biotransformation is due to increased synthesis or decreased degradation of the enzymes in the mixed function oxidase system (Cadwallader, 1983; Anadon, 1983).

On the other hand, metabolic enzymes may also be inhibited. Allopurinol inhibits xanthine oxidase, necessary for the biotransformation of 6-mercaptopurine and azathioprine. These drugs subsequently have longer half-lives (Mayer et al., 1980). Cimetidine decreases microsomal enzymes in the liver and has been shown to decrease the clearance of diazepam (Cadwallader, 1983).

4. Interaction at the receptor site

Drug action occurs when the drug binds to a specific receptor and evokes a response in the cell. However, many drugs lack specificity for a single receptor and may bind to other receptors eliciting an unexpected response (Gilman et al., 1980, Atchison et al., 1984). For example, phenothiazine and the tri-cyclic antidepressants have different desirable pharmacologic effects, yet both drugs are also anticholinergic agents. When given together the anticholinergic effects may be increased, a response not necessarily desired (Cadwallader, 1983).

5. Interactions associated with excretion

In the kidney, transport into the urine and subsequent excretion can be influenced at the passive and active transport system level. By altering the pH of the urine, ionizable drugs may be excreted more quickly or retained. As mentioned previously, tylosin, a weak base, will not be reabsorbed if the urine is acidic. Controlling urine pH is one method for increasing or decreasing the half life of some drugs (Cadwallader, 1983).

Active transport mechanisms appear to be specific, and some drugs have been shown to compete for these mechanisms. Probenecid has been shown to inhibit the excretion of penicillin. This fact could be used as an advantage to increase the half life of penicillin (Cadwallader, 1983).

6. Other aspects of drug interactions

In the racing horse industry, certain drugs have been used to either mask the presence of or alter the excretion of drugs deemed illegal for use. Thiamine, for instance, has been used in large doses to mask or confuse chemical detection of other drugs (Tobin, 1981). Furosemide, a diuretic has been used to dilute the concentration of drugs in the urine, the most common body fluid tested for the presence of illegal substances in racing horses. Tobin et al. (1977) demonstrated that furosemide can decrease the urinary concentrations of phenylbutazone and the pentazocine glucuronide metabolite by 40-50 fold. In another study, Gabel et al.

(1977) concluded that phenylbutazone will interfere with detection of other medications, depending on the analytical method used. However, the type of medication or methods of analysis were not detailed. Tobin (1981) states, "bute (phenylbutazone) masking depends entirely on the concentration of bute, the drugs that are being tested for, the diligence with which the search for other drugs is pursued and to some extent on the experience and attitudes of the analyst."

Outside of the racing industry, the presence of drugs in the urine or serum may be important in interpreting clinical laboratory tests (Forman and Young, 1976). For example, tests results for glucose and protein levels in the urine may be altered by the presence of penicillin or gentamicin (Yosselson and Sinai, 1986; Rotblatt and Koda-Kimble, 1987).

7. Summary

The pharmacokinetics and pharmacodynamics of drugs in the body are complex mechanisms of which understanding is necessary to predict the length of action, the mode of action and termination of action of drugs. When more than one drug is given concurrently, interactions may occur at any of the systems used for absorption, distribution, biotransformation or excretion. However, these interactions are frequently known only from the clinical response observed. The specific mechanisms for the observed response may not be known (Hansten, 1985). In determining the presence of drug interactions, observation of the expected response following

administration, subjective and objective measurements of animal health, and comparison of plasma, serum or tissue levels of the drugs to values obtained when the drugs are given separately may be used. *In vivo* animal systems are needed to determine changes in absorption, distribution and excretion. Some changes in biotransformation may be discovered via *in vitro* cultures of tissues, but in these systems, enzyme inducing or inhibiting drugs are not detected.

Drug interactions may allow for further understanding of the physiological processes inherent in the system by revealing receptors, enzymes, carriers or other molecules not previously known.

Chapter 2:

The Use of Drugs in the Swine Industry. Tylosin, Lincomycin and Fenbendazole: the Potential for Drug Interactions when Administered Concurrently.

A. Swine Industry and Feeding Practices

Pork production has, over the last few decades, intensified considerably with respect to land and animal management. This intensification has been possible in part by the use of growth promoting and disease preventing drugs. In the United States pigs are raised in confinement, on open lots and on pasture. In all three of these situations, a variety of drugs are used for treatment and prevention of bacterial and parasitic diseases, and for growth promotion. Several treatment modalities are possible: i.e. these compounds may be administered in the feed or water, or by intramuscular, subcutaneous or intravenous injection. Frequently, more than one drug is used at the same time. So far however little attention has been given to the effects, either synergistic or inhibitory of these combinations. In the swine industry most of the disease preventing or growth promoting drugs are administered as "in-feed" preparations. This method of treatment has advantages in that direct animal handling is not needed, saving time for the producer and reducing stress for the animals. However, exact dosage of the compound to individual animals cannot be guaranteed; sick animals may have reduced appetites, and competition at the feeder may reduce the intake of some animals.

Examples of growth promoting antibacterial compounds include virginiamycin, carbadox, copper sulfate, lincomycin and tylosin. The mechanism of action for improved average daily weight gain and feed efficiency is not fully known. It is suspected that these drugs alter the metabolic activity of intestinal bacteria in a manner that benefits the pig by decreasing the production of detrimental compounds, ie. ammonia (Visek, 1972, 1978). Some of these same compounds are also used at higher dose rates for disease prevention and treatment.

Anthelmintics are given to reduce the effect of two major parasitic diseases of pigs i.e., ascariasis and trichuriasis. Animals raised in open lots and pastures frequently suffer from these parasites due to ready access to dirt contaminated with parasite ova. But, even in complete confinement systems, the parasites which do not require intermediate hosts and have hardy eggs may be present at significant levels. Estimated losses in the swine industry due to internal parasites was $240,000,000 in 1981 (Stewart and Hale, 1988). The in-feed administration of anthelmintics has the same advantages and disadvantages as in-feed antibacterial compounds.

Concomitant administration of drugs is primarily for convenience of the pork producer. Swine are routinely maintained on low level antibiotics in the feed. Anthelmintics, however, have to be given at a relatively high level for a limited time; long low dose administration is not effective and may even induce anthelmintic resistance. As feed is frequently mixed in large batches intended to last one to two weeks, the administration of anthelmintics under current practices requires that

special diets be made for a short period of time, removing the pigs from the maintenance diet during anthelmintic treatment. Administration of anthelmintic superimposed on the routine antibiotic treatment would facilitate animal treatment and feed handling.

The objective of this study was to determine the effect of concomitant feed administration of fenbendazole (FBZ), an anthelmintic, with one of two antibacterial compounds, tylosin (TY) or lincomycin (LI), to pigs. Our hypothesis was that LI or TY would not interfere with the efficacy of FBZ against *Ascaris suum* or *Trichuris suis*. Additionally, we hypothesized that concurrent administration would not decrease the therapeutic index, or alter the tissue residue depletion patterns of fenbendazole, tylosin or lincomycin.

B. Introduction to Fenbendazole

1. Description

The benzimidazole (BZD) anthelmintics were first recognized with the discovery of thiabendazole in 1961 (Horton, 1990). A benzimidazole consists of a bicyclic ring in which benzene has been fused to the 4- and 5-position of the heterocycle. Modifications of the benzimidazole ring system at the 2- and/or 5-position have yielded the most active drugs. A carbamate addition at the 2-position yields the group of drugs most frequently used, the benzimidazole carbamates (Townsend and Wise, 1990) These structurally related compounds: flubendazole, parbendozole,

oxibendazole, albendazole, mebendazole, oxfendazole and fenbendazole (FBZ), showed greater anthelmintic activity than thiabendazole, which lacks the carbamate addition (Loewe, 1977). Fenbendazole is a methyl-5-phenylthio benzimadazol-2yl-carbamate (Figure 2). This compound is light brownish gray, odorless, tasteless, with a molecular weight of 299.35 and a melting point of 233° C. It is freely soluble only in dimethlysulphoxide and relatively insoluble in water. (Windolz et al, 1983).

2. Mode of action

The benzimidazoles as a group are thought to have a selective ability to inhibit microtubule formation. The selectivity for helminth tubulin, the microtubule subunit building block, over mammalian tubulin is not fully understood. Most of the basic work in this area has been performed with mebendazole (Lacey, 1990). Specific work with fenbendazole has been limited, but a similar mode of action is hypothesized.

In the nematode *Ascaris suum*, fenbendazole is thought to interfere with glucose absorption, with the metabolism of glucose to glycogen, and to decrease breakdown of endogenous glycogen via tubulin inhibition. Conflicting reports concerning the interference with fumarate reductase have been published. Prichard et al. (1978b) concluded that fumarate reductase in mitochondria of susceptible *Haemonchus contortus* was inhibited 31% by fenbendazole. However, Düwel (1977b) stated that inhibition of fumarate reductase does not occur as is found with thiabendazole. These conflicting reports on observed responses were made prior to the discovery of

the basic mechanism of microtubule inhibition. Fenbendazole is incorporated into the excretory ducts, dorsal and ventral nervous system of *A. suum*, and adults may take in FBZ orally. Overall, the activity is based on alterations of microtubule formation which in turn may affect energy metabolism in *A. suum* (Lacey, 1990). Additionally, there may be some neurotoxic affect on the cestode *Hymenolepis diminuta* (Booze and Oehme, 1982).

3. General uses in the swine industry

Fenbendazole (FBZ) has a broad spectrum of activity. Effective removal of >90% of adult worms with FBZ dosages of 3-15 mg/kg body weight has been shown against *Ascaris suum*, *Oesophagostomum* sp., *Hyostrongylus rubidus* and *Stephanurus dentatus* (Baeder et al., 1974; Enigk et al., 1974ab; Kirsch, 1974; Tiefenbach, 1974, 1977; Batte, 1977, 1978; Enigk et al., 1977; Marti et al., 1978; Romaniuk et al., 1978; Stewart et al., 1981;). Efficacy against adult *Trichuris suis* has ranged between 20-99% (Enigk et al., 1974; Marti et al., 1978; Romaniuk et al., 1978; Stewart et al., 1981; Corwin et al., 1984). Additionally, efficacy has been shown against migrating stages of *A. suum* (Stewart et al., 1984, 1986); a lower efficacy (44-96%) was established against *H. rubidus* and *Oesophagostomum* sp. (Kirsch, 1974; Kirsch and Düwel, 1975;). Extended treatment regimens, whereby the total dose of fenbendazole is the same, but using divided doses over several days, has been shown in pigs, sheep and cattle to be a useful method to increase the effectiveness of FBZ and facilitate

feed management (Enigk et al., 1977; Enigk, 1978; Gaenssler et al., 1978; Prichard et al., 1978a; Tiefenbach and Kirsch, 1978; Kirsch, 1980; Corwin et al., 1984).

Resistance to benzimidazole drugs has developed and studies using resistant populations of *Haemonchus contortus* showed the benzimidazole had reduced binding affinity for parasite tubulin, implying a structural change in the β-tubulin molecule of the parasite (Roos, 1990).

4. Pharmacokinetics

a. Absorption

Fenbendazole is generally administered orally, however it does not appear to be well absorbed from the rumen, with slightly better absorption in the abomasum of ruminants and stomach of monogastric animals (Christ et al., 1974; Booze and Oehme, 1982; Ngomuo et al., 1984). Studies on absorption have been primarily based on oral administration followed by measurement of plasma concentrations. These types of studies do not indicate the mechanism of, or factors affecting, absorption and may not allow for the anatomical and physiological differences between species. Prichard et al. (1981) found that 30% of an oral dose given to steers left the rumen, implying that 70% of the dose was absorbed or biotransformed, while 27% of the (oral) dose left the abomasum, this contradicts the previous research (Booze and Oehme, 1982) concluding that absorption was poor in the rumen.

Bogan and Marriner (1982) found that the bioavailability (area under the curve) of FBZ in the abomasum was 111-249% greater in sheep clinically affected by *Ostertagia circumcinta* infection than non-parasitized animals. They hypothesized that changes in the abomasal pH due to parasite damage to the endothelium may change the concentrations of the sulphoxide metabolite of FBZ in the abomasum. Prichard et al. (1978b) discovered that following intra-abomasal administration of fenbendazole, plasma levels were 2-4 times higher in animals infected with susceptible strains of *Haemonchus contortus* than animals infected with resistant strains. Apparently the gastrointestinal environment plays an important role in the absorption of drugs with respect to pH and subsequent ionization of the compound. Infection with *Ostertagia ostertagia* in cattle and *Trichuris* sp. in dogs and pigs are known to influence gastrointestinal tract pH, water balance and epithelial integrity (Soulsby, 1982). Changes in pH could alter the chemical nature of the drug, thereby promoting or inhibiting solubility and, subsequently, diffusion. Damage to the epithelium may decrease carrier mediated absorption or increase passive absorption. Therefore, infection with parasites may alter the absorption of the drug.

The rumen may serve as a reservoir for fenbendazole, effectively extending the period the anthelmintic is present for action against parasites. This "physiologic" prolonged administration contributes to the efficacy of the drug (Prichard et al., 1978a). The potential exists for drug metabolism by rumen microbes, however research in this area is lacking. Reduction of oxfendazole to fenbendazole has been

shown to occur in the rumen (Marriner and Bogan, 1981a). Prolonged administration has been shown to be an effective method for increasing the spectrum and effectiveness of anthelmintics, benzimidazoles in particular (Prichard et al., 1978a). In monogastric animals, physiological prolonged administration cannot occur, therefore external administration over time is needed.

b. Distribution

The distribution of fenbendazole is not well known. Tissue residue studies indicate that fenbendazole is found in the liver for as long as 14 days post-treatment, in sheep, cattle and swine. Residues are also present in the muscle, fat and kidney up to 7 days in the same species. Other tissues were not tested (Düwel, 1977a). Prichard et al. (1981) concluded that extensive recycling of FBZ occurs in the gastrointestinal tract of sheep. Using gastrointestinal cannulas, 27% of the FBZ dose was measured in the digesta leaving the abomasum, while 52% was present at the terminal ileum. They concluded that the biliary route was the most likely source for recycling and may contribute to the potency of FBZ. Peak plasma levels following oral administration occurred at 11 hours in goats (Short et al, 1987a), 24 hours in sheep (Marriner and Bogan, 1981b), 24 hours in cattle (Ngomuo et al., 1984), 24-30 hours in rabbits (Christ et al., 1974; Düwel, 1977a), and 6-12 hours in pigs (Düwel, 1977a). Half-lives in plasma or serum ranged from 6 to 15 hours in the rat and rabbit respectively (Christ et al., 1974; Düwel et al., 1975), 10 hours in the pig (Düwel et al., 1975; Düwel, 1977a) to 26 hours in sheep (Christ et al., 1974). Ruminants had

the longest retention times, up to 120 hours in the serum, as compared to pigs with a retention time of 48 hours (Düwel, 1977a). This difference in ruminants is probably due to the physiologic reservoir of the rumen. Christ et al. (1974) found that the half-life of FBZ in rats and dogs remained the same after intravenous administration as compared to oral application, however in sheep the half life was 50% lower after intravenous administration than after oral dosing.

c. Biotransformation

Biotransformation of FBZ occurs primarily in the liver and most oxidation is performed by the hepatic mixed function oxidase enzymes (Marriner and Bogan, 1981a,b). Fenbendazole is rapidly oxidized to the sulphoxide form (FBZ-SO), known as oxfendazole, also an effective anthelmintic. This biotransformation is unique in that it is reversible (Bogan and Marriner, 1982; Gottschall et al., 1990). There are few xenobiotic conversions that are known to be reversible; prednisone-prednisolone conversion occurs presumably because of it's similarity to endogenous cortisone-cortisol metabolism (Bogan and Marriner, 1982). A further oxidation step takes the FBZ-sulphoxide (oxfendazole) to a sulphone. Two other metabolites have been identified in animals; FBZ amine, a product of demethoxycarbonylation, and hydroxyfenbendazole (*p*-hydroxylated), produced following oxidation of the 4-position of the phenylthio ring. An *in vitro* study of oxidative metabolism using the S9 fraction of the liver from cattle, goats, sheep, chicken, turkeys, ducks, rats, rabbits and catfish, conducted by Short et al. (1988b) indicated that all species produced FBZ-SO and

FBZ-SO_2, although at somewhat different rates. Additionally, the S9 liver fractions from all species but goats produced FBZ-OH, however in much smaller quantities than FBZ-SO and -SO_2. The amine metabolite, FBZ-NH_2, was not produced in the liver of any species. The FBZ-NH_2 and FBZ-OH metabolites seen in urine probably do not originate from liver metabolism, but from kidney and gastrointestinal metabolism. Short et al., (1987a) concluded that in the goat, the FBZ amine (NH_2) may be formed by a plasma cholinesterase. Fenbendazole and it's metabolites are shown in Figure 3.

d. Excretion

Düwel (1977) reported that 44% of the total dose given to pigs was excreted in the feces as unchanged fenbendazole. Cattle and sheep excreted 48-50% in the feces. In urine the primary metabolite found is the *p*-hydroxy form (FBZ-OH) (Christ et al., 1984), pigs excreting 13% of the total dose via this route and metabolite. Additionally, in pigs, 7% of the total dose was found in the urine as FBZ-NH_2 (Düwel, 1977). In the urine of cattle, goats and sheep, the FBZ-OH metabolite is frequently found in a conjugated form, primarily with glucuronide or sulfate (Short et al, 1987a,b).

Excretion is almost complete 3-7 days following oral doses in cattle and sheep (Baeder et al., 1974; Christ et al., 1974), however residues can still be detected in the liver of pigs up to 21 days after dosing (Düwel, 1977a).

e. Toxicity

Toxicity studies have been performed in a variety of species. However, a dose which elicits adverse affects has not been determined, due to an inability to physiologically administer a high enough dose. The oral LD_{50} in rats is >10,000 mg/kg, in dogs >500 mg/kg, in pigs >5000 mg/kg, in cattle and sheep >2000 mg/kg (Baeder et al., 1974; Düwel, 1977a). Additionally, teratogenicity has not been identified in any species, a striking difference from other benzimidazoles (Baeder et al., 1974; Wilkins, 1974; Düwel, 1977a).

C. Introduction to Tylosin

1. Description

Tylosin (TY) is a member of the group of antibiotics called macrolides. The first in this group, pikromycin, was isolated in 1950 from a *Streptomyces* sp.. A common feature among all macrolides is the macrocyclic lactone structure; a large lactone ring with 13 to 16 carbons, one amino sugar and sometimes other sugar side chains (Burrows, 1980). Tylosin was isolated from *Streptomyces fradiae*. It is a 16 carbon macrolide (Figure 4), related to, in structure and isolation, relomycin and macrocin (Omura and Tanaka, 1984). It is soluble in lower alcohols, esters, ketones and water (Windolz, 1983), and has a pK_a of 7.1 (Burrows, 1980). The hydrochloride and tartrate salts of tylosin are water soluble (Windholz et al, 1983).

2. Mode of action

The mode of action among all macrolide antibiotics is assumed to be similar. Erythromycin is generally used as the representative compound for the group (Burrows, 1980) and the largest body of work concerning macrolide mode of action has taken place with erythromycin using *Bacillus subtilis* 168 as the test organism.

Macrolides inhibit ribosomal dependent protein synthesis by binding tightly and in a stoichiometric fashion (1:1) to the 50*S* ribosomal subunit. Some aspect of the sugar moiety and part of the lactone structure are similar to the peptidyl-tRNA, allowing for competitive binding (Corcoran, 1984). The overall effect is inhibition of translocation of the developing peptide chain from the acceptor site to the donor site required for elongation of the chain as the ribosome moves along the messenger RNA (Burrows, 1980). Small peptides may be formed, but not large highly polymerized chains (Monro et al., 1971).

This action is then selective for prokaryote 50*S* ribosomal subunits; mammalian ribosomes contain 80*S* subunits. However, mammalian mitochondrial ribosomes (which are made up of 50*S* subunits) are apparently not affected due to the inability of the macrolide to penetrate the mitochondrial membrane. Binding is dependent on NH_4^+ or K^+, and is competitive between macrolides (Corcoran, 1984). Activity is primarily against the Gram + bacteria and some *Mycoplasma* sp. Bacterial resistance against the macrolides has developed (Omura, 1984).

3. General uses in the swine industry

Tylosin is used at subtherapeutic levels in the feed as a growth promoter in swine. Increased feed efficiency and average daily gain may be seen in growing pigs fed levels of 22-100 g tylosin/ton (T) of feed (Wahlstrom and Libal, 1975; Cromwell et al., 1976; Tarrago et al., 1978; Hagsten et al., 1980). At same or higher levels, tylosin is used to prevent, decrease the severity of, or treat swine dysentery caused by *Treponema hyodysenteriae*, and arthritis and pneumonia, caused by *Mycoplasma hyosynoviae* and *M. hyopneumoniae* respectively. Tylosin has been used successfully to treat mycoplasmal pneumonia and arthritis via intramuscular injection and as an in-feed preparation (Ross, 1970; Ross and Duncan, 1970; Sampson et al., 1974; Zimmermann and Ross, 1975; Williams and Shively, 1978; Yonkers et al., 1979; Kunesh, 1981; Lukert and Mulkey, 1982; Ross, 1986).

4. Pharmacokinetics

a. Absorption

Tylosin is a weak base (pK_a 7.1), that has adequate water solubility and good lipid solubility. At pH less than 4, desmycosin, a metabolite with antibiotic properties, is formed (Wilson, 1984). Tylosin is usually administered orally or intramuscularly, but rarely intravenously. Absorption has been described as adequate in dogs, cats and pigs, tissue concentrations of tylosin are present at 10-60 minutes following administration (Wilson, 1984). Sauter et al. (1962) reported pigs given

tylosin tartrate or base intramuscularly, had peak serum levels of .9 - 1.1 units of tylosin/ml less than 2 hours after injection (Sauter et al., 1962). Moderate plasma protein binding occurs in sheep (38-45%) and cattle (34-44%) (Wilson, 1984). The half-life of tylosin in the dog is 55 minutes, in cattle 97 minutes (Wilson, 1984) and in quail, pigeons and cranes 90 minutes (Locke et al., 1982).

b. Distribution

The concentration of tylosin in the liver, kidney, spleen and reproductive tract are approximately 10 times higher than those of serum (Burrows, 1980; Wilson, 1984; Locke, 1982).

The liver and bile generally have higher levels than kidney or urine (Wilson, 1984). Because of the distribution, tylosin pharmacokinetics are best described by a two-compartment open model (Wilson, 1984).

c. Biotransformation

Metabolism occurs primarily in the liver, 5-6 metabolites being produced, however, the mechanism has not been described. Tylosin factor D is the major metabolite with smaller amounts of tylosin factor A and dihydrodescycosin. The latter two have some antimicrobial activity (Wilson, 1984).

d. Excretion

Approximately 99% of the metabolites of tylosin are found in the feces, most likely due to bile excretion and there is some concern that these levels may have an effect on soil bacteria (Wilson, 1984). Some excretion occurs via the prostate,

pancreas, small intestine, kidney and milk (4%) (Burrows, 1980). Pigs fed 100 g/T had no residues in the liver, fat, muscle, skin or kidney at 0, 24 or 48 hours; at 1000 g/T residues were detected in the liver at 24 hours (Kline and Waitt, 1971). Following intramuscular injection of tylosin base, blood, liver and kidney contained no detectable residues at 24-48 hours (Moats et al., 1985; Kietzmann, 1985).

e. Toxicity

Tylosin has a low order of toxicity; primary signs of toxicity include gastrointestinal dysfunction and allergy. It may have a direct irritating effect on the gastrointestinal mucosa (Burrows, 1980). The LD_{50} in rats and mice after intravenous administration is 580-695 mg/kg; dogs tolerated 100 mg/kg/day for two years without detectable side effects (Wilson, 1984).

D. Introduction to Lincomycin

1. Description

Lincomycin was first described in 1962, after discovery in fermentation broths of *Streptomyces lincolnensis* var. *lincolnensis* (Mason et al., 1962), isolated from soil samples from Lincoln, Nebraska (Herrell, 1969; Hornish et al., 1987;). The structure, illustrated in Figure 5, is unique from other classes of antibiotics. It is a free base with a pK_a of 7.6 and is soluble in water and most organic solvents other than the hydrocarbons (Herr and Slomp, 1967). The crystalline hydrochloride form is also freely soluble in water (Herrell, 1969). The structure is distinguished by the two

heterocycles, joined by an amide linkage (n-propylhygric acid) and a thioaminooctose (α-methylthiolincosamide)(Hornish et al., 1987).

2. Mode of action

Lincomycin binds to the 50*S* ribosomal subunit of bacteria, inhibiting the formation of all peptide bonds and the synthesis of small and large peptides. Although the specific process inhibited is slightly different than with the macrolides, this binding to the ribosome is competitive between lincomycin and the macrolides (Monro et al., 1971; Burrows, 1980). It is effective against Gram + bacteria, and mycoplasmas (Monro et al., 1971; Burrows, 1980).

3. General uses in the swine industry

Lincomycin has been used effectively to treat swine dysentery (*Treponema hyodysenteriae*), and mycoplasma pneumonia and arthritis (*Mycoplasma hyopneumoniae* and *M. hyosynoviae*, respectively)(Hamdy, 1978; Yonkers et al., 1979; Kunesh, 1981; Lukert and Mulkey, 1982;). Also, addition of lincomycin to the diets of growing pigs can improve feed efficiency and weight gain (DeGeeter and Harris, 1975).

4. Pharmacokinetics

a. Absorption

In rats, absorption of lincomycin following oral administration is approximately 40-60% of the total dose. In humans, absorption after oral application has been shown to be affected by the presence of food. Absorption of lincomycin following a dose given during fasting was 25-50%. Absorption dropped to 5% when lincomycin was administered with a meal (Hornish et al., 1987). In pigs, following an oral dose of 25 mg/kg lincomycin, peak serum levels of approximately .2 ppm were achieved in 2 - 4 hours (Hornish, 1987). In humans, peak blood levels were present at 2 hours after an oral dose of 1 gram (Herrel,1969).

b. Distribution

Tissue concentrations may be similar to tylosin due to a similar lipid solubility (Burrows, 1980). Distribution into various organs varies. In mice, following subcutaneous or intramuscular injection or oral administration, the highest levels were found in the kidney, followed, in descending order, by spleen, lung, liver, muscle and brain (Herrel, 1969). In pigs, the kidney and liver appear to be the organs with the highest concentrations, with muscle containing 10 - 14 times less lincomycin at the same time period (Hornish, 1987). Gyrd-Hansen and Rasmussen (1967) found that in cows following intravenous administration, plasma protein binding was 26-46%.

c. Biotransformation

The metabolism of lincomycin has not been fully characterized in mammals. The primary metabolite found in the urine of dogs and humans was unchanged lincomycin. No evidence of glucuronide or sulfate conjugation was found and less than 1.5% of the dose was *N*-demethyl lincomycin or lincomycin sulphoxide (Figure 6) (Hornish et al., 1987). Herrel (1969) reported no inactivation of lincomycin as measured by a microbiological assay after *in vitro* incubation with homogenized liver.

d. Excretion

In dogs, 8 hours after intravenous (IV) injection of lincomycin, 44% of the dose was excreted in the urine, 5.2% in the bile, .1% in the pancreatic juices. Urinary excretion peaked 30 minutes following IV injection (Wyman et al., 1968). The kidney is the primary route for excretion in the dog (Brown et al., 1975). In humans, urinary excretion totalled 12.7-15% of an intravenous dose given 24 hours earlier (Wyman et al., 1968). Gyrd-Hansen and Rasmussen (1967) concluded that lincomycin was excreted by both filtration and tubular secretion in the kidney of cattle. In swine, approximately 11% of the oral dose was excreted in the urine; fecal residue contained the bulk of excreted residue (Hornish et al., 1987). Muscle residues were zero at >120 hours; at 168 hours kidney residues had decreased to <.2 ppm and liver to .5 ppm (Hornish, 1987).

e. Toxicity

Diarrhea in humans has been associated with administration, presumably from preferential growth of toxin producing bacteria; a condition called pseudo-membranous colitis has been described (Leigh et al., 1980). Additionally, neuro-muscular blockade in conjunction with some anesthetics can occur; it is reversible by administration of calcium (Burrows, 1980). The acute oral LD_{50} of lincomycin in rats is greater than 4000 mg/kg; rats receiving 300 mg/kg for one year were unaffected (Herrel, 1969).

E. Conclusions

Predictions on potential drug interactions between fenbendazole and lincomycin or tylosin are difficult to make based on the pharmacokinetic data available in the literature. The chemical structures of FBZ, TY and LI are distinctly different and these differences may preclude any potential interactions of absorption, distribution, biotransformation or excretion. In particular, the good water solubility of TY and LI and the relative insolubility of FBZ would indicate differences in pharmacokinetics.

In the gut, chemical interactions between drugs may take place, which could interfere with the absorption of FBZ and LI or TY if the drugs complexed with one another. An added concern is the potential for interactions in the feed itself although this is less likely due to the generally crystalline form of the drugs when mixed into feeds.

It does not appear that the plasma protein binding, and subsequent distribution of the compound, evident with TY and LI, would be altered by the concurrent administration of FBZ as the latter is not significantly bound.

Because so little is known concerning the mechanism for biotransformation of TY and LI, few predictions can be made regarding the potential for interaction between FBZ and TY or LI metabolism. FBZ is extensively metabolized in the liver by the mixed function oxidase system, TY and LI are most likely metabolized in the liver as well, yet the specific mechanism is not known.

Excretion of TY and LI occurs primarily in the kidney, with some biliary and subsequent fecal excretion. FBZ is found in the urine, but the bulk of FBZ is excreted in the feces, and gastrointestinal recycling may play an important role in this excretion. It seems that FBZ and TY or LI would probably use different mechanisms for excretion based on the primary organ involved and differences in water solubility.

Residue patterns of TY, LI and FBZ are different in that FBZ is retained in liver and kidney for 2-12 days longer than LI or TY. Again this may be due to differences in water solubility, biotransformation and excretion patterns.

In conclusion, the limited data available do not allow a sound prediction on potential interactions, but it is hypothesized that interactions between FBZ and LI or TY would be minimal.

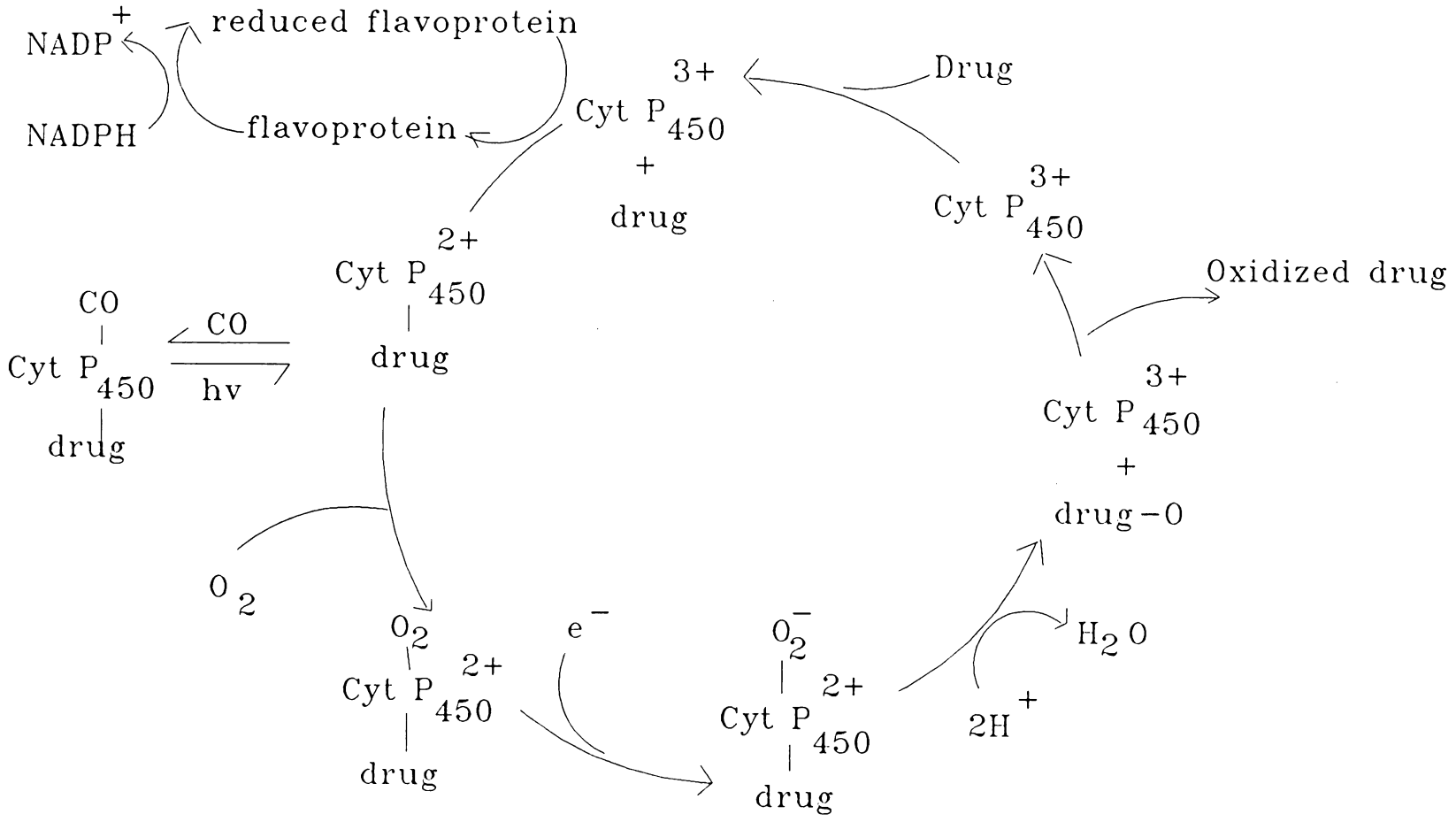


Figure 1. Cytochrome P_{450} Pathway

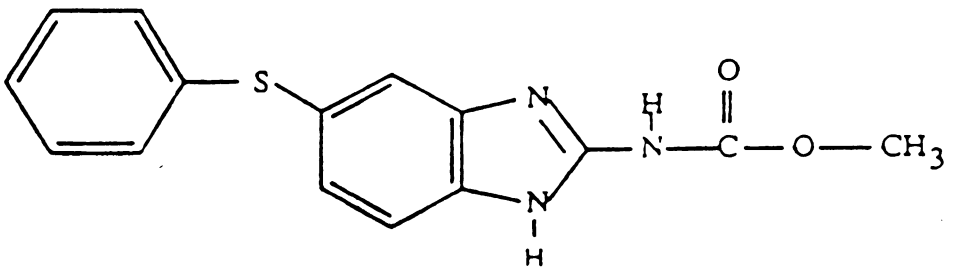


Figure 2. Chemical Structure of Fenbendazole

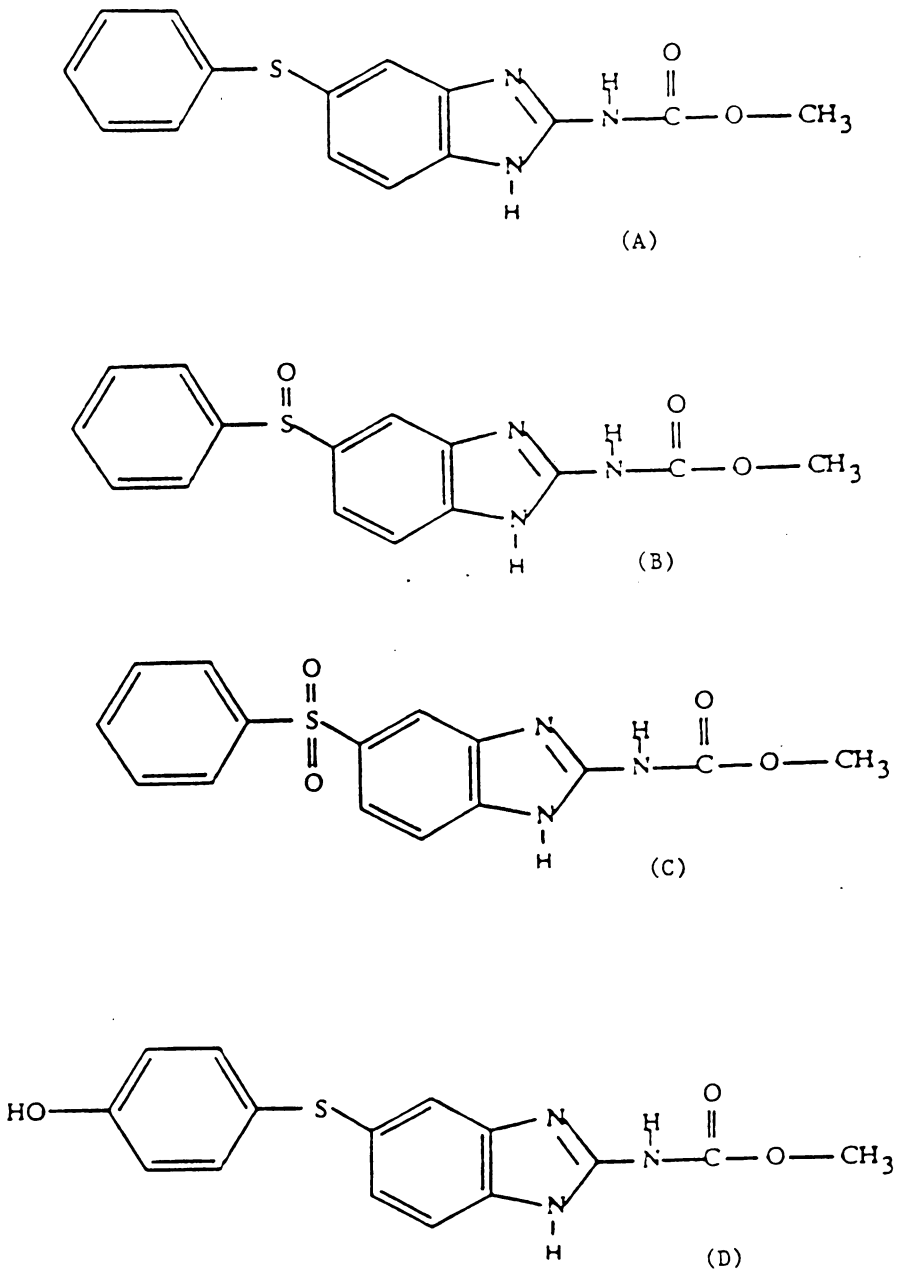


Figure 3. Fenbendazole (FBZ) (A), FBZ–sulphoxide (B), FBZ–sulphone (C), FBZ–OH (D).

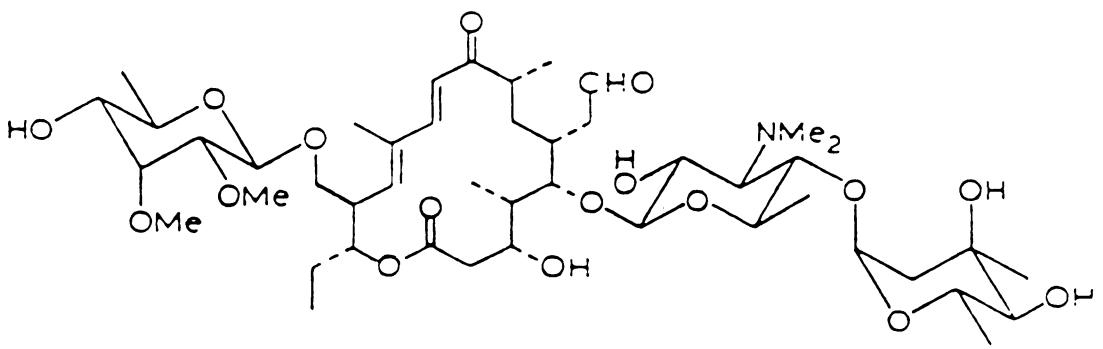


Figure 4. Chemical Structure of Tylosin

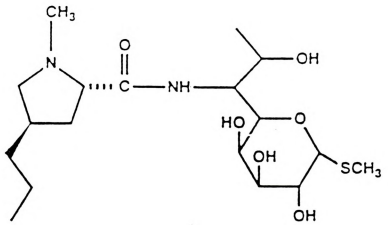
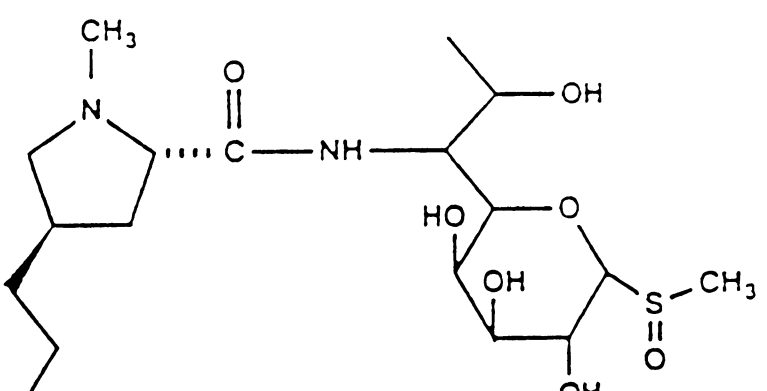
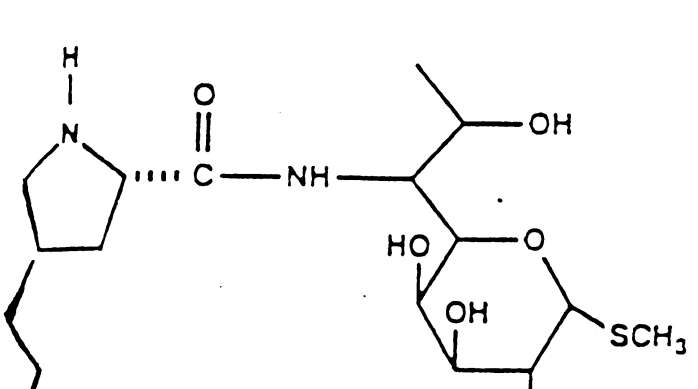


Figure 5. Chemical Structure of Lincomycin



(A)



(B)

Figure 6. Lincomycin sulphoxide (A),
N-demethyl lincomycin (B).

Chapter 3:

Study to Determine the Efficacy of Fenbendazole against *Ascaris suum* and *Trichuris suis* when Administered Concurrently with Tylosin or Linocmycin to Pigs

A. Introduction

Fenbendazole (FBZ) is a broad spectrum anthelmintic commonly administered in the feed. It has been shown to be effective in removing *Ascaris suum* (roundworm) and *Trichuris suis* (whipworm) infections in pigs (Batte, 1977; Corwin et al., 1984). In the United States, it is licensed for used in swine at a total dose of 9 mg/kg of body weight divided over 3 to 12 days (Muirhead, 1990).

Tylosin (TY) and lincomycin (LI) are antibiotics used to promote growth and reduce disease due to mycoplasmal and spirocheteal disease (Hamdy, 1978; Lukert and Mulkey, 1982). They are administered in low doses, over extended time periods, in the feed of young pigs. The are licensed for use at varying rates, from 10-200 g/ton of feed depending on indication (Muirhead, 1990).

In order to manage feed mixing, handling and delivery, concurrent administration of FBZ and TY or LI is desirable. Feed containing these antibiotics would not have to be withdrawn from pigs during treatment of parasite infections.

However, drug interactions may occur when more than one drug is administered at a time. These interactions may take the form of reduced efficacy of one the drugs.

The objective of this experiment was to determine the efficacy of fenbendazole when administered in the feed with lincomycin or tylosin and compare this efficacy to that of FBZ, LI and TY when administered singly.

B. Materials and Methods

1. Animals

The experiment was conducted using two replicates (A and B). For each replicate, 30 female and 30 male crossbred pigs were purchased from a commercial swine operation. At acquisition, the following procedures were performed on each pig:

1. weight taken
2. eartagged
3. vaccinated against erysipelas and pseudorabies
4. a fecal sample was obtained and examined
5. 500 larvated *Ascaris suum* eggs and 400 larvated *Trichuris suis* eggs were orally inoculated.

Pigs were maintained on a 13% protein diet for 10 days in order to promote parasite infection and then placed on a diet balanced for growing pigs, with no antibiotics or anthelmintics. For 42 days following inoculation, pigs were housed 12 animals per pen on cement floors bedded with straw. Water and feed were provided

ad libitum via nipple waterer and automatic feeder. This period of 42 days was to allow time for parasites to reach an adult patent stage and is referred to as the pre-patent period.

2. Treatment

At the end of the pre-patent period, the pigs were weighed and a fecal sample was obtained and examined. Pigs were then allotted to 12 treatment groups of 5 pigs per group, stratified by weight and sex. The treatment groups were as follows:

Group	Treatment
1	control
2	FBZ 3.0 mg/kg/day, days 10-12
3	FBZ 1.5 mg/kg/day, days 6-12
4	FBZ .75 mg/kg/day, days 1-12
5	FBZ 3.0 mg/kg/day, days 10-12 + LI 200 g/T
6	FBZ 1.5 mg/kg/day, days 6-12 + LI 200 g/T
7	FBZ .75 mg/kg/day, days 1-12 + LI 200 g/T
8	LI 200 g/T of feed
9	FBZ 3.0 mg/kg/day, days 10-12 + TY 100 g/T
10	FBZ 1.5 mg/kg/day, days 6-12 + TY 100 g/T
11	FBZ .75 mg/kg/day, days 1-12 + TY 100 g/T
12	TY 100 g/T of feed

The treatment period lasted for 12 days, with feeding of the fenbendazole medicated feed scheduled such that all FBZ treatment groups received the last dose on day 12. TY and LI medicated feed were present for 12 days in the automatic

feeder. Lincomycin was supplied as lincomycin hydrochloride in a premix which was added to the feed to attain a final concentration of 200g/T. Tylosin was supplied as tylosin phosphate, in premix, added to the finished feed to a concentration of 100 g/T. A 4% fenbendazole premix was separately mixed with control, TY or LI medicated feed in sufficient quanitity to provide each pen with the required daily dose of FBZ in approximately one pound of feed. The actual dose of FBZ (in mg/kg) to be fed was calculated for each pen based on actual weight and expected concentration of FBZ in the feed.

The treatment period lasted for 12 days. During this period, the FBZ mixture was fed in a wooden trough, measuring 30 cm X 183 cm, placed in each pen. Each day the automatic feeders, which contained control, TY or LI medicated feed only, were closed for 2-4 hours. Following this fasting period, FBZ medicated feed was placed in the trough. Pigs were observed until the FBZ-medicated feed was consumed; then the regular feeder was opened. Following the treatment period, all pigs were placed on control diet (no anthelmintic, no antibiotic) for 7-12 days.

3. Sample collection

From day 7 to day 12 after the end of the treatment period, an equal number of animals were randomly selected from each treatment group and euthanized with an intravenous injection of T-61® (Hoechst-Roussel Agri-Vet Co.). They were weighed, the abdomen was opened, the duodenum tied off at the pyloric region, the ileum tied

off at the ileocecal valve and the rectum tied off in the pelvic canal. The small and large intestines were stripped of their mesenteric attachments, removed from the animal and placed in a bucket with the animal's eartag. The liver was removed and any milk spots (scars from *A. suum* migration) on the diaphragmatic surface were counted.

The small intestine was opened with scissors and the contents allowed to drop into a liter calibrated bucket. The mucosa was scraped clean by pulling the intestine between two fingers. The material was added to the contents of the bucket. Any *A. suum* found were separated from the contents and counted, noting male and female numbers and recorded as a direct count. Two 10% sub-samples were taken from the small intestine contents in the bucket and each sample was placed in a separate one quart mason jar. Phosphate buffered formalin was added to each sub-sample for preservation of the sample. The jar and lid were labelled with the pig number, contents and volume.

The large intestine was cut into approximately one foot long sections. The last six inches of rectum (containing feces) was tied off at both ends and saved for fecal examination. The contents of each section of the large intestine were washed into a liter calibrated bucket. Each section was washed again with water, which was added to the contents bucket. Each section was opened with scissors and laid flat on the table with the mucosal surface facing up. The mucosal surface was grossly examined for adult *T. suis* still attached to the mucosa. These were collected,

counted, recorded as a direct count and discarded. Two 10% sub-samples were taken from the contents bucket and handled in the same manner as the small intestine sub-samples.

4. Sample testing

Each small intestine sub-sample was poured over a 2.0 mm and then a .840mm screen and washed. The residue left behind was examined grossly for adult *A. suum* which were counted.

Each large intestine sample was poured over a 2.0mm, .840mm and .177mm screen stacked in that order. The screens were washed with water. The residue left on the .177mm screen was examined under a dissecting microscope for the presence of *T.suis* adults, which were counted.

These results were recorded and total worm burden was calculated by multiplying the sub-sample count by the dilution factor for the sub-samples and adding the product to the direct count made at the time of necropsy and sample preparation.

5. Statistical Analysis

A one way analysis of variance (ANOVA) was performed to determine the effect of time on replicates. Replicates were not significantly different ($p<.05$) and the data from the two replicates were combined. Data were analyzed using linear ANOVA. Comparisons were made using the least significance difference method. Comparisons

were done for number of liver scars, number of *A. suum* and number of *T. suis* among all treatment groups.

C. Results

The number of scars on the diaphragmatic surface of the liver associated with *A. suum* migration varied between 0 and 57 per animal with an overall mean of 12. The number of scars on the liver associated with *A. suum* larvae migration did not differ among treatment groups ($p=.44$).

The total number of adult *A. suum* found in the gut varied between 0 and 66; the mean was 2. All groups receiving fenbendazole had a lower number of *A. suum* as compared to the control group ($p=.02$).

The total number of adult *T. suis* varied between 0 and 265 worms per animal. The overall average for all animals was 45. Treatment groups 1, 4, 8 and 12 had higher numbers of *T. suis* than all other treatment groups ($p<.01$). The other treatment groups were not different from one another ($p>.05$). The average number of *A. suum* and *T. suis* present in each treatment group are reported in Table 1. Percent reduction of worm burden and number of liver scars in each treatment group are listed in Table 2.

D. Discussion

Scars on the diaphragmatic surface of the liver were counted to evaluate the level of infection with *A. suum*. The larval stage of *A. suum* migrates through the parenchyma of the liver. In so doing, inflammation and subsequent scarring occurs. This scarring can be seen on the surface of the liver as pale white spots (Soulsby, 1982). Based on average number liver scars for each treatment group, *A. suum* infection rates did not differ among groups.

Moderate numbers of *A. suum* in treatment groups 1, 8 and 12 were present. No adult ascarids were found in any animals in the treatment groups either receiving FBZ alone or in combination with TY or LI, resulting in 100% efficacy. These results on efficacy of FBZ against *A. suum* concur with the results of others utilizing FBZ alone, at the same dosage, obtained elsewhere (Batte, 1978; Corwin, 1984).

At least one animal in each group harboured adult *T. suis* following treatment with FBZ. Complete removal was not achieved. Percent reduction in *T. suis* adults in the TY or LI alone treatment groups was 13 and 34% respectively as compared to the control group. These values represent normal variation in parasite numbers and were not statistically different from one another ($p>.05$).

Enigk et al. (1974) reported a 65%, 91% and 96.6% reduction in the number of adult *T. suis* in pigs following an oral dose of 15 mg/kg, 20 mg/kg and 30 mg/kg FBZ, respectively. Batte (1978) using a critical test (Gibson, 1964) found that 99.8% of *T. suis* were eliminated with an oral dose of 3 mg/kg for 3 days. Corwin et al. (1984)

in a similar experiment in which a total dose of 9 mg/kg was divided over 3, 6 or 12 days, obtained respectively 100%, 99% and 87% reductions when using fenbendazole alone. A 66% reduction of *T. suis* was achieved with FBZ 3 mg/kg/day for 3 days by Marti et al. (1978). The percent reductions obtained in the experiments reported here are similar to Corwin et al. (1984) in all treatment groups given FBZ, with and without TY or LI, except for FBZ alone divided over 12 days. Percent reduction was 25% when fenbendazole was given over 12 days and was not different ($p<.05$) from the control group.

The efficacy of FBZ against *T. suis* as reported in the literature is variable. All our treatment groups receiving FBZ with TY or LI showed reductions in worm counts similar to those reported by Corwin et al. (1984). Treatment groups receiving FBZ alone divided over 3 or 6 days were also similar to FBZ with TY or LI. Only the group receiving FBZ 9 mg/kg divided over 12 days without TY or LI did not show a significant reduction in the number of adult *T. suis*. The difference in *T. suis* numbers among groups receiving a dose of FBZ divided over twelve days given with TY or LI and the same dose of FBZ given without TY or LI are difficult to explain. This trial may have experienced variations in unmeasured parameters similar to those of other trials. These parameters may include competition at the feeder, differences in overall health of individual pigs and variation in infection rates. We were not able to use an objective measurement such as liver scar scores as with *A. suum* to measure overall infection rates.

From this trial, we can conclude that TY and LI do not interfere with the efficacy of FBZ against *A. suum* and *T. suis* when administered to pigs in feed as compared to control groups and efficacy levels reported in the literature. However, FBZ at a dose of .75mg/kg/day for 12 days was not effective in eliminating *T. suis* in the pig.

Table 1. Effect of Tylosin or Linomycin on the Efficacy of Fenbendazole against *Ascaris suum* and *Trichuris suis* in the pig.

		Number of worms recovered	
Treatment[1]	n	*Ascaris suum* [2]	*Trichuris suis* [2]
CO	10	9(18)[a3]	134(86)[a]
8	10	4(8)[a]	89(62)[a]
12	10	13(20)[a]	116(84)[a]
2	10	0[b]	6(7)[b]
5	10	0[b]	2(2)[b]
9	10	0[b]	7(13)[b]
3	10	0[b]	1(2)[b]
6	10	0[b]	6(18)[b]
10	10	0[b]	8(21)[b]
4	10	0[b]	100(81)[a]
7	10	0[b]	2(3)[b]
11	10	0[b]	16(29)[b]

[1]CO = control
2 = Fenbendazole (FBZ) 3 mg/kg/day, days 10-12
3 = FBZ 1.5 mg/kg/day, days 6-12
4 = FBZ .75 mg/kg/day, days 1-12
5 = FBZ 3 mg/kg/day, days 10-12; Lincomycin (LI) 200 g/T, days 1-12
6 = FBZ 1.5 mg/kg/day, days 6-12; LI 200 g/T, days 1-12
7 = FBZ .75 mg/kg/day, days 1-12; LI 200 g/T, days 1-12
8 = LI 200 g/T, days 1-12
9 = FBZ 3 mg/kg/day, days 10-12; Tylosin (TY) 100 g/T, days 1-12
10 = FBZ 1.5 mg/kg/day, days 6-12; TY 100 g/T, days 1-12
11 = FBZ .75 mg/kg/day, days 1-12; TY 100 g/T, days 1-12
12 = TY 100 g/T, days 1-12

[2]Average worms per pig (standard deviation)

[3]Values with different superscripts within columns are different $p<.05$

Table 2. Scars Present on the Diaphragmatic Surface of the Liver and Percent Reduction of *Ascaris suum* and *Trichuris suis* as Comparred to Respective Control Group.

Treatment[1]	Liver Scars	n	% *A. suum*	%*T. suis*
CO	14 (10)[2]	10	-----	-----
2	14 (16)	10	100	95.3
3	6 (9)	10	100	99.6
4	11 (11)	10	100	25.5
5	9 (5)	10	100	97.6
6	19 (16)	10	100	93.0
7	10 (7)	10	100	98.2
8	13 (16)	10	-----	-----
9	12 (15)	10	100	94.2
10	12 (11)	10	100	93.5
11	7 (8)	10	100	86.2
12	12 (9)	10	-----	-----

[1]CO = control
2 = Fenbendazole (FBZ) 3 mg/kg/day, days 10-12
3 = FBZ 1.5 mg/kg/day, days 6-12
4 = FBZ .75 mg/kg/day, days 1-12
5 = FBZ 3 mg/kg/day, days 10-12; Lincomycin (LI) 200 g/T, days 1-12
6 = FBZ 1.5 mg/kg/day, days 6-12; LI 200 g/T, days 1-12
7 = FBZ .75 mg/kg/day, days 1-12; LI 200 g/T, days 1-12
8 = LI 200 g/T, days 1-12
9 = FBZ 3 mg/kg/day, days 10-12; Tylosin (TY) 100 g/T, days 1-12
10 = FBZ 1.5 mg/kg/day, days 6-12; TY 100 g/T, days 1-12
11 = FBZ .75 mg/kg/day, days 1-12; TY 100 g/T, days 1-12
12 = TY 100 g/T, days 1-12

[2]Average number of scars (standard deviation)

Chapter 4:

Study to Determine the Safety of a Fenbendazole-Tylosin or Fenbendazole-Lincomycin Combination in Pigs.

A. Introduction

Evaluations of a drug are important to determine if a drug is safe to use at effective doses and also to determine the therapeutic index, i.e., the ratio of the dose at which toxicity occurs to the effective dose. For drugs with a low therapeutic index, the dose at which treatment is affected and the dose which is toxic are very near each other. This principle is important when considering the implications of inadvertent overdoses.

Fenbendazole (FBZ) has been administered to pigs at 5000 mg/kg with no adverse side effects (Düwel, 1977). In animals in which FBZ has been tested, doses which may produce adverse side effects have not been reached due to the inability to physically administer high enough doses (Baeder et al, 1974).

Tylosin also has a low order of toxicity, the LD_{50} in rats and mice after intravenous administration is 580-695 mg/kg; dogs tolerated 100 mg/kg/day for two years without detectable side effects (Wilson, 1984). Signs of intolerance include gastrointestinal disturbance and allergies.

Lincomycin has been given to rats at 300 mg/kg for one year with no adverse side effects; the acute oral LD_{50} in rats is >4000 mg/kg. In humans, diarrhea has been

noted in patients receiving lincomycin; this was felt to be due to an increase in the number of toxin producing bacteria in the gut. Instances of anal swelling and cutaneous reddening have been noted in pigs at a dose of 200 g/ton of feed. These reactions are usually transitory (Muirhead, 1990).

In evaluating the safety (or lack of toxicity) of drugs, studies measuring the overall health of the animals being tested may be performed. These studies may include measurement of enzymes, minerals and electrolytes in the serum, complete blood counts, and measurement of clotting ability of the blood.

This trial was conducted to determine the effect of concurrent administration of fenbendazole and tylosin or lincomycin. The objective method of measuring serum enzymes, electrolytes, minerals, white blood cell numbers, red blood cell numbers and blood clotting ability was used in order to compare the health of control animals with animals receiving 1, 3 or 5 times the normal level of the drugs.

B. Materials and methods

1. Animals

The trial was conducted in four replicates:

Replicate A - FBZ with TY

20 animals

Average weight = 33 lbs

Replicate B - FBZ with TY

20 animals

Average weight = 29 lbs

Replicate C - FBZ with LI

20 animals

Average weight = 34 lbs

Replicate D - FBZ with LI

20 animals

Average weight = 38 lbs

For each replicate, female pigs were obtained from a local swine producer. All animals were crossbred (Duroc, Landrace), large white type. Each animal was individually housed in a crate with wire flooring and provided with a self-feeder and nipple waterer. Upon acquisition, the pigs were eartagged, weighed and allotted to treatment groups, stratified by weight.

2. Treatment

The following treatment groups were assigned:

Group	Nunber of Animal	Treatment
CO-A	5	control, FBZ and LI
CO-B	5	control, FBZ and LI
CO-C	5	control, FBZ and TY
CO-D	5	control, FBZ and TY
1LI-A	5	FBZ 3 mg/kg BW/day, days 10-12 LI 200 g/T of feed, for 21 days
1LI-B	5	same as 1LI-A
3LI-A	5	FBZ 9 mg/kg BW/day, days 6-14 LI 600 g/T of feed, for 21 days
3LI-B	5	same as 3LI-A
5LI-A	5	FBZ 15 mg/kg BW/day, days 6-14 LI 1000 g/T of feed, for 21 days
5LI-B	5	same as 5LI-A
1TY-A	5	FBZ 3 mg/kg BW/day, days 10-12 TY 100 g/T of feed, for 21 days
1TY-B	5	same as 1TY-A
3TY-A	5	FBZ 9 mg/kg BW/day, days 6-14 TY 300 g/T of feed, for 21 days
3TY-B	5	same as 3TY-A
5TY-A	5	FBZ 15 mg/kg BW/day, days 6-14 TY 500 g/T of feed, for 21 days
5TY-B	5	same as 5TY-A

BW = body weight
FBZ = fenbendazole
TY = tylosin
LI = lincomycin

Each replicate of the trial lasted for 21 days. Tylosin was provided in a tylosin tartrate premix, lincomycin in the hydrochloride form. Appropriate amounts were added to a base diet balanced for growing pigs for each treatment group. The tylosin or lincomycin medicated feed was provided *ad libitum* in automatic feeders to the pigs. A fenbendazole premix was separately mixed with TY or LI medicated feed in sufficient quantity to provide each animal with the daily dose of FBZ in approximately one pound of feed. The actual dose of fenbendazole (in mg/kg) to be fed was calculated for each pig based on actual weight and expected concentration of FBZ in the feed. Prior to feeding FBZ medicated feed, pigs were fasted for 2-4 hours. After giving FBZ-medicated feed, they were observed to make sure the medicated feed was consumed. Pigs were observed daily for appetite, attitude and stool appearance.

3. Sample collection

Blood samples were obtained from the jugular vein using a Vacutainer® system. On days 0, 13 and 21 the following samples were taken:

1. 10 ml blood, allowed to clot
2. 3 ml whole blood in EDTA
3. 4.5 ml whole blood in citrate

4. Sample testing

The following determinations on the samples were made by the Clinical Pathology Laboratory, Veterinary Clinical Center, Michigan State University.

Parameters evaluated:

Serum chemistry:

total protein, albumin, globulin, sodium, potassium, chloride, total carbon dioxide, calcium, phosphorus, magnesium, glucose, blood urea nitrogen, creatinine, amylase, total bilirubin, alkaline phosphatase, sorbitol dehydrogenase, aspartate amino transferase; all values except electrolytes were determined using a Flexigem® serum chemistry analyzer and electrolytes were determined with a Beckman® electrolyte analyzer.

Complete blood count:

red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, total white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, platelet count, activated partial thromboplastin time; platelet, red and total white blood cell numbers, and hemoglobin values were determined using the H-1 Technicon® automated system. White blood cell differential counts were performed by hand, by examining a stained blood smear, counting 100 cells and determining the percentage of each white blood cell type. Total numbers of these cell types were calculated using the total white cell count.

5. Statistical Analysis

Effect of replicate (LI-A and B or TY-A and B) was tested using a one-way analysis of variance (ANOVA) and for parameters which were not significantly different ($p>.05$), data were combined. Analysis of the data was performed using analysis of variance (ANOVA). Least significant difference test was used for comparison of the means, using $p<.05$ for level of significance. Comparisons were made at each bleeding time point between treatment groups within TY or LI with FBZ. Comparisons between TY and LI were not performed.

C. Results

Overall, in all treatment groups, pigs consumed the medicated feed readily, had normal feces and appeared bright, alert and responsive throughout the treatment period.

Fenbendazole plus Tylosin

Summaries of the averages and standard deviation for each treatment group and parameter evaluated are presented in Tables 3 through 10.

At the first bleeding date, prior to drug administration, magnesium, glucose, amylase, chloride and potassium values differed ($p<.05$) between treatment groups.

At day 13, magnesium and globulin was different ($p<.05$) between treatment groups receiving 5 or 3 times the normal dose and the control group; amylase was

different ($p<.05$) between the control and the group receiving 3 times the normal dose.

At day 21, the 5 times and 3 times normal dose treatment groups had different ($p<.05$) total protein (replicate A and B); the 5 times normal treatment group globulin (replicate B only) levels differed ($p<.05$) from controls.

There were no differences between treatment groups ($p<.05$) for all red blood cell parameters, platelet numbers and activated partial thromboplastin time.

Fenbendazole plus Lincomycin

Average values and standard deviation for each treatment group and parameter evaluated are presented in Tables 11 through 18.

At the first bleeding date, prior to drug administration, albumin (replicate A only), sodium and amylase differed ($p<.05$) between treatment groups.

At day 13 bleeding, levels of blood urea nitrogen, calcium, magnesium (replicate A only), albumin (replicate B only), glucose (replicate A only) the treatment group receiving 5 times the normal dose differed ($p<.05$) from the control group. The treatment group receiving 3 times the normal dose had different levels of magnesium (replicate A only), glucose (replicate A only), blood urea nitrogen, and albumin (replicate A only) than the control group. Glucose levels (replicate A only) in the 1 times normal dose treatment group differed ($p<.05$) from the control group.

At day 21 the 1, 3, and 5 times normal dose treatment group had different ($p<.05$) levels from the control group of alkaline phosphatase.

At day 0, prior to drug administration, hemoglobin values (replicate A only) differed ($p<.05$) between groups.

At day 13, groups receiving 3 or 5 times the normal dose had different ($p<.05$) numbers of red blood cells and hemoglobin (replicate A only) from the controls. Eosinophil numbers in the treatment group receiving 1 times the normal dose differed ($p<.05$) from the controls.

At day 21, red blood cell numbers (replicate B only) in treatment groups receiving 1, 3 or 5 times the normal dose all differed ($p<.05$) from the control. Mean corpuscular hemoglobin concentration differed in treatment groups receiving 3 or 5 times the normal dose differed ($p<.05$) from the control.

All other parameters measured were not different ($p>.05$) from the control at any time point.

D. Discussion

Statistically different values were noted between the treatment groups and the control group, however, these changes may not be biologically significant. Jain (1986) reported normal blood values for pigs with ranges in which to expect normal values to fall. Tumbleson et al. (1986) also reported normal ranges of serum chemistry and blood for pigs of different age groups and stages of life (pregnant, in estrus, postpartum). These values may vary widely and can be affected by age, environment, feeding, stress, pregnancy or genetics (Tumbleson et al., 1986). This variation

between pigs was also evident in our trial by the differences between treatment groups at day 0, prior to drug administration. The values we obtained were within the ranges for pigs 4 to 8 weeks of age as reported by Tumbleson et al. (1986).

In a study by Hayes et al. (1983b), treatment with fenbendazole at 75 mg/kg/day or 125 mg/kg/day for 5 days resulted in a transient lymphopenia, and moderate increase in serum levels of sorbitol dehydrogenase and phosphorus. In a another study by Hayes et al. (1983a), daily increasing doses of FBZ beginning at 250 mg/kg up to 1000 mg/kg over 6 days resulted in slight elevations of sorbitol dehydrogenase and leukopenia. Hayes et al. (1983a,b) concluded that fenbendazole alone was safe for pigs. In our experiment, the combination of FBZ with TY or LI did not produce any ill effects on the health of young pigs as measured by serum chemistry, red blood cell, platelet, activated partial thromboplastin time or white blood cell parameters.

Table 3. Influence of Tylosin and Fenbendazole on Serum Levels of Total Bilirubin, Sorbitol Dehydrogenase, Alkaline Phosphatase and Aspartate Aminotransferase

		Treatment[1]				
Item	n	CO	1TY	3TY	5TY	P
Total Bilirubin (mg/dl)						
Day 0	10	.2(.1)[2]	.2(.1)	.2(.1)	.2(.1)	.97
Day 13	10	.1(.1)	.2(.1)	.2(.1)	.1(.1)	.52
Day 21	10	.2(.2)	.2(.1)	.2(.2)	.2(.1)	.44
Alkaline Phosphatase (IU/l)						
Day 0	10	248(40)	234(59)	235(75)	240(38)	.95
Day 13	10	220(39)	217(37)	197(38)	207(37)	.58
Day 21	10	198(43)	188(30)	178(55)	198(41)	.65
Sorbitol Dehydrogenase (IU/l)						
Day 0	10	3(2)	3(2)	3(1)	3(1)	.97
Day 13	10	3(1)	4(4)	8(10)	5(2)	.27
Day 21	10	5(5)	3(2)	3(2)	3(2)	.36
Aspartate Aminotransferase (IU/l)						
Day 0	10	34(9)	34(10)	30(7)	28(6)	.08
Day 13	10	27(5)	29(7)	41(30)	29(6)	.25
Day 21	10	29(8)	27(8)	27(7)	25(6)	.61

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days
[2]Standard deviation

Table 4. Influence of Tylosin and Fenbendazole on Serum Levels of Glucose, Amylase, Blood Urea Nitrogen and Creatinine

		Treatment[1]				
Item	n	CO	1TY	3TY	5TY	P
Glucose (mg/dl)						
Day 0	10	122(12)[a3]	104(13)[b]	109(12)[b]	113(21)[ab]	.03
Day 13	10	111(10)[2]	134(36)	116(6.5)	116(10)	.08
Day 21	10	111(20)	117(10)	108(23)	116(18)	.65
Amylase (IU/l)						
Day 0, rep A	5	2122(765)[ab]	2842(337)[a]	2752(460)[a]	1754(231)[ab]	.03
Day 0, rep B	5	1870(601)	1546(319)	2212(466)	1744(339)	.18
Day 13	10	1919(704)[a]	1996(491)[ab]	2403(454)[b]	1666(360)[a]	.03
Day 21	10	1946(741)	1943(582)	2320(540)	1679(443)	.14
Blood Urea Nitrogen (mg/l)						
Day 0	10	12(3.8)	12(2.9)	12(2.1)	14(4.3)	.14
Day 13	10	12(1.8)	15(3.1)	13(3.0)	12(2.8)	.15
Day 21	10	12(2.2)[a]	14(2.9)[b]	14(2.5)[ab]	12(2.7)[a]	.04
Creatinine (mg/dl)						
Day 0	10	1.3(.2)	1.2(.2)	1.2(.2)	1.2(.2)	.23
Day 13	10	1.0(.1)	1.1(.2)	1.1(.2)	1.1(.1)	.54
Day 21	10	1.1(.4)	.98(.2)	.97(.3)	.91(.8)	.57

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 5. Influence of Tylosin and Fenbendazole on Serum Levels of Total Protein, Albumin and Globulin

		Treatment[1]				
Item	n	CO	1TY	3TY	5TY	P
Total Protein (gm/dl)						
Day 0	10	5.9(.4)[2]	5.7(.4)	5.9(.3)	5.7(.3)	.28
Day 13	10	5.8(.4)	6.0(.5)	6.3(.5)	6.0(.5)	.15
Day 21	10	6.5(.5)[a3]	6.6(.3)[a]	6.5(.4)[a]	5.8(1)[b]	.01
Albumin (gm/dl)						
Day 0	10	3.6(.4)	3.4(.4)	3.5(.2)	3.5(.4)	.57
Day 13	10	3.3(.5)	3.5(.4)	3.8(.4)	3.6(.3)	.22
Day 21	10	3.8(.4)	4.1(.4)	4.1(.5)	4.0).5)	.26
Globulin (gm/dl)						
Day 0	10	2.2(.4)	2.3(.5)	2.4(.3)	2.2(.4)	.62
Day 13, rep A	5	2.3(.3)	2.5(.5)	2.5(.3)	2.5(.2)	.97
Day 13, rep B	5	2.2(.5)[ab]	2.3(.3)[ab]	2.7(.2)[a]	1.8(.2)[b]	.05
Day 21	10	2.7(.6)[a]	2.5(.4)[a]	2.4(.3)[ab]	2.1(.3)[b]	.03

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 6. Influence of Tylosin and Fenbendazole on Serum Levels of Calcium, Phophorus, and Magnesium

Item	n	Treatment[1] CO	1TY	3TY	5TY	P
Calcium (mg/dl)						
Day 0	10	10.0(1.7)[2]	9.6(1.2)	10.0(.38)	10.0(.75)	.66
Day 13	10	10.3(.69)	10.5(.55)	10.9(.58)	10.7(.62)	.11
Day 21	10	9.9(.32)	10.0(.78)	10.0(.30)	10.2(.49)	.64
Phosphorus (mg/dl)						
Day 0	10	9.0(1.6)	9.3(2.0)	10.0(1.5)	9.2(1.2)	.32
Day 13	10	10.5(.79)	10.4(.79)	10.0(.50)	10.0(.71)	.27
Day 21	10	10.6(.95)	10.3(.90)	10.3(.96)	10.7(.79)	.68
Magnesium (mg/dl)						
Day 0, rep A	5	2.0(.31)^{ab}[3]	1.9(.08)^{b}	2.2(.19)^{a}	2.3(.17)^{a}	.04
Day 0, rep B	5	2.1(.25)	2.1(.19)	2.4(.22)	2.4(.08)	.08
Day 13	10	2.1(.23)^{a}	2.2(.26)^{ab}	2.4(.19)^{b}	2.4(.10)^{b}	.01
Day 21	10	2.2(.41)	2.1(.32)	2.3(.32)	2.2(.30)	.11

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 7. Influence of Tylosin and Fenbendazole on Serum Levels of Sodium, Potassium, Chloride and Total Carbon Dioxide

		Treatment[1]				
Item	n	CO	1TY	3TY	5TY	P
Sodium (mEq/l)						
Day 0	10	144(5.2)[2]	144(5.5)	145(5.5)	144(5.1)	.69
Day 13	10	143(1.8)	144(3.4)	145(3.4)	146(3.0)	.70
Day 21	10	143(2.4)	143(1.9)	144(2.5)	144(1.8)	.79
Potassium (mEq/l)						
Day 0	10	6.4(1.0)[a3]	5.6(1.0)[b]	6.5(.78)[a]	5.8(.60)[ab]	.02
Day 13	10	5.7(.42)	5.8(.70)	5.9(.69)	5.8(.60)	.79
Day 21	10	5.5(.52)	5.5(.56)	5.6(.63)	5.8(.46)	.41
Chloride (mEq/l)						
Day 0, rep A	5	105(.98)[a]	102(.89)[b]	102(2.1)[b]	102(1.5)[b]	.03
Day 0, rep B	5	109(4.2)	109(1.9)	110(1.7)	108(1.6)	.46
Day 13	10	105(2.1)	106(2.1)	106(1.9)	104(2.0)	.08
Day 21	5	105(2.2)	106(2.2)	105(2.7)	105(1.9)	.57
Total Carbon Dioxide (mEq/l)						
Day 0	10	28(3.9)	30(2.7)	30(3.5)	30(2.7)	.27
Day 13	10	32(2.6)	30(4.9)	32(2.0)	34(1.7)	.15
Day21	10	32(2.4)	31(3.2)	32(2.2)	34(1.9)	.14

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 8. Influence of Tylosin and Fenbendazole on Total White Blood Cell, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil Numbers.

Item	n	Treatment[1] CO	1TY	3TY	5TY	P
Total White Blood Cells ($x10^3$)						
Day 0, rep A	5	17.7(3.6)[2]	18.1(2.1)	17.1(3.3)	18.3(4.5)	.98
Day 0, rep B	5	12.7(2.3)	14.6(4.2)	13.6(4.4)	16.4(1.8)	.39
Day 13, rep A	5	17.5(1.5)	17.5(.88)	17.0(3.7)	17.1(.50)	.53
Day 13, rep B	5	13.4(3.4)	15.4(3.9)	15.4(2.9)	13.5(1.5)	.53
Day 21, rep A	5	15.3(1.2)	16.6(1.8)	16.1(2.5)	16.8(2.2)	.80
Day 21, rep B	5	12.4(.89)	14.3(2.4)	12.5(1.5)	12.9(2.0)	.52
Neutrophils ($x10^3$/ul)						
Day 0, rep A	5	5.7(1.7)	7.5(1.7)	6.0(1.9)	8.0(2.8)	.38
Day 0, rep B	5	4.1(.95)	5.2(2.1)	2.9(1.1)	4.3(.79)	.14
Day 13	10	6.0(.98)	5.9(1.8)	6.1(1.8)	4.7(1.1)	.22
Day 21, rep A	5	5.2(1.2)	5.1(2.2)	4.6(.54)	6.1(1.6)	.57
Day 21, rep B	5	4.6(1.4)	3.7(1.4)	3.8(1.2)	3.1(1.1)	.34
Lymphocytes ($x10^3$/ul)						
Day 0	10	9.3(2.1)	9.2(2.0)	9.8(3.2)	8.2(3.0)	.55
Day 13, rep A	5	10.6(1.2)	11.2(.60)	10.6(3.2)	10.8(.62)	.78
Day 13, rep B	5	7.3(3.4)	8.7(2.9)	7.7(3.2)	8.3(1.8)	.86
Day 21, rep A	5	9.3(.73)	10.9(2.5)	10.5(2.0)	9.6(.37)	.60
Day 21, rep B	5	7.2(1.4)	10.1(1.8)	8.0(1.9)	9.1(2.3)	.24
Monocytes ($x10^3$/ul)						
Day 0	10	.81(.61)	.52(.42)	.76(.60)	.86(.47)	.50
Day 13	10	.51(.36)	.64(.49)	.58(.31)	.69(.51)	.87
Day 21	10	.49(.25)	.41(.21)	.59(.31)	.69(.51)	.53
Eosinophils ($x10^3$/ul)						
Day 0, rep A	5	.18(.16)	.41(.33)	.45(.28)	.22(.09)	.37
Day 0, rep B	5	.17(.15)	.05(.06)	.06(.04)	.17(.16)	.39
Day 13	10	.16(.11)	.22(.26)	.25(.28)	.27(.14)	.72
Day 21	10	.25(.17)	.18(.13)	.19(.22)	.18(.17)	.70
Basophils ($x10^3$/ul)						
Day 0, rep A	5	.04(.08)	.10(.13)	.10(.10)	.13(.21)	.80
Day 0, rep B	5	.03(.05)	0.0	.04(.05)	0.0	.41
Day 13	10	.04(.11)	.05(.11)	.09(.13)	.04(.07)	.62
Day 21	10	.01(.04)	0.0	.02(.04)	.07(.11)	.18

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days
[2]Standard deviation

Table 9. Influence of Tylosin and Fenbendazole on Erythrocyte Numbers, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration.

		Treatment[1]				
Item	n	CO	1TY	3TY	5TY	P
Erythrocytes (x10^6/ul)						
Day 0	10	6.6(.70)[2]	6.5(.40)	6.4(.48)	6.5(.45)	.93
Day 13	10	6.5(.37)	6.5(.39)	6.4(1.0)	6.7(.38)	.66
Day 21	10	6.6(.52)	6.8(.52)	6.7(.61)	6.6(.46)	.82
Hemoglobin (g/dl)						
Day 0	10	12(.94)	12(.75)	12(.95)	12(.93)	.93
Day 13	10	12(.65)	12(.76)	12(.78)	12(.46)	.15
Day 21	10	12(.89)	13(.94)	12(.86)	12(1.0)	.25
Mean Corpuscular Volume (fl)						
Day 0	10					.07
Day 13, rep A	5	60(2.3)	60(2.7)	61(2.0)	59(.88)	.09
Day 13, rep B	5	60(2.3)	30(2.7)	61(2.0)	59(.88)	.60
Day 21, rep A	5	53(3.4)	55(2.3)	55(1.7)	54(.96)	.36
Day 21, rep B	5	60(2.5)	62(2.9)	60(1.9)	59(1.2)	.54
Mean Corpuscular Hemoglobin (pg)						
Day 0	10	18.5(1.1)	18.6(.50)	18.8(.90)	18.1(.86)	.29
Day 13, rep A	5	17.3(.97)	18.0.31)	18.4(1.0)	17.6(1.1)	.14
Day 13, rep B	5	18.8(.75)	19.0(.64)	18.8(.69)	18.4(.78)	.60
Day 21, rep A	5	17.5(1.1)	18.2(.43)	18.4(.89)	18.1(1.1)	.32
Day 21, rep B	5	18.8(.96)	19.3(.61)	18.8(.92)	18.4(.32)	.47
Mean Corpuscular Hemoglobin Concentration (g/dl)						
Day 0, rep A	5	32(.77)	33(.35)	32(.66)	33(.47)	.30
Day 0, rep B	5	33(.26)	33(.62)	33(.57)	33(.54)	.55
Day 13	10	31(.54)	31(.70)	31(.65)	31(.67)	.40
Day 21, rep A	5	33(.29)	33(1.0)	33(.47)	33(.70)	.59
Day 21, rep B	5	31(.57)	31(.65)	31(.70)	31(.52)	.86

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days

[2]Standard deviation

Table 10. Influence of Tylosin and Fenbendazole on Platelet Numbers and Activated Partial Thromboplastin Time

Item	n	Treatment[1] CO	1TY	3TY	5TY	P
Platelets ($x10^3$/ul)						
Day 0	10	478(117)[2]	455(198)	513(131)	517(128)	.80
Day 13	10	451(95)	479(162)	500(140)	555(98)	.40
Day 21, rep A	5	442(65)	451(149)	368(95)	450(98)	.69
Day 21, rep B	5	260(132)	351(71)	309(50)	291(113)	.32
Activated Partial Thromboplastin Time (seconds)						
Day 0	10	14.1(1.5)	15.0(1.0)	14.1(1.1)	14.1(5.1)	.87
Day 13	10	14.9(.61)	14.1(1.5)	14.3(1.3)	14.2(1.2)	.56
Day 21, rep A	5	15.3(.52)	15.3(1.2)	14.7(1.2)	14.5(.60)	
Day 21, rep B	5	13.3(1.0)	13.0(.39)	13.3(1.1)	12.2(.81)	.19

[1]CO = control
1TY = fenbendazole 3 mg/kg/day, days 10-12; tylosin 100 g/T, 21 days
3TY = fenbendazole 9 mg/kg/day, days 6-14; tylosin 300 g/T, 21 days
5TY = fenbendazole 15 mg/kg/day, days 6-14; tylosin 500 g/T, 21 days
[2]Standard deviation

Table 11. Influence of Lincomycin and Fenbendazole on Serum Levels of Total Bilirubin, Sorbitol Dehydrogenase, Alkaline Phosphatase and Aspartase Aminotransferase

		Treatment[1]				
Item	n	CO	1LI	3LI	5LI	P
Total Bilirubin (mg/dl)						
Day 0	10	.12(.1)[2]	.22(.2)	.22(.1)	.18(.1)	.28
Day 13	10	.11(.1)	.14(.1)	.17(.1)	.15(.1)	.50
Day 21	10	.13(.1)	.15(.1)	.17(.1)	.11(.1)	.43
Alkaline Phosphatase (IU/l)						
Day 0	10	210(38)	229(36)	219(61)	233(48)	.62
Day 13	10	178(37)	213(46)	199(32)	205(46)	.28
Day 21	10	159(29)[a3]	216(40)[b]	195(42)[b]	199(23)[b]	.02
Sorbitol Dehydrogenase (IU/l)						
Day 0	10	6(3)	5(2)	5(3)	9(9)	.29
Day 13	10	4(4)	2(1)	27(44)	31(64)	.29
Day 21	10	4(2)	3(1)	5(3)	4(2)	.72
Aspartate Aminotransferase (IU/l)						
Day 0	10	33(12)	32(7.4)	30(9.0)	32(5.1)	.91
Day 13	10	29(3.4)	26(5.4)	118(131)	142(108)	.10
Day 21	10	29(6.1)	26(6.3)	26(3.6)	25(5.0)	.52

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 12. Influence of Lincomycin and Fenbendazole on Serum Levels on Glucose, Amylase, Blood Urea Nitrogen and Creatinine

		Treatment[1]				
Item	n	CO	1LI	3LI	5LI	P
Glucose (mg/dl)						
Day 0	10	109(9.2)[2]	112(11)	109(14)	109(8.8)	.92
Day 13, rep A	5	110(10)[a3]	128(16)[b]	135(7.0)[b]	124(13)[ab]	.04
Day 13, repB	5	110(8.3)	109(14)	111(15)	124(13)	.34
Day 21	10	109(8.3)	116(6.4)	110(9.2)	109(8.1)	.23
Amylase (IU/l)						
Day 0	10	2400(485)[a]	1728(337)[b]	1910(443)[b]	2115(498)[ab]	.02
Day 13	10	2358(459)	1824(694)	1916(381)	2018(525)	.21
Day 21	10	2517(471)	1981(512)	2132(439)	2222(608)	.18
Blood Urea Nitrogen (mg/l)						
Day 0	10	13(4)	13(2)	14(4)	12(2)	.58
Day 13	10	12(3.7)[a]	14(3)[ab]	16(3)[b]	17(5)[b]	.02
Day 21	10	13(2)	14(3)	14(3)	14(6)	.79
Creatinine (mg/dl)						
Day 0	10	1.1(.2)	.9(.2)	1.0(.2)	1.0(.1)	.09
Day 13	10	1.2(.3)	1.1(.1)	1.2(.1)	1.2(.1)	.94
Day 21	10	1.2(.1)	1.1(.1)	1.2(.2)	1.1(.1)	.30

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 13. Influence of Lincomycin and Fenbendazole on Serum Levels of Total Protein, Albumin and Globulin

		Treatment[1]				
Item	n	CO	1LI	3LI	5LI	P
Total Protein (gm/dl)						
Day 0	10	5.6(.38)[2]	5.7(.22)	5.8(.32)	5.9(.33)	.28
Day 13	10	6.5(.46)	6.2(.40)	6.2(1.1)	6.8(.40)	.19
Day 21	10	6.5(.55)	6.5(.59)	6.6(.53)	6.2(.37)	.49
Albumin (gm/dl)						
Day 0, rep A	5	3.2(.20)[a3]	3.5(.14)[b]	3.8(.17)[c]	3.6(.26)[bc]	.003
Day 0, rep B	5	2.9(.16)	3.3(.17)	3.1(.28)	3.3(.47)	.37
Day 13, rep A	5	3.7(.50)[a]	4.0(.45)[a]	4.3(.14)[b]	4.7(.23)[b]	.001
Day 13, rep B	5	3.5(.18)	3.6(.10)	3.8(.23)	4.0(.39)	.07
Day 21	10	3.6(.24)	3.8(.21)	4.0(.34)	3.9(.31)	.08
Globulin (gm/dl)						
Day 0	10	2.5(.41)	2.3(.27)	2.4(.36)	2.5(.40)	.69
Day 13	10	2.8(.46)	2.5(.40)	2.6(.19)	2.4(.44)	.08
Day 21	10	2.9(.46)	2.7(.49)	2.6(.69)	2.4(.39)	.26

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 14. Influence of Lincomycin and Fenbendazole on Serum Levels of Calcium, Phosphorus and Magnesium

Item	n	Treatment[1] CO	1LI	3LI	5LI	P
Calcium (mg/dl)						
Day 0	10	9.9(.45)[2]	10.1(.55)	10.1(.71)	10.5(.68)	.12
Day 13	10	10.3(.73)[a3]	10.3(1.0)[a]	10.8(.89)[ab]	11.0(.87)[b]	.04
Day 21	10	10.5(.59)	10.7(.66)	10.4(.65)	10.7(.75)	.51
Phosphorus (mg/dl)						
Day 0	10	9.7(.80)	9.4(.88)	9.3(.94)	9.6(1.2)	.71
Day 13	10	10.4(1.0)	9.9(.90)	10.3(.99)	10.4(1.4)	.64
Day 21	10	10.0(.80)	10.2(.43)	10.3(.90)	10.6(.80)	.45
Magnesium (mg/dl)						
Day 0	10	2.1(.14)	2.2(.21)	2.1(.10)	2.2(.22)	.80
Day 13, rep A	5	2.6(.40)[a]	2.7(.26)[a]	3.1(.18)[b]	3.1(.33)[b]	.003
Day 13, rep B	5	2.1(.11)	2.0(.16)	2.3(.18)	2.3(.33)	.24
Day 21	10	2.1(.25)	2.2(.27)	2.3(.31)	2.2(.40)	.36

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 15. Influence of Lincomycin and Fenbendazole on Serum Levels of Sodium, Potassium, Chloride and Total Carbon Dioxide

		Treatment[1]				
Item	n	CO	1LI	3LI	5LI	P
Sodium (mEq/l)						
Day 0	10	146(2.2)[a23]	144(2.0)[bc]	144(1.1)[b]	146(2.5)[ac]	.02
Day 13	10	143(2.3)	144(3.0)	143(2.8)	144(2.4)	.64
Day 21	10	143(2.8)	145(1.9)	143(1.6)	143(2.1)	.20
Potassium (mEq/l)						
Day 0	10	6.0(.69)	9.1(11.3)	5.4(.65)	6.2(.71)	.53
Day 13	10	5.5(.54)	5.8(.39)	5.8(.40)	5.9(.61)	.35
Day 21	10	5.6(.82)	5.9(.59)	5.5(.67)	5.7(.59)	.57
Chloride (mEq/l)						
Day 0	10	108(1.8)	107(2.3)	106(2.1)	107(2.1)	.28
Day 13	10	107(2.0)	106(2.3)	106(2.7)	104(2.2)	.15
Day 21	10	105(1.8)	106(1.5)	105(1.6)	105(1.5)	.14
Total Carbon Dioxide (mEq/l)						
Day 0	10	30(1.7)	31(2.0)	30(2.5)	29(3.4)	.43
Day 13	10	31(2.3)	32(2.9)	31(2.6)	33(3.2)	.31
Day 21	10	32(1.7)	31(1.2)	31(1.5)	33(2.8)	.48

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 16. Influence of Lincomycin and Fenbendazole on Total White Blood Cell, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil Numbers.

Item	n	Treatment[1] CO	1LI	3LI	5LI	P
Total White Blood Cells ($x10^3$)						
Day 0	10	19.5(2.6)[2]	20.6(4.4)	18.2(4.4)	22.0(5.7)	.24
Day 13, rep A	5	22.4(5.0)	24.4(3.5)	19.4(4.2)	15.7(4.2)	.08
Day 13, rep B	5	13.9(3.1)	16.4(2.8)	15.4(2.3)	17.0(3.2)	.52
Day 21, rep A	5	23.2(4.1)	24.7(1.1)	20.4(3.5)	21.8(4.5)	.33
Day 21, rep B	5	16.1(.87)[ab3]	17.4(2.3)[a]	13.9(1.5)[b]	15.1(1.2)[b]	.03
Neutrophils ($x10^3$)						
Day 0	10	6.5(2.3)	8.1(3.0)	6.4(2.1)	8.5(3.5)	.33
Day 13	10	6.7(4.1)	7.1(3.1)	5.9(1.6)	5.5(2.7)	.72
Day 21, rep A	5	11.0(3.3)	6.7(2.2)	6.7(1.8)	6.6(3.0)	.11
Day 21, rep B	5	5.0(2.3)	5.0(1.2)	3.9(1.4)	4.1(1.5)	.70
Lymphocytes ($x10^3$)						
Day 0	10	10.5(2.5)	10.5(2.3)	10.0(4.2)	11.2(3.5)	.85
Day 13, rep A	5	10.7(2.5)	13.7(2.5)	10.3(2.8)	9.2(2.3)	.12
Day 13, rep B	5	8.1(1.7)	8.2(1.5)	8.2(1.4)	9.1(.64)	.72
Day 21	10	9.4(2.8)	12.7(3.3)	9.8(2.5)	11.0(3.4)	.09
Monocytes ($x10^3$)						
Day 0	10	2.0(.76)	1.7(.90)	1.5(.67)	1.9(.72)	.64
Day 13, rep A	5	1.9(.65)	2.2(.91)	2.3(.98)	1.6(.27)	.56
Day 13, rep B	5	1.4(.60)	1.1(.52)	1.1(.53)	1.4(.40)	.84
Day 21	10	1.5(.57)	1.5(.40)	1.4(.56)	1.5(.42)	
Eosinophils ($x10^3$)						
Day 0	10	.34(.16)	.25(.32)	.21(.21)	.26(.36)	.78
Day 13	10	.30(.27)[a]	.57(.30)[b]	.34(.28)[ab]	.20(.13)[a]	.04
Day 21	10	.44(.27)	.80(.48)	.57(.40)	.48(.34)	.25
Basophils ($x10^3$)						
Day 0, rep A	5	.16(.31)	.21(.20)	.03(.06)	.22(.20)	.55
Day 0, rep B	5	.04(.07)	0.0	.03(.07)	.06(.12)	.74
Day 13	10	.14(.10)	.08(.12)	.15(.16)	.07(.09)	.47
Day 21	10	.17(.20)	.17(.21)	.04(.07)	.16(.14)	.33

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 17. Influence of Lincomycin and Fenbendazole on Erythrocyte Number, Hemoblobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration

		Treatment[1]				
Item	n	CO	1LI	3LI	5LI	P
Erythrocytes (x10^6/ul)						
Day 0, rep A	5	5.9(.33)[2]	6.0(.18)	6.3(.34)	5.9(.38)	.39
Day 0, rep B	5	6.6(.17)	6.8(.36)	6.6(.31)	6.9(.64)	.54
Day 13	10	6.6(.41)[a3]	6.9(.16)[ab]	7.2(.62)[b]	7.3(.52)[b]	.01
Day 21, rep A	5	7.0(.54)	7.5(.50)	7.8(.56)	7.3(.39)	.29
Day 21, rep B	5	6.4(.25)[a]	7.1(.21)[b]	6.7(.26)[c]	6.7(.44)[c]	.001
Hemoglobin (g/dl)						
Day 0, rep A	5	11.1(.2)[a]	11.4(.26)[ab]	11.9(.28)[b]	11.6(.35)[b]	.02
Day 0, rep B	5	11.8(.8)	11.9(.39)	11.7(.12)	12.4(.80)	.44
Day 13, rep A	5	11.9(.38)[a]	12.5(.35)[ab]	13.4(.55)[c]	13.1(.67)[bc]	.01
Day 13, rep B	5	11.3(.85)	12.0(.65)	11.9(.54)	12.8(.61)	.09
Day 21, rep A	5	12.4(.67)	13.5(.83)	13.8(.46)	13.3(.88)	.12
Day 21, rep B	5	11.3(..68)	12.4(.49)	11.9(.34)	12.0(.76)	.06
Mean Corpuscular Volume (fl)						
Day 0, rep A	5	59(1.7)	59(.72)	59(2.7)	61(3.6)	.53
Day 0, rep B	5	55(2.5)	53(2.9)	54(2.6)	55(3.7)	.90
Day 13, rep A	5	57(1.2)	58(1.3)	57(1.9)	58(3.0)	.85
Day 13, rep B	5	54(1.6)	44(20)	54(1.8)	54(3.9)	.38
Day 21	10	56(2.3)	56(3.1)	56(1.6)	53(7.6)	.32
Mean Corpuscular Hemoglobin (pg)						
Day 0, rep A	5	19.0(.85)	19.2(.35)	19.0(.81)	19.5(.86)	.72
Day 0, rep B	5	17.9(.79)	17.6(.80)	17.7(.72)	17.9(1.2)	.91
Day 13	10	17.6(.65)	17.7(.64)	17.7(.75)	17.7(1.0)	.98
Day 21	10	17.6(.60)	17.8(.67)	17.8(.70)	18.0(1.0)	.90
Mean Corpuscular Hemoglobin Concentration (g/dl)						
Day 0	10	32(.79)	33(.92)	33(.50)	32(.78)	.26
Day 13	10	32(.98)	32(.93)		32(.81)	.41
Day 21, rep A	5	31(.64)[ab]	31(.41)[a]	32(.65)[bc]	32(.30)[c]	.004
Day 21, rep B	5	33(.29)	33(.81)	32(.25)	33(.43)	.89

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Table 18. Influence of Lincomycin and Fenbendazole on Platelet Numbers and Activated Partial Thromboplastin Time

Item	n	Treatment[1] CO	1LI	3LI	5LI	P
Platelets ($x10^3$/ul)						
Day 0, rep A	5	535(83)[2]	483(93)	427(70)	469(91)	.40
Day 0, rep B	5	522(144)	617(69)	585(98)	477(58)	.26
Day 13	10	521(68)	515(90)	418(150)	471(97)	.14
Day 21	10	523(124)	463(88)	446(105)	414(57)	.18
Activated Partial Thromboplastin TIme (seconds)						
Day 0	10	14.4(2.1)	14.9(1.2)	14.5(1.7)	13.5(4.4)	.73
Day 13	10	14.6(1.0)	15.2(3.6)	13.5(.83)	13.1(4.1)	.49
Day 21	10	14.4(.68)	13.4(1.0)	13.0(4.1)	13.7(1.4)	.62

[1]CO = control
1LI = fenbendazole 3 mg/kg/day, days 10-12; lincomycin 200 g/T, 21 days
3LI = fenbendazole 9 mg/kg/day, days 6-14; lincomycin 600 g/T, 21 days
5LI = fenbendazole 15 mg/kg/day, days 6-14; lincomcyin 1000 g/T, 21 days

[2]Standard deviation

[3] Values with different superscripts within rows are different $p<.05$.

Chapter 5.

Study to Determine the Tissue Residue Patterns of Fenbendazole, Tylosin and Lincomycin in the Liver and Kidney of the Pig.

A. Introduction

Prevention of the occurrence of tissue residues of drugs used to treat animals is an important part of food production. In general, drug residues are unacceptable and studies to determine the residue patterns are carried out for all drugs used in food animals. Based on this pattern a decision is made regarding the time needed between cessation of treatment and slaughter to ensure that unacceptable drug residues are not present in the tissues.

Residue patterns have already been established for the drugs used in this study when administered singly. Fenbendazole given to pigs in a single oral dose of 5 mg/kg was found in the liver at .67 μ/g 2 days after administration and at 14 days at .11 μ/g. In the kidney, the level obtained was .53 μ/g at 2 days after dosing, by five days FBZ was not detected (detection limit .05 μ/g) (Düwel, 1977).

Tylosin residues, following intramuscular injection of 8.8 mg/kg, were not detected at 24 hours in the liver or kidney (Moats et al., 1985).

Lincomycin was given in 10 oral doses of unspecified amounts to pigs; at 4 hours after treatment, liver and kidney residues, measured by C^{14} radiolabelling, were 14 ppm and 10.1 ppm respectively. At 48 hours after treatment, liver and kidney

residues had declined to 3.7 ppm and 2.5 ppm respectively. By 168 hours after treatment, liver and kidney residues had declined further to .53 ppm and .16 ppm respectively (Hornish et al., 1987).

Drug residue patterns are a function of pharmacokinetics. The potential for changes in the pharmacokinetics of drugs when administered concurrently requires that residue patterns be established for the drugs when they are administered at the same time.

The objective of this experiment was to determine the residue patterns of fenbendazole, tylosin and lincomycin in the liver and kidney when fenbendazole was fed to growing pigs with either tylosin or lincomycin.

B. Materials and Methods

Two trials were conducted separately; Trial 1 - FBZ + TY, Trial 2 - FBZ + LI.

Trial 1

1. Animals, Trial 1

In Trial 1, 36 male and female Yorkshire, Duroc, Hampshire purebred or crossbred pigs were selected from the Michigan State University Swine Research Farm. Pigs were weighed and eartagged upon acquisition. Animals were kept in pens with slatted concrete floors, provided with automatic feeders and nipple waterers.

2. Treatment, Trial 1

Pigs were randomly assigned to the following treatment groups, which were stratified by weight, sex, litter and breed.

Group No.	Avg. Wt.	Treatment
20	4	control
25	16	FBZ 3 mg/kg BW/day, days 19-21 TY 100 g/T of feed for 21 days
26	16	FBZ .75 mg/kg BW/day, days 10-21 TY 100 g/T of feed for 21 days

BW=body weight
FBZ = fenbendazole
TY = tylosin

Groups 25 and 26 consisted of 2 pens, 8 animals per pen.

Tylosin premix (Tylan 40®, Eli Lilly Co., South Bend IN) was added to a base corn-soybean type diet balanced for growing pigs to a concentration of 100 g/T. Fenbendazole (Safe-Guard®, Hoechst-Roussel Agri-Vet Co., Somerville, NJ) premix was added to a separate portion of TY medicated diet, at a rate to provide the daily dose of FBZ in approximately 1 pound of feed. Actual amounts fed were calculated on a mg FBZ/kg of body weight based on actual weights of all pigs in the pen. Prior to feeding FBZ-TY-medicated feed, pigs were fasted for 2-4 hours. FBZ-TY-medicated feed was placed in a wooden trough in the pen. Pigs were observed to insure all animals ate the FBZ-TY-medicated feed. The treatment period lasted for

21 days. Pigs were placed on antibiotic/anthelmintic free feed at 8 am at the end of the treatment period.

3. Sample collection and testing, Trial 1

Animals were randomly selected from each pen and euthanized via electrocution and exsanguination. The schedule for euthanasia and tissue collection was as follows:

Time	# animals/treatment
12 hours after treatment	4
24 hours after treatment	4
3 days after treatment	4
6 days after treatment	4

One animal from the control pen was euthanized at each time period.

The liver and kidney from each animal was collected, placed in a plastic bag and frozen within 3 hours of euthanasia. Samples were sent to commercial laboratories for analyses. Tylosin levels in tissue were detected using an agar plate system using *Sarcina lutea* as the assay organism. Tylosin levels were estimated by comparing the zones of inhibition (average zone diameters) from experimental samples to those from standard recoveries. Fenbendazole levels were determined using a high pressure liquid chromatography system utilizing UV detection, Waters μ-Bondapack $C18_R$ column and 65/35 methanol/glacial acetic acid-water (99:1) mobile phase.

Liver samples from the 12 and 24 hour time periods were analyzed for tylosin residues. Fenbendazole residue analysis was performed on only liver samples from pigs euthanized 12 hours after treatment ceased.

Trial 2

4. Animals, Trial 2

Pigs were obtained from the same source as in Trial 1. All handling and allotment procedures were the same.

5. Treatment, Trial 2

The treatment groups were as follows:

Group No.	Avg. Wt.	Treatment
20	4	control
21	16	FBZ 3 mg/kg BW/day, days 19-21 LI 200 g/T of feed for 21 days
22	16	FBZ .75 mg/kg BW/day, days 10-21 LI 200 g/T of feed for 21 days
23	16	FBZ 3 mg/kg BW/day, days 19-21 LI 100 g/T of feed for 21 days
24	16	FBZ .75 mg/kg BW/day, days 10-21 LI 100 g/T of feed for 21 days

BW=body weight
LI = lincomycin (Linco®, The Upjohn Co., Kalamazoo MI)

Two pens per treatment group were used for groups 21-24, 8 pigs per pen. Feed mixing, treatment method and schedule were conducted in the same manner as trial 1.

6. Sample collection and testing, Trial 2

Tissues were collected and tested in the same manner as in trial 1. Samples were tested in commercial laboratories. Lincomycin levels were determined on liver and kidney 12 hour, 24 hour, 3 day and 6 day samples using a microbiological assay in a similar manner as tylosin, except the test agent was *Micrococcus luteus*. Fenbendazole levels were determined on 12 hour liver samples in the same manner as trial 1.

C. Results

1. Trial 1

Tylosin was not detected in any sample (detection limit .1 ppm). Table 19 lists the fenbendazole results.

2. Trial 2

The levels of fenbendazole found are presented in Table 20. Lincomycin results are presented in Tables 21 and 22. The detection limit for lincomycin was .1 ppm.

D. Discussion, Trial 1 and Trial 2

Tylosin was not detected in any of the samples tested. These data concur with Kline and Waitt (1971), Moats et al. (1985), and Kietzmann (1985).

Lincomycin levels in the liver at all time periods were much lower than those found by Hornish et al. (1987). At 48 hours, 3.7 ppm FBZ were identified in the

liver. In our study, at 24 hours, levels ranged from .00-.02, approximately >185 times less.

Fenbendazole levels in the liver were measured 12 hours after withdrawal from treated feed. Düwel (1977) reported values for 48 hours withdrawal time. For animals treated with LI and FBZ, the values we obtained were similar to those found by Düwel (1977) at 48 hours.

In the 3 mg/kg/day for 3 days FBZ + TY treatment group, one animal had a level of 7.43 ppm FBZ in the liver, accounting for the large variation in this group. Individual animal variation in drug pharmacokinetics may account for this higher level. Measurement of the 24 hour or 72 hour samples for FBZ may provide a more information on tissue depletion rates and overall residue pattern, however, this was not done.

E. Conclusions Trial 1 and 2

Tylosin residues in the liver and kidney were similar to levels found by other researchers (Moats et al., 1985). It does not appear that FBZ affects the residue pattern of TY. FBZ residues however, when administered concurrently with TY was highly variable in one treatment group and the levels found at 12 hours were 4 times higher than those found by Düwel (1977). Further analysis of tissues collected at other time points are needed to determine the overall pattern of FBZ residues in the liver when administered with TY.

Lincomycin residues were less than those reported by Hornish et al. (1987), however, the dose they used was not specified. Additionally, these researchers used a radiolabel method for detection of LI residues. This method may detect LI metabolites containing the C^{14} atom, whereas the method employed in this study detects microbiologically active LI and presumably not the metabolites. This may account for the difference between the two studies. FBZ in liver was similar to the levels found by Düwel (1977)(using a non-radiolabel, but otherwise unspecified method), although direct comparison is difficult due to the time periods evaluated. Analysis of FBZ residues in the 72 hour samples would more closely approximate Düwel's 48 hour sample.

It appears that residue patterns for FBZ, LI and TY are similar to those reported in the literature.

Table 19. Average Fenbendazole Residues Recovered from the Liver of Pigs, Trial 2

Treatment[1]	n	12[2]
CO	1	.00(.00)[3]
1	4	2.87(3.1)
2	4	.260(.164)

[1] CO= control
1 = Fenbendazole 3 mg/kg/day, days 10-12; Tylosin (TY) 100 g/T, days 1-12
2 = FBZ .75 mg/kg/day, days 1-12; TY 100 g/T, days 1-12

[2] Fenbendazole residues 12 hours after withdrawal from medicated feed, ppm.

[3] Standard deviation

Table 20. Average Fenbendazole Residues Recovered from the Liver of Pigs, Trial 1.

Treatment[1]	n	12[2]
CO	1	.00(.00)[3]
1	4	.116(.07)
2	4	.049(.03)
3	4	.992(.90)
4	4	.113(.04)

[1] CO= control
1 = Fenbendazole 3 mg/kg/day, days 10-12; Lincomycin (LI) 200 g/T, days 1-12
2 = FBZ .75 mg/kg/day, days 1-12; LI 200 g/T, days 1-12
3 = FBZ 3 mg/kg/day, days 10-12; LI 100 g/T, days 1-12
4 = FBZ .75 mg/kg/day, days 10-12; LI 100 g/Y, days 1-12

[2] Fenbendazole residues 12 hours after withdrawal from medicated feed, ppm.

[3] Standard deviation

Table 21. Average Linocmycin Residues Recovered from the Liver of Pigs, Trial 2.

Treatment[1]	n/time period	12[2]	24	72	144
CO	1	.00(.00)[3]	.00(.00)	.00(.00)	.00(.00)
1	4	.02(.04)	.00(.00)	.06(.07)	.00(.00)
2	4	.06(.04)	.02(.03)	.15(.20)	.00(.00)
3	4	.02(.03)	.00(.00)	.04(.06)	.00(.00)
4	4	.00(.00)	.00(.00)	.00(.00)	.00(.00)

[1] CO= control
1 = Fenbendazole 3 mg/kg/day, days 10-12; Lincomycin (LI) 200 g/T, days 1-12
2 = FBZ .75 mg/kg/day, days 1-12; LI 200 g/T, days 1-12
3 = FBZ 3 mg/kg/day, days 10-12; LI 100 g/T, days 1-12
4 = FBZ .75 mg/kg/day, days 10-12; LI 100 g/Y, days 1-12

[2] Hours after withdrawal from medicated feed, ppm.

[3] Standard deviation

Table 22. Average Lincomycin Residues Recovered from the Kidney of Pigs, Trial 2.

Treatment[1]	n/time period	12[2]	24	72	144
CO	1	.00(.00)[3]	.00(.00)	.00(.00)	.00(.00)
1	4	.15(.10)	.06(.04)	.11(.11)	.00(.00)
2	4	.09(.06)	.08(.01)	.27(.22)	.00(.00)
3	4	.10(.03)	.00(.00)	.11(.10)	.00(.00)
4	4	.08(.01)	.01(.02)	.09(.06)	.03(.05)

[1] CO= control
1 = Fenbendazole 3 mg/kg/day, days 10-12; Lincomycin (LI) 200 g/T, days 1-12
2 = FBZ .75 mg/kg/day, days 1-12; LI 200 g/T, days 1-12
3 = FBZ 3 mg/kg/day, days 10-12; LI 100 g/T, days 1-12
4 = FBZ .75 mg/kg/day, days 10-12; LI 100 g/Y, days 1-12

[2] Hours after withdrawal from medicated feed, ppm.

[3] Standard deviation

Chapter 6:

Overall Conclusions.

Humans have used drugs since before written history for the alleviation of pain and to treat disease. However, the study of drugs and their actions was not able to progress beyond empirical discoveries until the 19th century when techniques were developed for purifying and analyzing the compounds producing the desired response. At the same time discovery and isolation of pure compounds was occurring; the study of pharmacokinetics was developing with Francois Magendie and his pupils, who outlined the basic principles of absorption, distribution, biotransformation and excretion of drugs in the body. Their work led to further developments in drug action.

Drug interactions may occur when the concurrent administration of more than one drug results in responses not seen when the drugs are administered individually. These unexpected responses may be the result of changes in absorption, distribution, receptor binding, biotransformation and/or excretion. The mechanisms involved in drug interactions are frequently not known. To study drug interactions, a basic knowledge of the pharmacokinetics of the drugs when administered singly is needed, followed by controlled experiments to measure the responses seen with concurrent administration. *In vitro* and *in vivo* studies may be needed before the actual mechanisms for the response seen can be defined.

Studies to determine the efficacy of a drug when administered concurrently with another, studies to evaluate the safety of the drug combination and studies to determine the tissue drug residues (particularly in food animals) may be used to estimate drug interactions.

In the experiments outlined in this thesis, we evaluated the efficacy, safety and tissue residues of three commonly used drugs in swine production.

Fenbendazole (FBZ) is a benzimidazole anthelmintic, used over short periods of time to treat pigs with *Ascaris suum* and *Trichuris suis* (among others) infections.

Tylosin (TY) and lincomycin (LI) are antibiotics used over long periods of time to prevent disease caused by *Mycoplasma hyosynoviae*, *M. hyopneumoniae* and *Treponema hyodysenteriae*. These antibiotics are also used to promote feed efficiency and weight gain.

Fenbendazole, tylosin and lincomycin are commonly administered in the feed of pigs. Current regulations require that the drugs be used separately, necessitating removal of feed containing antibiotics during treatment with anthelmintics. In order to facilitate feed management, concurrent administration is desirable.

Based on the pharmacokinetic data available, we hypothesized that drug interactions would not occur with the combination of FBZ with TY or LI administered orally to pigs. To test this hypothesis, we conducted three trials.

In the first trial, the efficacy of fenbendazole when administered with tylosin or lincomycin was compared to the efficacy of fenbendazole alone. Pigs (120) were

artificially infected with *A. suum* and *T. suis* and then placed on medicated diets. Following treatment, total worm counts were performed. The efficacy of fenbendazole was not decreased by the concomitant administration of lincomycin or tylosin.

The second trial was conducted to evaluate the safety of fenbendazole and tylosin or lincomycin when fed at 1, 3 or 5 times the recommended dose. Animal appetites and attitudes, serum chemistry profiles, red blood cell indices, white blood cell indices and blood clotting time were measured to evaluate animal health. The combination of FBZ and TY or LI did not have any adverse affects on the health of pigs based on the parameters measured.

In the third trial, animals were fed recommended doses of FBZ and LI or TY. At 12, 24, 72 and 144 hours after removal from medicated feed, the animals were euthanized and kidney and liver samples obtained and analyzed for FBZ, TY and LI residues. Based on comparisons reported in the literature, the concurrent administration of FBZ and TY or LI did not appear to alter the residue patterns of the three drugs.

In conclusion, tylosin or lincomycin do not appear to interact with fenbendazole when administered in the feed to growing pigs as measured by fenbendazole efficacy, animal health and tissue residue patterns.

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