MICROFLORA MANIPULATION OF ARTIFICIALLY REARED PIGLETS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

Introduction

Establishing and maintaining a heathly swine herd is a major factor in modern pork production. In herds that have a serious problem with mycoplasma pneumonia and atrophic rhinitis, production efficiency is greatly reduced. Mycoplasma pneumonia of growing and finishing swine is common and is considered to be the most costly swine disease in the United States. Atrophic rhinitis is also a cause of reduced growth and poor feed conversion. Surveys conducted in a variety of countries indicate that lesions from chronic pneumonia occur in 30 to 80% of all slaughter hogs (Switzer and Ross, 1975). Loss caused by chronic pneumonia was estimated by Betts (1955) to depress growth rate by 16% and feed conversion by 22%. Young et al. (1959) found that pigs with atrophic rhinitis and pneumonia required about one month longer to reach market weight than pigs free from these conditions. Swine producers are aware of the possible losses due to mycoplasma pneumonia and atrophic rhinitis and many have asked if they should repopulate their herds or try to minimize losses by medicating with antimicrobial compounds. With today's production practices in high-investment confinement systems, these problems are of greater significance than a few decades ago.

The experiments reported herein were conducted to determine the effect of a method which is easy to employ, cost-efficient, and applicable to the present management practices of the swine industry. It is a practical alternative to the SPF (Specific Pathogen Free) program for breaking the cycle of respiratory problems and producing a minimal disease herd. The use of early-weaned, artificially-reared piglets combined with treatment of lincomycin and long-acting oxytetracycline was evaluated as a system of manipulation of the microflora to eliminate some major pathogens endemic in swine herds.

Literature Review

Incidence and Economic Effects of Mycoplasma Pneumonia

Chronic mycoplasma pneumonia (MP) has worldwide distribution. Pullar (1948) indicated occurence of MP in a survey in Australia that showed 68% of market hogs were infected with the pneumonia whereas only 32% of the adult animals exhibited similar lesions. Betts (1952) of England, examined two separate groups of one thousand pigs. Mycoplasma pneumonia was found in 42 and 61 % of each respective group. He believed that MP was the most common disease in England. MacPherson and Shanks (1955) of Scotland reported that of 1000 market hogs examined 55.4% showed lesions typical of MP and 40% had extensive MP lesions. Occurences of MP in Canada were reported by Carter and Shroeder (1956). Rees (1964) of New Zealand observed pneumonia in 85% of slaughter weight pigs with 80% of the lungs diagnosed as typical cases of MP. Kono et al. (1967) in Japan reported the presence of pneumonia lesions from four different herds. Seventy-six diseased pigs were examined and 84% showed lesions of MP with varying degrees of lung involvement.

Pneumonia in swine caused more loss in Sasketchewan than did all other disease as shown by Fulton et al. (1953). In the United States, MP has been estimated by Beveridge (1953) to be present in 50% of the swine in Ohio, by Young and Underdahl (1955) between 50 and 70 % in midwestern swine, and by Betts (1956) as high as 74% of a day's slaughter at Rochester, New York. Using both gross and histopathological methods for detection, Young and Underdahl (1960) found MP in eastern Nebraska and western Iowa was from 34 to 43% among 3,500 market hogs. Switzer (1967) stated the incidence of MP in Iowa to be between 35

and 60 percent.

Lam (1971) tested 433 swine serum samples from various field herds for antibodies against MP and found that 291 samples were serologically positive (67.2%). Slaughter data were available on 169 pigs and 87 (51.5%) pigs had lungs that were pneumonic versus 82 with normal lungs. Antibodies were detected in 78.1% of the pigs with microscopic lung lesions and in 69% without lesions. Huhn (1970) reported that 62% of market weight swine had gross and microscopic lesions of MP. The herd of origin significantly affected the incidence and severity of MP lesions.

Mycoplasma pneumonia caused an estimated 5% reduction in rate of gain (Betts, 1952). In a further study, Betts et al. (1955) estimated that the loss caused by MP in swine depressed growth rate by 16% and feed conversion by 22%. Betts and Beveridge (1953) determined the effect of MP on rate of gain and feed efficiency in both summer and winter months. From these trials they found that the reduction in weight gain and feed efficiency for pigs with MP was approximately 25%.

Young et al. (1959) observed that pigs with atrophic rhinitis and MP required about one month longer to reach market weight than pigs from a herd free of both diseases. The incidence and severity of atrophic rhinitis seemed to have little relationship to the growth rate of affected pigs, while the rate of gain was closely correlated with the incidence and extent of MP lesions. Pearce and Row (1967) reported that of these two diseases, only MP has adverse economic effects.

Goodwin (1963) estimated the cost of MP per affected pig in England at about 34 shillings. Goodwin (1966) reported that eradication of MP should result in a 20% improvement in feed efficiency.

It has been found in some studies that MP exerts little economic effect. Eikmeier and Mayer (1965) found that a 40% incidence of MP had no economic

effect on either rate of weight gain or feed efficiency, possibly because of good management. However, economic loss becomes increasingly more apparent as severity or extent of the lesion increases as shown by Englert and Eisenack (1964).

Huhn (1970) reported the determination of the economic effect of MP is clouded somewhat by the different experimental conditions used by various investigators. There is a general concensus that management is important in stemming the detrimental effects of MP. He also reported the rate of weight gain was decreased in proportion to severity of MP lesions. The effect of environment combined with the decreased gain accountable to severity of MP indicated that the economic effect of MP may be abated by good management procedures.

Braude and Plonka (1975) compared rate of weight gain and feed efficiency in a herd free of MP for three years and for a subsequent three year period following introduction of the disease. The incidence of MP following the outbreak ranged from 20 to 47 percent. They associated the loss of decreased feed efficiency at .75 pound of extra feed for the herd infected with MP.

Diagnosis and Evaluation of Mycoplasma Pneumonia

Betts (1952) described MP as a chronic disease with a high morbidity and low mortality. The gross lesions were characterized as well demarked plum colored to grayish pneumonia areas in the apical and cardiac lobes. Other lobes may be affected but are usually less frequently involved. Another typical lesion is enlargement of lymph nodes involved in drainage of the lungs (Pullar, 1948).

Pattison (1956) found consistent histopathological changes in lungs from pigs infected experimentally with MP. The gross lesions on the lungs typical of MP were that described by Betts (1952). The lungs of nine experimentally infected pigs were removed at intervals from 24 h to 33 d post-inoculation. The first change

from normal lung tissue was an increase in cellularity of the interaveolar tissue with some edema and a few large mononuclear cells free in the aveolar spaces. Later pigs showed extensive lymphoid hyperplasia related to the bronchi and bronchioles. The most severe lesions had obliteration of large areas of aveolar tissue by dense accumulations of lymphocytes associated with the bronchi and bronchioles. Macroscopic evidence of MP was confined to the animals killed on or after the eleventh day after inoculation.

Developmental microscopic changes in the lungs of pigs inoculated with MP have also been described by Livingston (1972). Descriptions are in agreement with Pattison (1956) on morphological changes but sequence and severity differ. Initial evidence of peribronchiolar lymphoid hyperplasia was observed at 6 d post-inoculation. More advanced lesions were observed at 11 d post-inoculation and gross evidence at 14 d.

Mare' and Switzer (1966) reported the microscopic examinations of hemotoxylin and eosin-stained sections from experimentally produced lesions of MP indicated peribronchiolar and perivascular lymphoid hyperplasia. The peribronchiolar lesions were consistently more predominant than the perivascular ones and the blood vessels associated with the lymphoid hyperplasia were usually those in close proximity to the bronchioles.

MacKenzie (1963) showed that in the presence of swine lungworm infection, MP lesions were greatly increased. Underdahl and Kelley (1957) observed a ten-fold increase in the size of lesions in pigs that were infected simultaneously with MP and ascarid larvae as with those pigs infected with MP alone.

Mycoplasma pneumonia must be differentiated from other pneumonias of swine, especially lungworm infestation and swine influenza. Classical swine influenza is an acute disease with an incubation period of 2-4 d, followed by elevated temperature and extreme prostation (Shope, 1931). By contrast, MP has an

incubation period of 10-16 days and causes little or no elevation of temperature with few signs of illness. Urman (1958) differentiated the lungs of pigs infected with swine influenza virus histopathologically from those of MP. The chronic, dry, nonproductive cough is characteristic of both MP and lungworm pneumonia. MacKenzie (1959) has distinguished between the histopathological lesions of MP and those caused by lungworms.

Duncan (1966) described the pathology of experimentally induced Bordetella bronchiseptica pneumonia in pigs. He reported that Bordetella bronchiseptica pneumonia has considerably different tissue changes from those associated with MP. Grave et al. (1963) used clinical history, gross lesions, and microscopic lesions to diagnose MP. He felt it was feasible to accurately diagnose MP on a herd basis with this technique.

Serological methods for diagnosis have been developed in the last 15 years. Indirect hemagglutination (IHA) was used by Goodwin et al. (1969) as a method to diagnose MP. Although it has been shown to be a specific and sensitive procedure, the test has not been employed to a great extent because of it's lengthy procedure. Goodwin et al. (1969) also used complement fixation (CF) for MP diagnosis. Pigs inoculated with MP developed detectable CF antibodies by 1-4 weeks after exposure. Titers were still found at 30 weeks post-inoculation, but by 60 weeks the titers were reduced. L'Ecuyer and Boulander (1970) utilized immunofluorescent staining as a diagnostic tool. Best results were found when large amounts of MP cells were present or at least 25 d after exposure to MP. Isolating the causitive organism by microbiological assays has been enhanced by Friis (1975). One of the major drawbacks of this technique is that 2-6 weeks are required for isolation and identification because of the difficulty in culturing mycoplasma.

Underdahl et al. (1980) utilized a culture method, scanning electron microscope, and light microscopy for evaluation of lung lesions. Light microscopy

and scanning electon microscopy verified gross lesion analysis. They found that culturing for MP did not always coincide with visual analysis of lesions.

Control and Eradication of Mycoplasma Pneumonia with Nonchemotherapeutic Means

Isolation and controlled rearing of breeding stock have been used successfully to obtain pneumonia-free herds of swine. Pullar (1948) found that the occurence of MP was much less in sows than in young pigs. MacPherson and Shanks (1955) pointed out the advantage in using old sows because of the decreased incidence of pneumonia in older animals as a source of new stock to be reared in isolation. Barber et al. (1955), Betts et al. (1955), and Whittlestone and Betts (1955) all reported the possibility of eradicating pneumonia through an isolation-controlled rearing scheme. Their method of eradication consisted of the following stages: 1. Farrowing sows in isolation to insure that any infection of the litter came only from the dam; 2. Determination of whether the litter was infected or not as judged by clinical examination supplemented by the necropsy of one or more pigs per litter, if necessary and possible; 3. Grouping of litters judged to be free from the disease but retaining the groups in isolation; 4. Examination at slaughter of lungs from a considerable proportion of the group as a further check when they reached market weight; 5. Replacement of the original breeding stock with the healthy progeny as soon as possible. Goodwin and Whittlestone (1960) also used this method for controlling pneumonia.

O'Brien (1961) discussed the concept of selection of pneumonia-free stock and the problems that arise with isolation procedures. He summarized that utilizing this method was more sound than continuing to live with MP.

Goodwin and Whittlestone (1967) proposed a more stringent program for health

control of herds. The program included certification of breeding herds subjected to veterinary inspection, rigid isolation, and periodic examination of lungs from slaughter pigs reared under close confinement. A registery was maintained to list herds free of MP.

The use of the complement fixation (CF) test combined with farrowing of older sows was a method designed by Preston (1976) as a means of deriving pigs free of MP. This method utilized culling of CF positive sows which initially reduced clinical signs of MP. After one year of testing nine out of ten herds that had been established with CF negative sows had CF positive females.

Obtaining piglets by surgical techniques has been an effective means to derive animals free from disease. Specific Pathogen Free (SPF) piglets were removed from their dam 2-4 d prematurely by hysterectomy (Young et.al., 1955) or by caesarotomy (Whitehair and Thompson, 1956); reared on colostrum free diets, and used to repopulate vacated, cleaned swine facilities. Young (1964) reported on the basic principles and use of SPF swine for repopulation. He found successful use of swine repopulation with SPF swine in Nebraska. Pneumonia was one of the diseases controlled by this method. SPF boars have also been successfully introduced into conventional swine herds. The SPF program has found wide application in the control of MP as reviewed by Twiehaus and Underdahl (1975).

Although very expensive initially, repopulation with SPF stock is particularly effective for controlling MP. The most difficult problem for the SPF producer is maintaining a minimally diseased herd since chances are quite high an infectious organism will eventually gain access to the herd. The greatest risk involves the introduction of new blood lines into the herd. Clinical and slaughter inspections have been used to monitor the disease-free status of the herd accredited. Goodwin (1965) felt the costs and time associated with the method of SPF repopulation were inhibitive when the chance for disease outbreaks were high and introduction of SPF

pigs to a normal herd would mean greater susceptibility to normal swine microorganisms.

Various other techniques have been used to derive piglets with minimal contact to the dam at birth. Done (1955) utilized a sterile basin to catch newborns during natural birth. The piglets were then raised artificially encountering the same rearing problems of colostrum deprived motherless pigs. Shuman et al. (1956) used a similar method as Done (1955) except sterile canvas towels were used to catch the piglets at natural birth. Pond et al. (1967) condoned the isolation of young pigs after birth to break the cycle of sow to piglet transmission of MP.

Chemotherapeutic Control of Mycoplasma Pneumonia

Various approaches have been used in attempts to clear single infections from herds by use of antibiotics such as Atrophic rhinitis (Farrington and Switzer, 1977), swine dysentery (Tasker, 1981), and MP (Schuller and Glawischnig, 1972).

Tylosin treatments were utilized by Schuller and Glawischnig (1972) with an early weaning scheme. Piglets were medicated twice daily with 20 mg tylosin per kg body weight starting immediately after birth until 8 d after early weaning at 4 d of age. Alexander (1980) has developed a method similar to this called medicated early weaning. In this program sows are given a combined parenteral-oral regimen using tiamulin and trimethoprim prior to farrowing and continuing until piglets are weaned at 5 d of age. Piglets are given the same antimicrobials from birth until 10 d of age. Pigs derived by this method were free of MP, and revealed no presence of MP or Bordetella bronchiseptica when examined from 5 weeks of age to market weight.

Chemotherapy with Lincomycin. The antibiotic, lincomycin, methyl 6,8 dideoxy-6-(1-methyl-4-galacto-octopyranoside) · HCl · H_2O, is produced by Streptomyces

lincolnensis. It was discovered by Mason et al. (1963) and characterized by Herr and Bergey (1963). Initial in vitro and in vivo studies (Lewis et al., 1963) demonstrated activity against gram positive organisms.

Lincomycin has been used either through feed or administered parenterally to minimize or prevent the effect of MP. Wilson et al. (1970) used lincomycin at 11 mg per kg of body weight parenterally for 2-5 d in ten 45-136 kg pigs with MP. There was clinical improvement in 24-48 h and the pigs were normal in 3-5 d. Rapid improvement was also noted in a litter of 16-kg pigs which were treated for 2 days. Kjar (1977) found that lincomycin given in the feed at 200 mg per kg of feed for 3 weeks in 5-6 week old pigs led to a 46% increase in the growth rate and an improvement in feed conversion of 27%. The number of pigs with gross lesions of MP was the same for both treated and control animals, although there was a 22% reduction in the average number of lobes with gross lesions in those pigs fed lincomycin. The physical condition of the lincomycin treated pigs was better than the controls and fewer watery stools were observed in the treated group.

Yonkers et al. (1979) treated pigs from a herd diagnosed to contain MP with lincomycin at 11 mg per kg of body weight for 3 consecutive days at birth and weaning. Treatment with lincomycin improved rate of gain in pigs during their first eight weeks of life and tended to have improved feed efficiency.

Neri et al. (1980) administered lincomycin at 11 mg per kg of body weight for three consecutive days at birth and weaning. The mean weight gain and feed efficiency from birth to eleven weeks of age were not different among treatments. A coughing score was evaluated and treatment with lincomycin did not change the score.

DeGeeter (1979) fed three levels of lincomycin (110, 220 and 330 mg/kg of feed) to swine with clinical signs of MP for three weeks. Lincomycin fed at the 220 mg level reduced the incidence and severity of gross lesions associated with

MP. The suppression of the disease process was also reflected in a lower incidence or absence of acute lesions, 5% or 0% in the lungs of pigs fed diets fortified with 220 or 330 mg per kg, respectively, versus 49% in controls. Daily gain and feed efficiency of pigs fed lincomycin were better when compared to nonmedicated pigs.

Kunesh (1981) administered lincomycin parenterally at 11 mg per kg of body weight for three consecutive days to piglets at birth and at 39 d of age. Treatment with lincomycin tended to increase average daily gain but did not affect feed efficiency. The incidence of coughing was measured four times during the post-weaning phase and the average coughing index did not change with the lincomycin treatment.

Several in vitro studies have shown the sensitivity of MP to lincomycin. Arai et al. (1966) demonstrated that lincomycin was not effective in inhibiting the growth of mycoplasma pneumoniae Mac. Mardh (1975) tested the sensitivity of various human mycoplasmas. The strains tested were found to be resistant to 20-80 mg/ml of lincomycin. Williams (1978) tested 9 strains of Mycoplasma hyopneumoniae and one strain of Mycoplasma hyorhinis for their in vitro susceptibility to 51 antimicrobial agents. Based on determining minimal inhibitory concentrations of .09 µg/ml for MP and .03 µg/ml for M. hyorhinis, all strains were susceptible to low concentrations of lincomycin.

Chemotherapy with tetracycline. Betts and Beveridge (1955) recognized a marked prophylactic effect of chlortetracycline on MP but not with penicillin or sulfonamides. Wesslen and Lannek (1954) isolated and cultivated MP which was found to be susceptible in vitro to chlortetracycline and oxytetracycline. Bornfors and Lannek (1955) examined the therapeutic activity of sulfamethazine, sulfanilimide, penicillin, oxytetracycline, and chlortetracycline on MP in two trials. In the first trial the treated animals had acquired their pneumonia in an infected herd where they had spent at least two months. In the second trial the treated pigs

had not been exposed until they were inoculated with a suspension of pneumonic lungs 25 d pretreatment. Therapeutic levels of all antimicrobials were given for ten and fourteen days, respectively. The antimicrobials were of equal therapeutic value and growth was improved with each treatment compared to the control. It was found in both trials that the treatment did not make the pigs non-infective. It was also observed that the treatments did not reduce the pneumonic lesions in comparison with the control animals which were treated with penicillin-streptomycin. The prophylactic activity of the tetracyclines on MP is in marked contrast to their lack of therapeutic action.

Lannek and Bornfors (1956) found that treatment with tetracycline or oxytetracycline, given to 6-7 week old pigs before they were exposed to the causative agent of MP, will prevent establishment of infection. Eight pigs weighing 16-17 kg were inoculated intranasally with a suspension of pneumonic lungs and continuously exposed to pigs suffering from MP. The pigs were slaughtered four weeks after the beginning of the trial and the lungs were examined histopathologically. Daily doses of 20, 15, and 10 mg per kg body weight effectively inhibited infection, whereas, 5 and 1 mg resulted in incomplete inhibition. In a field trial, 60 pigs with an average initial weight of 29 kg were exposed only to natural infection of MP in the herd of origin. Chlortetracycline was administered during the first three months of the trial but not during the last few weeks before slaughter. A dose of 5 mg per kg of body weight resulted in complete inhibition of MP, whereas, doses of 1 mg and 0.2 mg gave partial inhibition.

Hupka and Hutten (1956) indicated that MP-infected pigs fed chlortetracycline over a six-month period gained more than controls. The amount of additional gain, 8-13 pounds, did not warrant costs of the antimicrobial agent.

Betts and Campbell (1956) reported on the effects of sulfamethazine,

penicillin, streptomycin, chloromycetin, chlortetracycline, oxytetracycline, and tetracycline on the causal agent of MP. It was found that infection could be prevented in 8 to 16 week old pigs with chlortetracycline and oxytetracycline, but that neither of these antimicrobials, nor the other drugs tested, had any effect upon established lesions. Goodwin and Whittlestone (1960) believed that the tetracyline group of antibiotics was the only means of preventing the development of MP in susceptible pigs.

Mare and Switzer (1966) compared the effect of tylosin to chlortetracycline on MP. Thirteen weanling pigs were fed high levels of either tylosin or chlortetracycline at the time of inoculation with MP. Treatment was started 7 d before the pigs were infected and was continued until the pigs were killed and necropsied 21 d postinoculation. They found the causitive agent to be resistant to tylosin but it was highly sensitive to the action of chlortetracycline at 440 ppm/ton of feed. The agent that induced MP in the respiratory tissue of animals treated prophylactically with the tetracyclines was not found bacteriologically.

Eggert et al. (1980) fed three levels of chlortetracycline to 50 weanling pigs seven days prior to and 42 days following a challenge of MP. Pigs fed 220 ppm of chlortetracycline had no MP as determined by CF and had the best average daily gain compared to a control challenged with MP. In a second trial, 173 pigs were fed from 6.1 to 90 kg on medicated feed and a like number on non-medicated feed. All lungs were scored at slaughter for severity of gross pneumonic lesions of MP. Pigs fed chlortetracycline at 220 ppm had less lung pathology and gained faster than control pigs.

The use of antibiotics prophylactically in man was reported by Jensen et al. (1967) who demonstrated that when oxytetracycline was given to all members of families in which there was a case of MP, clinical disease could be reduced. In this study, equal numbers of patients were included in the drug and placebo groups, and

there were approximately equal numbers of proven infections in both groups. The groups of patients who received oxytetracycline had fewer numbers of clinical illnesses, but the antibiotic may have acted in a therapeutic rather than prophylactic manner.

The possibility that the agent inducing MP cannot be eliminated by antimicrobial therapy has been well researched. Slotkin et al. (1967) showed that chlortetracycline delayed colonization of MP in hamsters but failed to eradicate the organism. Using an in vitro system Larin et al. (1967) confirmed this result. Huhn (1971) demonstrated that chlortetracycline given at 50-200 g per ton of feed prevented development of pneumonia. The antimicrobial did not prevent establishment of infection, and lesions developed after cessation of medication. Etheridge (1979) found the chlortetracycline fed at levels suggested by Huhn (1971) did not prevent the infection of MP.

Smith et al. (1967) could not explain the persistance of MP by insufficient levels of antibiotic in the blood or in the respiratory tract of humans. However, therapeutic doses of erythromycin and tetracycline did suppress the number of MP organisms. The organisms isolated before and after therapy were found to have equivalent tetracycline sensitivities in vitro.

In vitro studies have confirmed the sensitivity of MP to tetracyclines. Arai et al. (1966) examined the effect of antimicrobials on a human mycoplasma pneumonia. The highest antimicrobial activity among the tetracyclines was found with oxytetracycline which was followed by chlortetracycline and tetracycline. Perlman et al. (1967) found that 7 of 8 strains of MP were sensitive to tetracycline. The tetracycline activities against MP were the same as reported by Arai (1966). Chlortetracycline was found effective in inhibiting growth of six strains of mycoplasma P as shown by Hoshino et al. (1970). Five strains of MP which were isolated from swine were examined for antimicrobial sensitivity by Ogata et al.

(1970). The sensitivity of MP to tetracyclines was lower than the antitumor antibiotics which would normally not be used for treatment. Mardh (1975) determined the sensitivity of various human mycoplasmas to different tetracycline analogues. The strains tested were most susceptible to doxycycline, somewhat less susceptible to methacycline, minocycline, and tetracycline, and least susceptible to oxytetracycline per unit of weight. Denny et al. (1971) found MP extremely sensitive to erythromycin and to most members of the tetracycline group. Sensitivity was variable to other well known antibiotics, including lincomycin, and was completely resistant to the penicillins.

Subsequent Effects of Chemotherapy

The use of antimicrobials for mycoplasmal control can also affect growth, feed efficiency, and the intestinal flora population.

Braude et al. (1953) compiled 337 swine growth comparisons and found overwhelming evidence that certain antibiotics, including chlortetracycline and oxytetracycline, were very effective in stimulating growth and improving feed efficiency.

Coates et al. (1955) observed that chicks fed rations containing an antibiotic have thinner intestinal walls than the comparable control animals. This they believed to be the result of inhibition of certain microorganisms in the gastro-intestinal tract, which have an irritant effect on the mucosa. Their elimination leads to a more efficient absorption of nutrients. Braude et al. (1955) found a tendency for chlortetracycline to reduce the weight of the small intestine attributed to a thinning of the gut. Pigs fed chlortetracycline had an increased body weight at slaughter compared to the control.

Fuller et al. (1960) observed a lack of effect of chlortetracycline on bacterial

counts of weanling pigs. Better gain and feed efficiency was produced in treated pigs compared to the control. Rapid development of resistance to the antimicrobial was, as expected, very evident and shows that chlortetracycline had some effect on the flora. The effect of feeding diets containing tetracyclines at levels of 4-30 ppm on the non-pathogenic E.coli population was shown by Smith and Crabb (1957). Practically all the strains of coliforms isolated from the pigs in which tetracycline containing diets were fed were resistant to tetracycline. These strains of E.coli were either absent or formed only a small proportion of the E.coli flora of the pigs fed diets without antibiotics. In a study carried out by Langlois et al. (1976), comparisons were made between the Coldstream swine herd fed chlortetracycline continuously since May of 1972 and the herd at Princeton, which had not received antibiotic therapeutically or in the feed since 1972. No difference in total coliform counts were observed, however, chlortetracycline resistant E.coli decreased markedly from 1974 to 1975 in the Princeton herd, dropping from 81 to 55 to 22%. In the three years after removal of chlortetracycline from the Princeton herd, tetracycline resistance averaged about 40% while remaining almost 85% in the Coldstream herd.

Smith (1970) reviewed the effect of the tetracyclines on the bacterial ecology of several animal species. Resistance to E.coli increased in all species on account of treatment with the tetracyclines.

The effect of the tetracyclines on the nutrition of the human is discussed by Gabuzda et al. (1958). Chlortetracycline given to undernourished adult men in doses of 2.5 or 3.0 g orally and daily resulted in losses of body weight. Direct gram stained smears of feces usually showed very little if any consistent change in relation to antibiotic administration and failed to reflect changes in cultures. The average total number of organisms per gram of feces usually varied from 10^6 to 10^9 and showed no consistent relation to antibiotic administration. Kikuchi et al.

(1973) administered tetracycline to infants and found that fecal bacterial counts did not change.

Lincomycin has also been shown to have effects on growth, feed efficiency and the intestinal flora population. Clinical studies were done on humans soon after lincomycin was developed. Holloway et al. (1963) and Harnecker et al. (1963) both performed clinical studies and found that treatment with lincomycin had good in vivo activity against several gram postive bacteria. Of the side effects observed, 5 of 55 and 2 of 60 patients in each study, respectively, experienced diarrhea. In a study to compare the antibacterial activity of lincomycin and clinimycin, McGehee et al. (1968) found that one-third of the subjects reported loose bowel movements after four doses of 500 mg of lincomycin. Finegold et al. (1966) treated 18 patients with 1.5-4.0 g of lincomycin per day. Four of the eighteen had some gastrointestinal side effects including loose stools. Bacterial cultures of all stools found consistent elimination of anaerobes from the fecal flora with relatively little modification of the aerobic flora.

Gorgach et al. (1969) utilized five normal human subjects and four individuals with long established, well-functioning ileostomies to investigate the effect of lincomycin on the human intestinal flora. The microflora of the small intestine, feces, and ileostomy effluent were determined before and after treatment with the antimicrobial. The upper gastrointestinal tract of normal individuals was found to contain small numbers of gram positive organisms. After seven days of treatment, lincomycin caused little change in these microbial populations. The microbial population of the distal ileum, ileostomy effluent, and feces were profoundly altered by lincomycin. There were significant reductions in the numbers of anaerobes, fecal streptococci, and E.coli in nearly all subjects. In some individuals, however, resistant strains of Enterobacter, Candida, and Proteus emerged after the drug was given. Two of the three normal subjects had an increase in the average

daily fecal water output and three of the four ileostomized individuals tended to have increased ileostomized water output.

Two of fourteen patients who took oral lincomycin had diarrhea, which responded to reduction in dosage as reported by Bartlett et al. (1972). In vitro sensitivity studies showed that lincomycin was effective against anaerobic bacteria although it was less effective than clindamycin.

DeGeeter et al. (1976) determined that exposure of ten E.coli isolates in vitro to a concentration of lincomycin found in the intestine of swine fed 110 mg of lincomycin per kg of feed did not affect sensitivity of eight antimicrobials, nitrofuran, and sulfonamide when compared with sensitivity of E.coli not exposed to lincomycin. An in vivo study was also conducted with 36 crossbred weaned pigs. Pigs fed diets fortified with lincomycin gained more weight and converted feed to gain more efficiently than did non-medicated controls. Less diarrhea was observed in medicated pigs than in non-medicated. Sensitivity of E.coli to tetracycline, dihydrostreptomycin, clindamycin, spectinomycin, or triple sulfa was not affected by feeding a diet medicated with lincomycin.

DeGeeter (1980) reported on the addition of lincomycin as a growth promotant for swine in two trials. In the first trial feeder pigs fed lincomycin for the first 21 d at 220 mg per kg of feed improved daily gain compared to the control. Lincomycin fed for 160 d in another treatment increased daily gains but did not affect feed utilization. In trial 2 pigs fed with 44 or 88 mg/kg of feed improved average daily gain and feed efficiency compared to the non-medicated control. Lincomycin was also evaluated as a feed additive by Dewilde and Vanhemelrijck (1980). Average daily gain tended to be improved, daily feed intake was greater, and feed efficiency did not change for pigs fed 20 ppm lincomcyin. Pollmann et al. (1980) showed that the addition of lincomycin for 30 d at 110 mg/kg of feed to a starter diet improved average daily gain and feed conversion compared to the

control in a 4-week old pig. Veloso et al. (1982) reported in two trials that lincomycin did not affect gains of gilts but did affect barrows with an average initial weight of 25 kg. Feed consumption and efficiency were not affected by lincomycin addition.

The research reviewed shows that both lincomycin and the tetracyclines have been used as feed additives for improved animal growth and production. There is little doubt about the possible beneficial effect, but there is no general agreement on how this positive result is obtained. Henderickx et al. (1980) summarized a series of experiments dealing with the effect growth promoting agents have on the intestinal gut flora. The effect has been limited to two hypotheses: 1. The effect is due to an influence on cell metabolism, or, 2. The effect is on the intestinal flora. The main contention against an influence on cell metabolism is that most growth promoting agents are not absorbed across the intestinal wall in sufficient enough quantities to effect intermediate metabolism. The result of the experiments summarized by Henderickx et al. (1980) is that the effect is on the intestinal flora. Feed additives can change the topographical and qualitative distribution of the intestinal flora. They can change the metabolic activity of the intestinal flora, leading to a reduction in the waste of energy and to increased availability of amino acids to the host.

Materials and Methods

Three experiments were conducted to evaluate the effects of manipulating the microflora of artificially reared piglets. The first trial was designed to determine a dosage rate for lincomycin and long-acting oxytetracycline. The second experiment examined the effect of antimicrobial therapy on the intestinal flora and isolation after therapy. The third experiment was designed to challenge the artificially reared piglet with a live culture of mycoplasma hyopneumoniae and evaluate the effect of antimicrobial therapy on that challenge.

ARTIFICIAL REARING PROCEDURE

Newborn piglets from crossbred sows were weighed, needle teeth clipped, ears notched, tails docked and given a one cc iron dextran injection. All piglets were allowed to nurse sows for 12-24 h before they were weaned. Piglets were reared as described by Hsu (1980) in individual wire cages (60x30x30 cm) in a 6x9 m environmentally controlled room located at the University Swine Farm. Each cage was equipped with a plastic cup with a 120 ml capacity. A gas heater was used to maintain the room temperature at 32 C and an overhead exhaust fan was operated at all times. Piglets were removed from sows at a minimum of 12 h of age and allotted by litter and weight to the individual cages. Initially, the piglets were not given water or milk replacer for the first 6 h to stimulate appetite. On the first d piglets were fed every 6 h, the second day every 8 h, and the remaining days they were fed every 12 hours. Every piglet had consumed milk by the second feeding on the first day. The milk left in the cups at the end of each feeding was discarded

and the cups were washed. Piglets were fed to appetite a milk replacer[1] medicated with neomycin (275 ppm) mixed with three parts water and individual consumption was recorded. Water was given once between feedings.

Scour scores were recorded twice daily and were based on a score of 1 to 4 (1=no scour, 2=loose feces, 3=liquid and some solids together, 4=severe watery scour). Feed intake and feed efficiency were calculated on a dry matter basis. Piglets were weighed at the beginning and end of the artificial rearing stage.

Trial 1. Fifty-five piglets from eleven litters were assigned by litter and weight to one of five treatments to determine the dosage rate for manipulating the microflora of the artificially reared piglet. Two levels of lincomycin were 11 mg (LILO) and 22 mg (LIHI) injection per kg of body wt, and two level of long-acting oxytetracycline were 100 mg (LALO) and 200 mg (LAHI) per injection compared to a non-medicated control (CTRL). Lincomycin was administered intramuscularly for three days after birth and from d 14 to 16 of the trial. Long-acting oxytetracycline was administered intramuscularly on d 1 and 14.

Blood samples were collected on d 3 and 17 via anterior vena cava puncture for blood analyses. Blood samples for cell counts and hematocrit were collected into evacuated glass tubes that contained sodium heparin. Blood collected was assayed for total leukocyte and erythrocytes counts, hemoglobin, hematocrit and differential leukocyte counts. Leukocyte and erythrocyte counts were done with an automatic counter[2].

[1] Coop Pig Milk Replacer had the following composition: Protein, 28%; Fat, 10%; Fiber, .15%; Vitamin A, 20,000 IU/lb; Vitamin D_3, 5,000 IU/lb; Vitamin E, 20 IU/lb.

[2] Coulter Counter, Model ZBI, Coulter Electronics, Inc. Hialeah, FL.

On d 21 of the trial the piglets were removed from the individual cages and sacrificed via electrocution. The chest cavity was opened and the lungs were examined for macroscopic lesions of mycoplasma pneumonia (MP). The lobes of the lung were evaluated and graded for MP by a subjective score (0=no lesions, 1=25% of the lobe involved in MP lesions, 2=50% of the lobe involved in MP lesions, 3=75% of the lobe involved in MP lesions, 4=100% of the lobe involved in MP lesions). The lobes were removed and fixed in 10% buffered formalin. Samples of each lobe were collected for microscopic examination. The tissues were embedded in paraffin and stained with hematoxylin and eosin. The specimens were evaluated and graded by the method described by Livingston (1972) to verify scoring of gross lesions.

All data were analyzed as a randomized complete block design using litter as the block by least square analysis of variance according to the General Linear Models procedures (Goodnight et al., 1982) of the Statistical Analysis System. Response curves (linear and quadratic) were determined for the effect of dosage level on all growth and blood parameters.

Trial 2. The effect of the antimicrobial therapy on the intestinal flora was evaluated in 20 piglets from four litters which were assigned to one of five treatments. The five treatments were: 1) control with non-medicated milk replacer (CTO), 2) control with neomycin medicated milk replacer (CTN), 3) lincomycin at 22 mg per kg of body weight (LIN), 4) long-acting oxytetracycline at 100 mg injection (LAO), and 5) a combination of LIN and LAO (L+L). Pigs on treatments 3,4 and 5 also consumed neomycin (275 ppm) medicated milk replacer. Piglets were reared, treated, and the same parameters were evaluated as in Trial 1. Piglets were bled as in the previous trial but only cell counts were evaluated.

Cell-mediated Immunity. In vivo cellular immunity was evaluated by the intradermal response to the mytogen phytohemagglutinin (Blecha et al. 1983) on d 20 of the trial. A double skin-folk thickness was measured on both flanks of all pigs with a constant-tension micrometer. Phytohemagglutinin (250 g in 1 ml sterile physiologic saline) was injected intradermally into the medial aspect of one flank; saline (control) was injected into the other flank. Measurements were taken 24 h postinjection and recorded. Data were expressed as change in flank thickness (24 h thickness minus preinjection thickness).

Bacterial Counts. One litter was killed each day, from d 20-23, by electrocution and a necropsy was performed to excise samples from five predetermined locations along the gastrointestinal tract for microbiological evaluation. The five sites were: stomach (cardiac region), duodenum (150 mm from the pylorus posterior to bile and pancreatic ducts), the ileum (300 mm from ileo-cecal junction), the entire cecum and a portion of the colon (100 mm anterior and posterior to apex). In addition, a fecal sample was removed from the posterior colon.

A sample (approximately 1 g) of tissue and digesta was placed in a plastic bag and 99 ml of sterile phosphate buffer was added. The samples were homogenized in a stomacher[3] for 1 minute.

Approximately 8 ml of each sample was poured into a sterile screw cap test tube which had been gassed with carbon dioxide to produce an anaerobic environment. Samples were then gassed again and 1 ml was placed into 9 ml of an anaerobic rumen fluid dilution blank. They were then vortexed and run through serial dilutions. The samples were diluted in 100-fold steps for enumeration of the organisms in roll tubes containing pre-reduced rumen fluid medium to determine

[3] Stomacher Lab-Blender, Tekmar Co., Cincinatti, OH.

total anaerobic count. Each sample was spun and incubated at 37 C for 5 d.

One half ml was taken from each of the above dilutions and added to 1 ml of sterile 0.75% agar at 55 C. Each sample was vortexed and inoculated on violet red bile agar[4] used to enumerate Escherichia coli and MRS agar, used to enumerate lactobacilli. The violet red bile agar plates were incubated aerobically at 37 C for one day and the MRS agar plates were anaerobically incubated at 37 C for three days. Plates with 30-300 colony-forming units (CFU) were counted for each medium and recorded. Data were expressed as logarithm of CFU per g of sample (wet basis) for statistical analyses.

Isolation in the Nursery Phase. Forty piglets from five litters were assigned by litter and wt to one of four treatments (2-5 as previously described) to evaluate the effect of isolation after antimicrobial therapy. Piglets were artificially reared and treated as described above. On d 21 of the trial the piglets were randomly assigned within litter to either the nursery at the research farm or an isolation room. Pigs were allowed access to a sorghum grain-soybean meal-whey diet (table 1) with no medication until they weighed approximately 20 kg.

Pigs were reared in a totally confined environmentally controlled nursery with woven wire floors over a Y-flush gutter at the Swine Research Farm. Pens were 1.2 m by 1.5 m and each was equipped with a nipple waterer and a five-hole stainless steel feeder. Temperature was controlled to approximately 32 C.

The isolation room was totally confined and environmentally controlled. Each pen was 1.2 m by 1.2 m with plastic coated expanded metal flooring raised above a concrete floor. Each pen was equipped with a nipple waterer and a four-hole galvanized steel feeder. Temperature was controlled to approximately 27 C.

[4] BBL, Cockeysville, MD.

TABLE 1. COMPOSITION OF STARTER DIET, TRIAL 2.

Ingredient	%
Sorgum grain, ground (IFN 4-04-383)	48.95
Soybean meal (44%) (IFN 5-04-612)	27.50
Dried whey (IFN 4-01-182)	20.00
Dicalcium phosphate (IFN 6-01-080)	1.55
Ground limestone (IFN 6-01-069)	1.10
Salt (IFN 6-04-151)	.10
Trace mineral[a]	.10
Vitamin premix [b]	.50
L-Lysine HCl [c]	.20

[a]Contained 5.5% Mn, 10% Fe, 1.1% Cu, 20% Zn, .15% I, .1% Co.

[b]Each kg of premix contained: Vitamin A, 880,000 IU; vitamin D_3 66,000 IU; riboflavin, 990 mg; choline, 88 g; d-pantothenic acid, 2640 mg; niacin, 5500 mg; vitamin E, 4400 IU; vitamin B_{12}, 4.84 mg; menadione dimethylpyrimidinal bisulfite, 550 mg; ethoxyquinone, 6270 mg.

[c]L-Lysine HCl 78% lysine activity.

At the end of the seven week nursery period the pigs were evaluated for growth, feed efficiency, and lobes of the lung were scored for MP as previously described.

All data were analyzed as a randomized complete block using litter as the block by least square analysis of variance according to the General Linear Models procedures (Goodnight et al., 1982) of the Statistical Analysis System.

Trial 3. Forty piglets from ten litters were assigned by litter to one of four treatments to evaluate the influence of a challenge of MP after antimicrobial therapy. Piglets were reared artificially and parameters evaluated were the same as in Trial 1 except no bleeding data were collected. The four treatments were the same as treatment 2-5 in Trial 2. All piglets were exposed at d 3 to a virulent culture of MP inoculated intranasally. Control piglets were reared in a separate room to prevent continuous exposure of MP to the other treatments. Piglets were sacrificed on d 21 of the experiment and lobes of the lung were evaluated for MP lesions as previously described.

All data were analyzed as a randomized complete block design using litter as the block by least square amalysis of variance according to the General Linear Models procedure (Goodnight et al, 1982) of the Statistical Analysis System.

Results

Trial 1. The performance and scours of the piglets in Trial 1 are shown in table 2. Treatment with LAHI reduced ($P<.05$) final weight, average daily gain and feed efficiency compared to LALO. The LAHI tended to depress final weight ($P=.08$), average daily gain ($P=.11$) and feed efficiency ($P=.09$) compared to CTRL treatment. The LALO had the highest means for performance though they were not different ($P>.05$) than the control. Treatment with lincomycin did not alter ($P>.05$) performance compared to the control. The LIHI had a tendency ($P=.10$) to improve feed efficiency.

There was a significant ($P<.05$) quadratic effect on final, average daily gain gain and feed efficiency by treatment with long-acting oxytetracycline. This is seen in figures 1-3, respectively.

Lincomycin gave the highest ($P<.05$) average scour scores compared to CTRL or both long-acting oxytetracycline treatments. A linear effect ($P<.05$) was found for incidence of scours to increase with increasing dosages of lincomycin.

In Trial 1 LALO had the greatest amount of lung lesions in the right apical lobe, the right cardiac lobe and in the total lung average (table 3). The LALO was not significantly higher ($P>.05$) than the control, but it was higher ($P<.05$) than both lincomycin dosage levels and LAHI.

The blood data for Trial 1 are found in tables 4 and 5 for the two respective bleedings. The LAHI significantly reduced ($P<.05$) white blood cell count (WBC), red blood cell count (RBC), hemoglobin, hematocrit, and neutrophils in bleeding 1. In bleeding 2 hemoglobin and hematocrit are different ($P<.05$) for LAHI. All other parameters are similar although LAHI had the lowest value for WBC, RBC, and neutrophils. The LILO depressed ($P<.05$) WBC in bleeding 1 which is reflected in a lower ($P<.05$) number of neutrophils compared to CTRL. In bleeding 1 monocytes

TABLE 2. EFFECT OF DOSAGE LEVEL ON PERFORMANCE AND SCOURS, TRIAL1.[ab]

Criterion	Treatments[c]					SEM
	CTRL	LILO	LIHI	LALO	LAHI	
Number of piglets-start	11	11	11	11	11	
Initial wt,kg	1.58	1.58	1.54	1.53	1.55	.06
Final wt,kg[de]	3.76	3.66	3.79	4.07	3.32	.39
Average daily gain,gm[de]	109	104	113	126	90	8
Feed:Gain[de]	1.05	0.99	0.93	0.94	1.18	.05
Average scour score[df]	1.37	2.08	2.14	1.39	1.54	.15
Number of piglets survived	10	10	11	11	11	

[a]Each value is the mean of the number of surviving piglets.

[b]Trial endpoint at 20 days.

[c]CTRL=control, LILO=lincomycin 11mg/kg body wt, LIHI=lincomycin 22mg/kg body wt, LALO=long-acting oxytetracycline 100mg/injection, LAHI=long-acting oxytetracycline 200 mg/injection.

[d]Treatment effect ($P<.05$).

[e]Significant ($P<.05$) quadratic effect of long-acting oxytetracycline.

[f]Significant ($P<.05$) linear effect of lincomycin.

FIGURE 1.
EFFECT OF LONG-ACTING OXYTETRACYCLINE ON AVERAGE DAILY GAIN IN TRIAL 1.

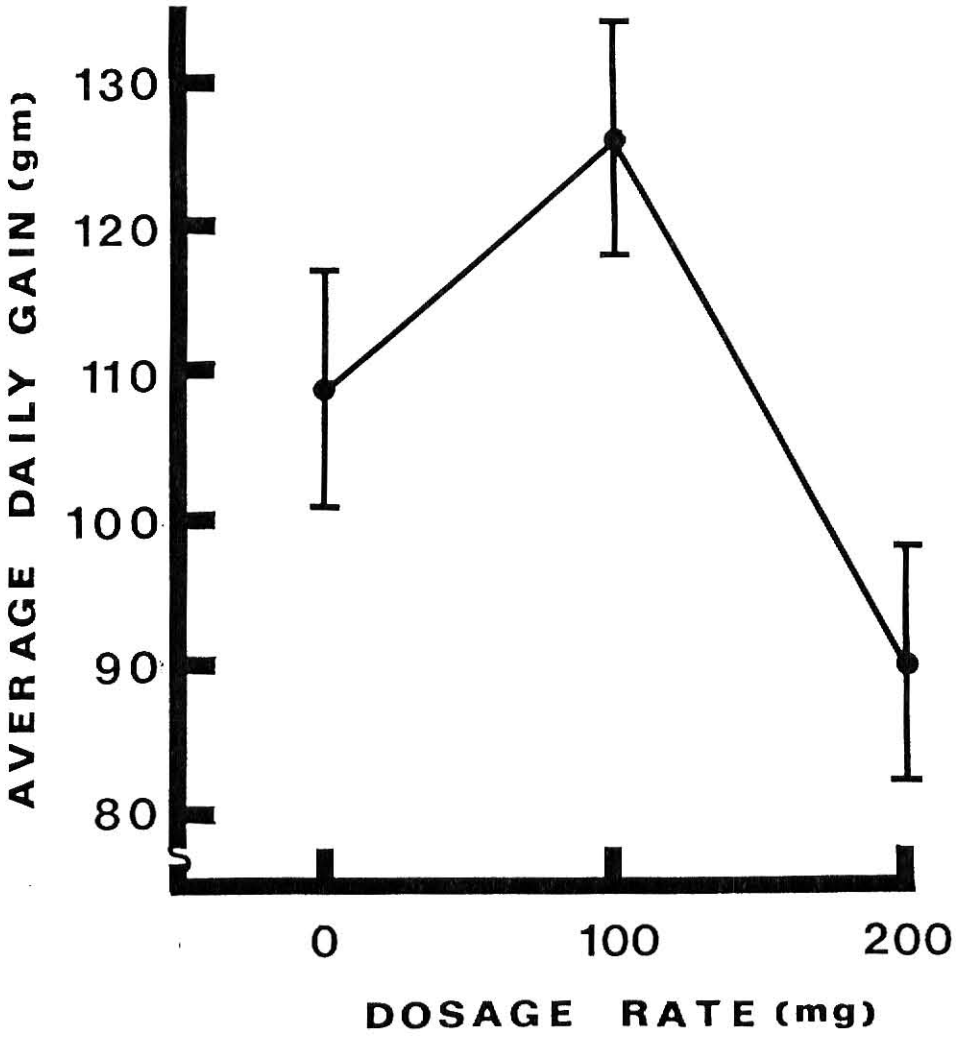

FIGURE 2.
EFFECT OF LONG-ACTING OXYTETRACYCLINE ON FEED:GAIN IN TRIAL 1.

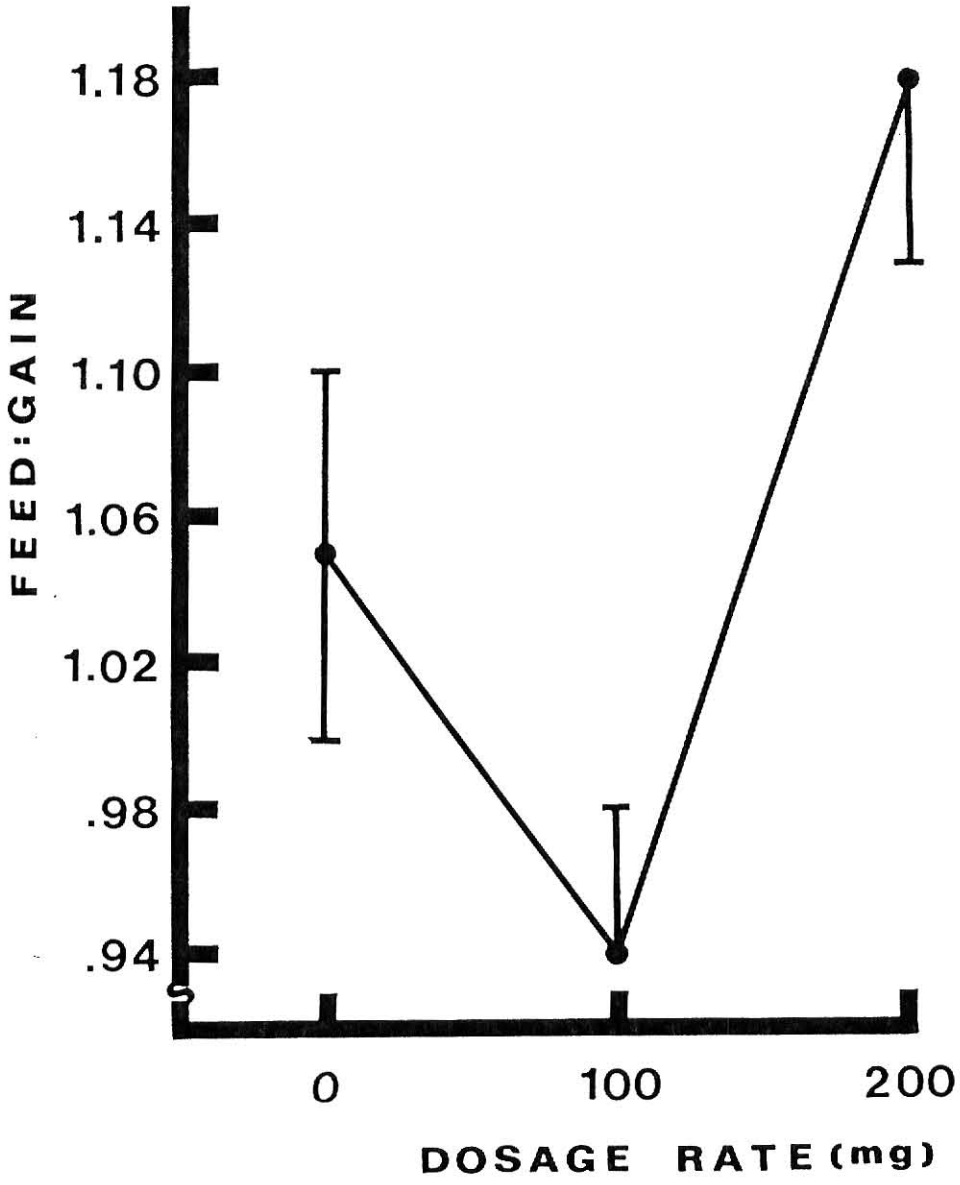

FIGURE 3.
EFFECT OF LINCOMYCIN
ON AVERAGE SCOUR SCORE
IN TRIAL 1.

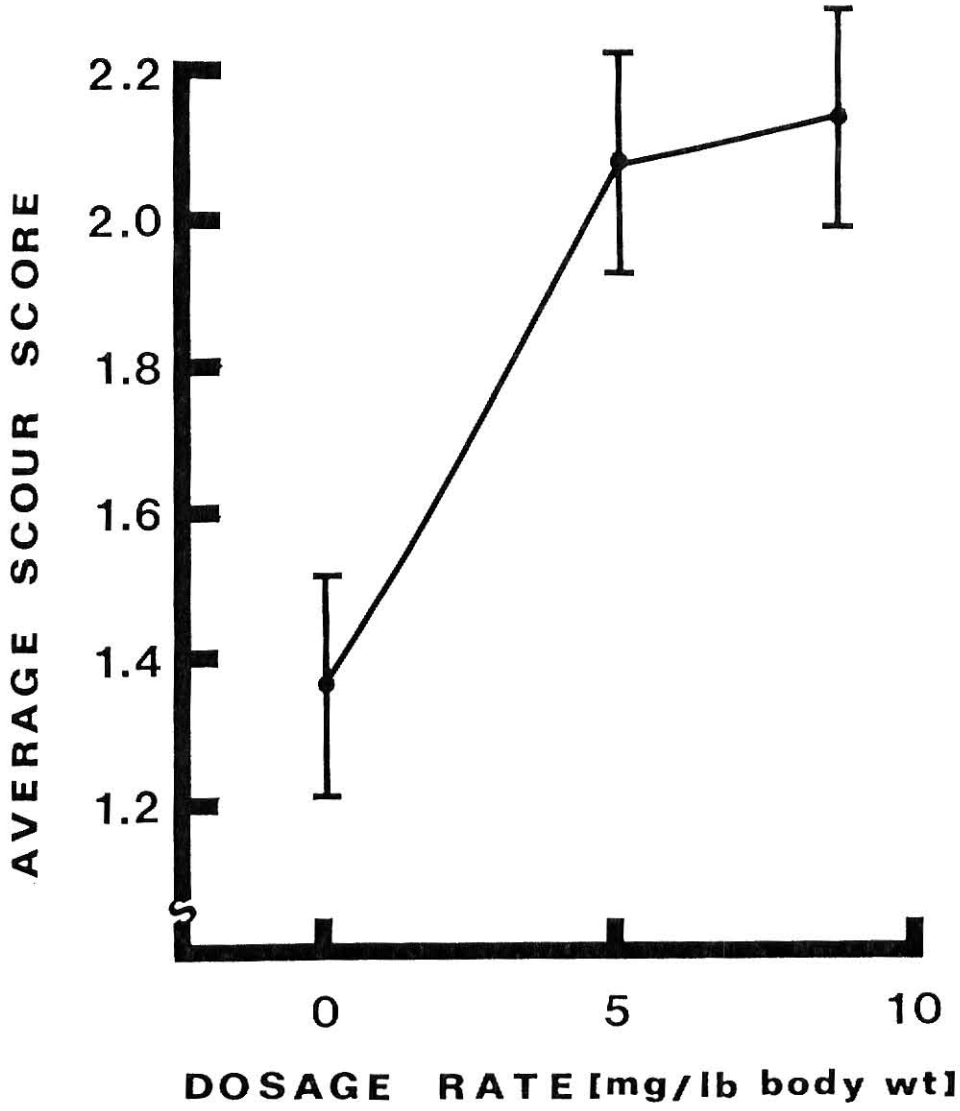

TABLE 3. EFFECT OF DOSAGE LEVEL ON MACROSCOPIC LUNG LESIONS, TRIAL 1.[ab]

Criterion	Treatments[c]					
	CTRL	LILO	LIHI	LALO	LAHI	SEM
Right cardiac lobe[d]	.305	.175	.112	.727	0.00	.21
Right apical lobe[d]	.405	0.00	.118	.727	0.00	.24
Left cardiac lobe	.097	0.00	0.00	0.00	0.91	.06
Left apical lobe	.061	.061	0.00	.182	0.00	.08
Total lung avg.[de]	.217	.057	.055	.409	.023	.12

[a]Each value is the mean of the number of surviving piglets.

[b]Trial endpoint at 20 days.

[c]CTRL=control, LILO=lincomycin 11mg/kg body wt, LIHI=lincomycin 22mg/kg body wt, LALO=long-acting oxytetracycline 100mg/injection, LAHI=long-acting oxytetracycline 200 mg/injection.

[d]Treatment effect ($P<.05$).

[e]Total lung avg. = Summation of lobe lung lesion means for each treatment/4.

TABLE 4. EFFECT OF DOSAGE LEVEL ON BLOOD PARAMETERS - FIRST BLEEDING, TRIAL 1.[ab]

Criterion	Treatments[c]					
	CTRL	LILO	LIHI	LALO	LAHI	SEM
Leukocytes[dfg] x 10^3	16.6	11.5	15.1	15.7	10.9	1.1
Erythrocytes[d] x 10^6	5.91	6.15	6.11	5.94	5.48	.18
Hemoglobin[dg] g/dl	11.8	11.8	11.6	11.3	10.2	.3
Hematocrit[dg] %	37.8	37.4	36.3	36.2	33.5	.9
Neutrophils[dfg]	10866	6236	9406	9801	5046	900
Band cells	526	671	532	558	494	100
Lymphocytes[defgh]	3994	4146	4467	4690	4556	400
Monocytes	993	358	524	469	599	100
Eosinophils	128	56	73	135	160	34
Basophils[g]	52	8	23	17	5	15
Myelocytes	0	0	0	0	5	2
Metamyelocytes	87	53	56	37	80	28

[a]Each value is the mean of the number of surviving piglets.

[b]Piglets bled on d 4 of trial.

[c]CTRL=control, LILO=lincomycin 11mg/kg body wt, LIHI=lincomycin 22mg/kg body wt, LALO=long-acting oxytetracycline 100mg/injection, LAHI=long-acting oxytetracycline 200mg/injection.

[d]Treatment effect ($P<.05$).

[e]Significant ($P<.05$) linear effect of lincomycin.

[f]Significant ($P<.05$) quadratic effect of lincomycin.

[g]Significant ($P<.05$) linear effect of long-acting oxytetracycline.

[h]Significant ($P<.05$) quadratic effect of long-acting oxytetracycline.

TABLE 5. EFFECT OF DOSAGE LEVEL ON BLOOD PARAMETERS - SECOND BLEEDING, TRIAL 1[ab]

Criterion	Treatments[c]					SEM
	CTRL	LILO	LIHI	LALO	LAHI	
Leukocytes x 10^3	14.2	13.5	15.3	13.1	11.9	1.1
Erythrocytes x 10^6	7.13	6.88	6.84	6.93	6.79	.22
Hemoglobin[d] g/dl	12.6	12.2	12.1	12.0	11.2	.3
Hematocrit[d] %	38.4	37.6	36.7	36.8	35.5	.9
Neutrophils	6748	5900	8104	6301	4951	1200
Band cells	240	130	272	83	182	94
Lymphocytes	6449	6626	5848	6062	6024	380
Monocytes[d]	541	528	815	444	463	120
Eosinophils	151	125	206	171	196	36
Basophils	28	0	25	11	7	19
Myelocytes	10	0	0	0	0	3
Metamyelocytes	39	46	66	0	7	31

[a]Each value is the mean of the number of surviving piglets.

[b]Piglets bled on d 18 of trial.

[c]CTRL=control, LILO=lincomycin 11mg/kg body wt, LIHI=lincomycin 22mg/kg body wt, LALO=long-acting oxytetracycline 100mg/injection, LAHI=long-acting oxytetracycline 200 mg/injection.

[d]Treatment effect ($P<.05$).

were increased (P<.05) in the CTRL compared to all other treatments and in bleeding 2 were highest in LIHI which was significantly higher (P<.05) than the long-acting oxytetracycline treatments.

There was a significant (P<.05) linear effect of long-acting oxytetracycline found for WBC (figure 4), hemoglobin, hematocrit, neutrophils (figure 5), monocytes and basophils in bleeding 1. A quadratic effect of lincomycin was also found for WBC, neutophils and monocytes. The monocytes gave a significant (P<.05) linear effect of lincomycin and quadratic effect of long-acting oxytetracycline.

Trial 2. This trial was done twice. The first attempt was stopped after one week because of E.coli scours. Over 50% of the piglets in two of the treatments died since the causitive organism could not be treated with antimicrobials other than the ones used in this trial. The strain of E.coli was resistant to both lincomycin and oxytetracline upon testing, but was susceptible to gentamycin. The second attempt also had E.coli scour problems so the piglets were left on their dam four days to gain additional resistance.

The performance and scours of the piglets in Trial 2 are shown in table 6. Piglets treated with lincomycin (LIN) had the highest (P<.05) final weight, total gain, and average daily gain. Feed efficiency tended (P=.07) to improve by treatment with LIN compared to the control (CTN). Adding lincomycin to long-acting oxytetracycline (L+L) tended to improve average daily gain (P=.11), total gain (P=.11) and tended to improve (P=.20) feed efficiency compared to CTN. Long-acting oxytetracycline (LAO) at the 100 mg level did not change (P>.05) performance parameters.

Lincomycin gave the highest (P<.05) average scour score and L+L tended (P=.08) to increase scours. The LAO treatment did not change (P>.05) the incidence of scours.

FIGURE 4.
EFFECT OF TREATMENT ON
LEUKOCYTES IN TRIAL 1.

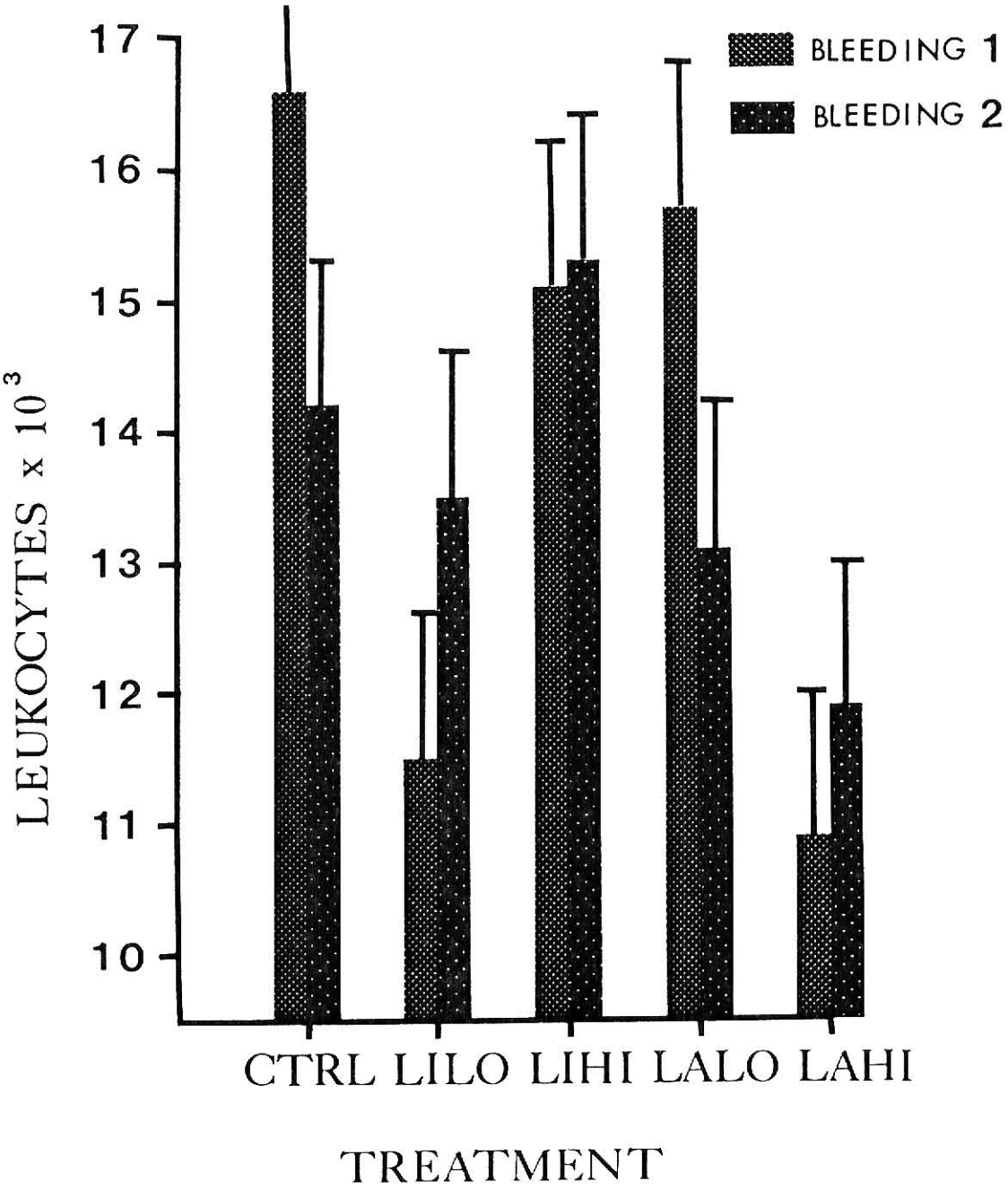

FIGURE 5.
EFFECT OF TREATMENT ON NEUTROPHILS IN TRIAL 1.

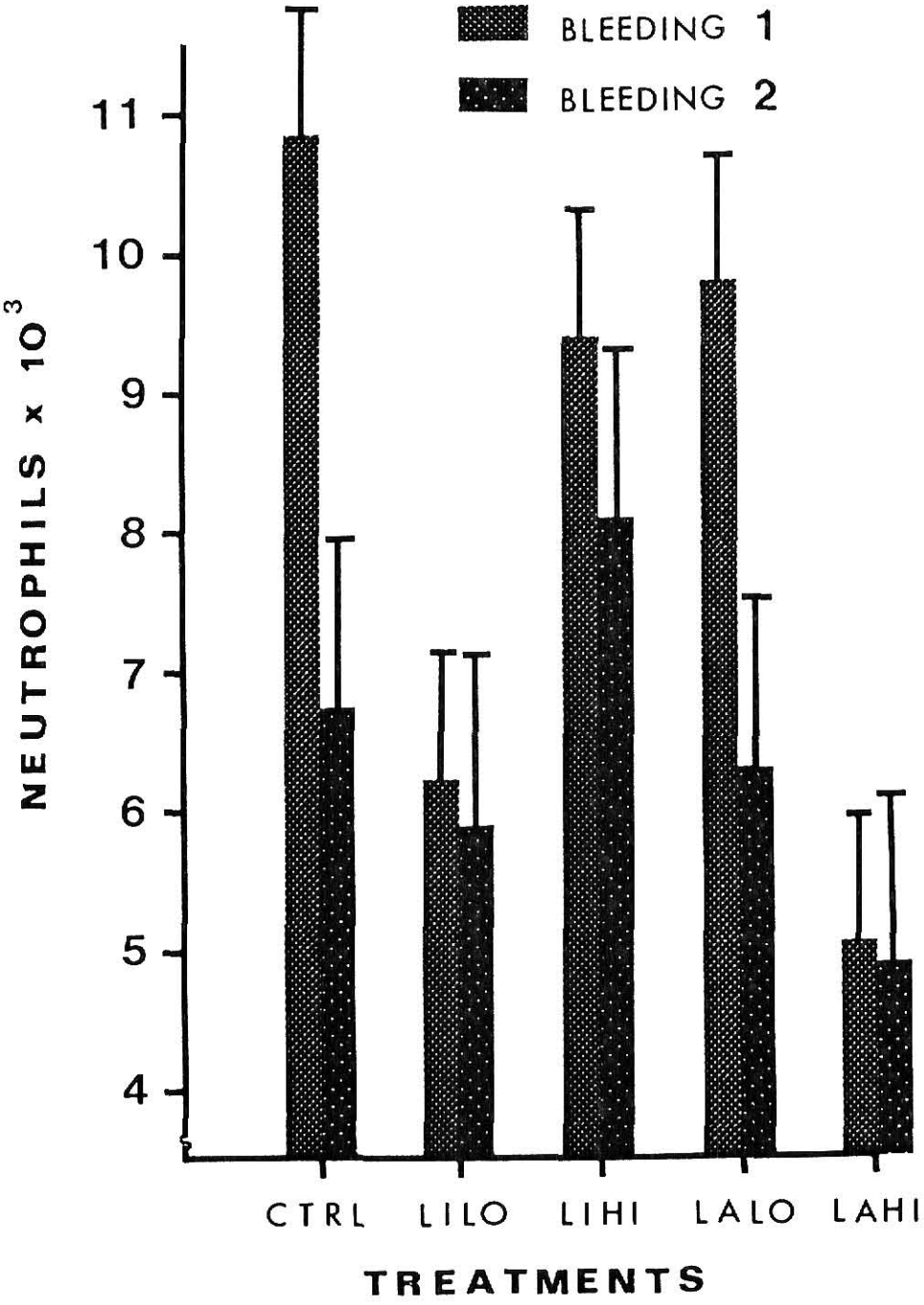

TABLE 6. EFFECT OF ANTIMICROBIALS ON PERFORMANCE AND SCOURS, TRIAL 2.[ab]

Criterion	Treatments[c]					
	CTO	CTN	LIN	LAO	L+L	SEM
Number of piglets start	4	14	14	14	14	
Initial weight, kg	1.85	1.84	1.87	1.69	1.89	.13
Final weight, kg	4.32	4.23	4.80	4.15	4.61	.41
Average daily gain, gm	112	107	131	110	122	7
Feed:Gain	1.00	1.10	0.91	0.98	0.96	.08
Average scour score	1.41	1.29	1.63	1.40	1.51	.08
Number of piglets survived	4	14	14	13	12	

[a]Each value is the mean of the number of surviving piglets.

[b]Trial endpoint at 22 days.

[c]CTO=control with nonmedicated milk replacer, CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetacycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Treatment effect ($P<.05$).

Cell-mediated immunity as evaluated by skin fold differences of piglets injected with phytohemagglutinin is shown in table 7. Control piglets had the highest skin fold difference although it was not different (P>.05) from the other treatments.

Lesions of each lobe were scored microscopically since no gross lesions were seen. In Trial 2 no differences (P>.05) were seen among treatment groups for severity of lung lesions (table 7) although there was a litter effect (P<.05). All piglets showed some degree of MP microscopically (figures 6-9). A lesion-free lung is shown (figure 6), lesions from piglets in Trial 2 are shown (figure 7 and 8) and extensive lesions typical of MP are illustrated (figure 9).

Blood data for Trial 2 is found in table 8. Control (CTN) piglets had the highest (P<.05) WBC count in the first bleeding, this is reflected in a higher (P=.08) total number of neutrophils and eosinophils compared to all treatments. A higher (P<.05) count of lymphocytes and band cells was also found for CTN compared to LIN and L+L treatments. The RBC count did not differ (P<.05) among treatments.

Bacterial counts of the piglets in Trial 2 are shown in tables 9 and 10. The LAO increased (P<.05) coliforms (EC) and lactobacillus (LB) in the stomach. L+L increased (P<.05) LB in the duodenum and depressed (P<.05) EC in the colon. No differences in EC and LB were found among treatments in the ileum, cecum and feces. No treatment effect (P>.05) was found for EC in the jejunum or for LB in the colon.

The effect of isolation on nursery performance is shown in table 11. Since there was no significant location effect on performance, data were pooled by locations to evaluate the treatment effects. All treatments performed similarly (P>.05) during the nursery phase in final weight and average daily gain. Feed efficiency was improved (P<.05) for piglets previously treated with LIN.

TABLE 7. EFFECT OF ANTIMICROBIALS ON CELL-MEDIATED IMMUNITY AND MICROSCOPIC LUNG LESIONS, TRIAL 2.

Criterion	Treatments[a]					
	CTO	CTN	LIN	LAO	L+L	SEM
Skin fold Difference, mm[bc]	5.94	7.06	6.79	6.88	6.54	.78
Total lung avg.[d]	-	1.30	1.10	1.40	1.20	.20

[a]CTO=control with nonmedicated milk replacer, CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100 mg/injection, L+L=combination of LIN and LAO.

[b]Each value is the mean of all artificially reared piglets.

[c]Cell-mediated immunity from d 20-21.

[d]Each value is the mean of piglets in the nursery.

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Figure 6. Tissue from Lung of Pig Considered Free of Mycoplasma Pneumonia

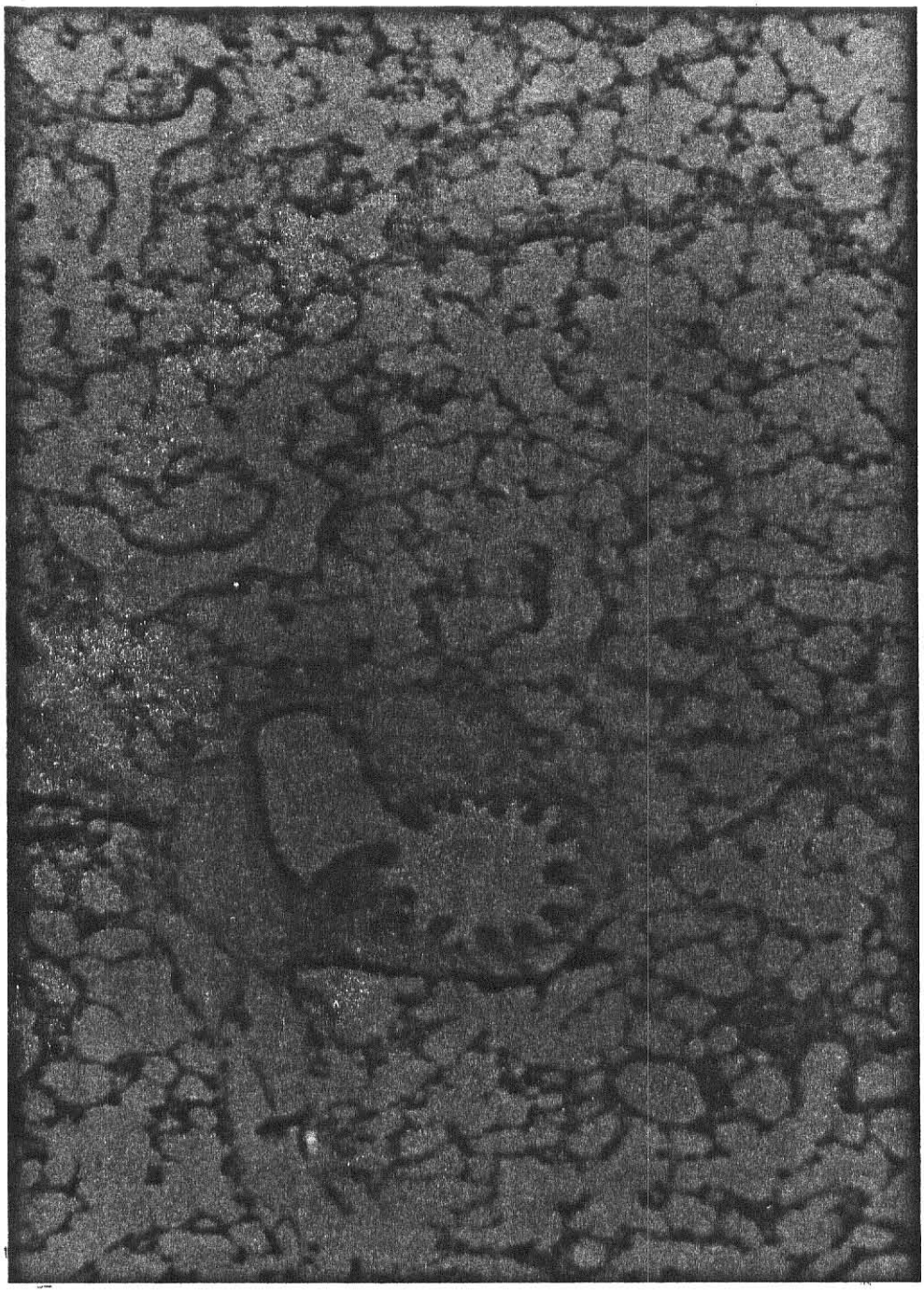

Figure 7. Tissue from Lung of Pig with Mycoplasma Pneumonia in Trial 2

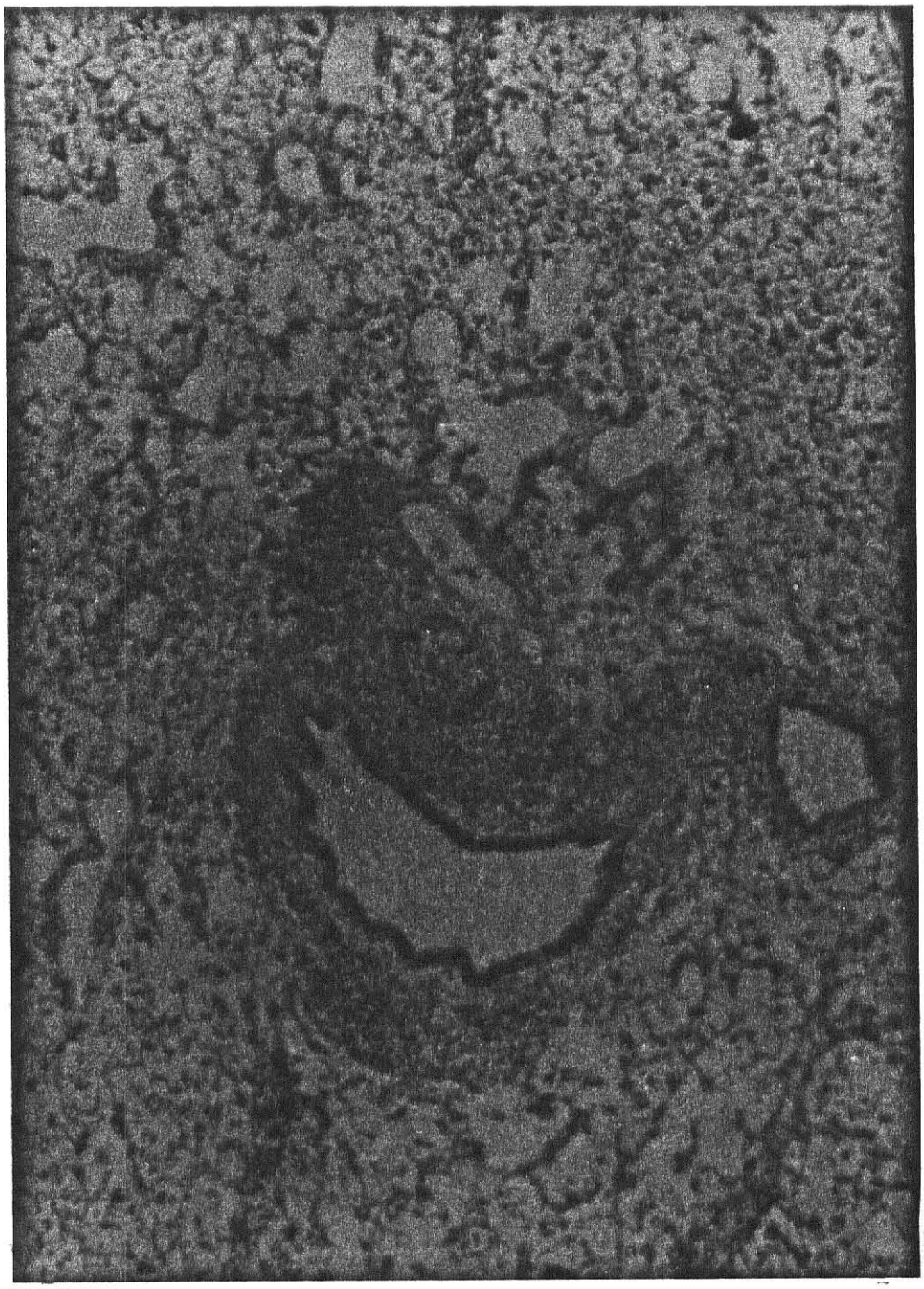

Figure 8. Microscopic Lesions of Lung Tissue of a Pig with Mycoplasma Pneumonia, Trial 2

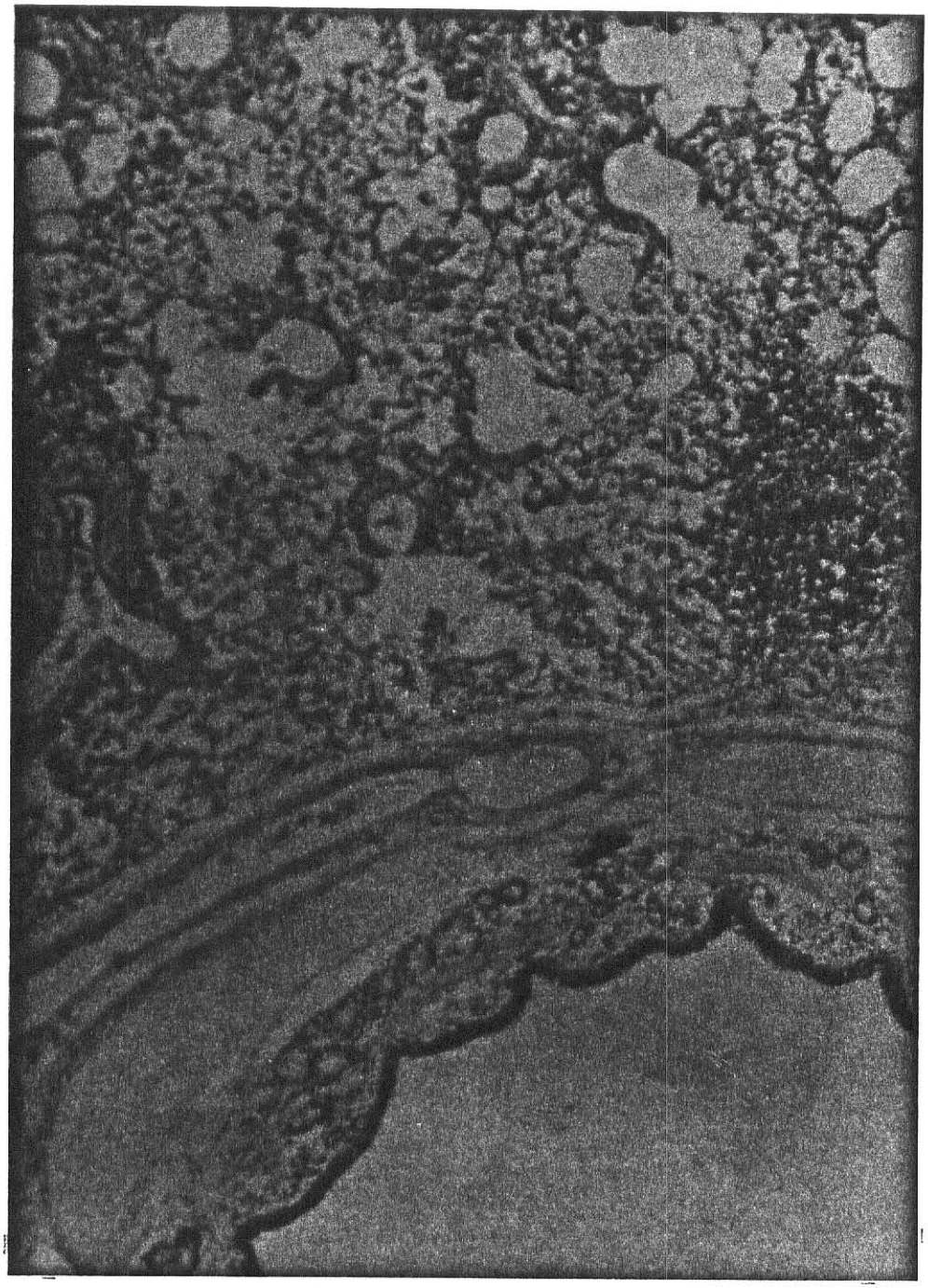

Figure 9. Tissue from Lung of Gnotobiotic Pig with Extensive Mycoplasma Pneumonia Infection

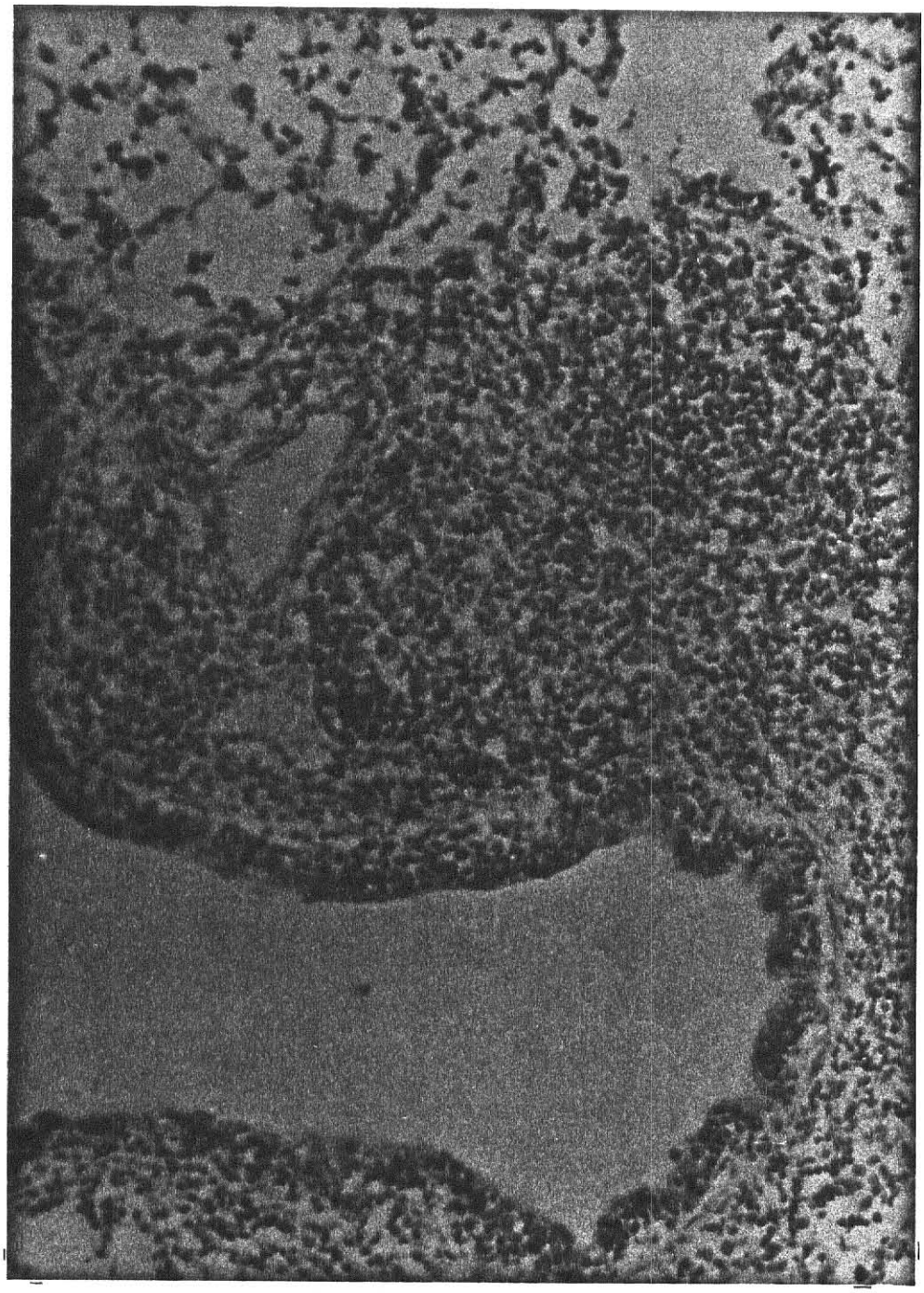

TABLE 8. EFFECT OF ANTIMICROBIALS ON BLOOD PARAMETERS, TRIAL 2.[ab]

Criterion	Treatments[c]					
	CTO	CTN	LIN	LAO	L+L	SEM
Leukocytes[d] x 10^3	9.80	13.82	9.48	11.69	9.67	.9
Erythrocytes x 10^6	6.75	6.89	6.82	6.43	6.78	.2
Neutrophils[d]	2836	5122	3450	4348	3571	490
Lymphocytes[d]	6170	6887	5157	5960	5138	610
Band cells[d]	786	1039	622	895	573	130
Eosinophils[d]	82	307	109	143	186	50
Monocytes	189	225	106	284	130	55
Basophils[d]	0	49	22	17	23	13

[a]Each value is the mean of all artificially reared piglets.

[b]Piglets bled on d 4 of trial.

[c]CTO=control with nonmedicated milk replacer, CTN=control wth neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetraycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Treatment effect ($P<.05$).

TABLE 9. EFFECT OF ANTIMICROBIALS ON COLIFORMS, TRIAL 2.[ab]

Item	Treatments[c]					
	CTO	CTN	LIN	LAO	L+L	SEM
	Log CFU per g of tissue[d]					
Stomach[e]	Nil	2.87	Nil	5.25	1.16	.9
Duodenum	Nil	1.82	1.23	3.69	2.44	1.5
Ileum	2.51	4.89	2.72	2.79	1.98	1.7
Cecum	6.99	7.72	5.34	5.32	6.74	1.4
Colon[e]	7.49	8.20	7.20	7.99	7.04	.3
Feces	7.89	8.44	7.46	7.03	5.31	1.7

[a]Four litters sacrificed on consecutive days, d 20 - 23 of the trial.

[b]Each value is the mean of four piglets.

[c]CTO=control with nonmedicated milk replacer, CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Log of colony-forming units.

[e]Treatment effect ($P<.05$).

TABLE 10. EFFECT OF ANTIMICROBIALS ON LACTOBACILLUS, TRIAL 2.[ab]

Item	Treatments[c]					SEM
	CTO	CTN	LIN	LAO	L+L	
	Log CFU per g of tissue[d]					
Stomach[e]	7.62	7.52	7.77	8.17	6.61	.4
Duodenum	7.10	6.88	6.17	7.31	7.78	.4
Ileum	7.03	8.18	7.81	7.90	8.12	.6
Cecum	8.54	9.32	9.35	7.60	9.35	1.2
Colon[e]	9.59	9.31	9.54	9.60	9.22	.3
Feces	9.33	9.63	10.17	10.04	9.77	.4

[a]Four litters sacrificed on consecutive days, d 20 - 23 of the trial.

[b]Each value is the mean of number of piglets.

[c]CTO=control with nonmedicated milk replacer, CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Log of colony-forming units.

[e]Treatment effect ($P<.05$).

TABLE 11. EFFECT OF ANTIMICROBIALS AND ISOLATION ON SUBSEQUENT NURSERY PERFORMANCE, TRIAL 2.[ab]

Criterion	Treatments[c]				SEM
	CTN	LIN	LAO	L+L	
Number of piglets start	9	10	9	10	
Weaning wt, kg	4.47	4.97	4.41	4.64	.5
Final wt, kg	22.35	21.51	21.88	22.07	2.4
Average daily gain, kg	.75	.69	.73	.74	.04
Feed:Gain[d]	2.03	1.92	2.00	2.03	.13
Number of piglets finish	9	10	9	10	

[a]Since no location difference (P>.05) on performance was observed each value is the pooled mean of two pens with five pigs in each pen.

[b]Trial endpoint at 52 d.

[c]CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Treatment effect (P<.05).

Trial 3. The performance of the piglets in Trial 3 are shown in table 12. Realizing the location was confounded with treatment in this trial, it was necessary to avoid cross-contamination of MP. Piglets reared in the artificial rearing room at the University Swine Farm contracted E. coli scours and twelve out of thirty piglets died amongst the treatments there. Although the animals that lived performed similarly to the control piglets which were isolated, the environmental difference is confounded.

Lung lesion scores are shown in table 13. No differences ($P>.05$) were observed for any lobe or as an average of the total lung among treatments.

TABLE 12: EFFECT OF CHALLENGE OF MYCOPLASMA PNEUMONIA ON PERFORMANCE, TRIAL 3.[ab]

	Treatments[c]				
Criterion	CTN	LIN	LAO	L+L	SEM
Number of piglets start	10	10	10	10	
Initial weight, kg	1.61	1.63	1.63	1.69	.05
Final weight, kg	4.94	4.73	4.19	4.96	.69
Average daily gain, gm	145	135	111	142	14
Feed:Gain[d]	.83	1.08	1.17	1.00	.09
Number of piglets finish	9	8	6	4	

[a]Each value is the mean of the number of surviving piglets.

[b]Trial endpoint at 23 days; Challenge given on d 5.

[c]CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Treatment effect ($P<.05$).

TABLE 13. EFFECT OF CHALLENGE OF MYCOPLASMA PNEUMONIA ON MACROSCOPIC LUNG LESIONS, TRIAL 3.[ab]

	Treatments[c]				
Criterion	CTN	LIN	LAO	L+L	SEM
Right cardiac lobe	0.06	0.00	0.43	0.13	.17
Right apical lobe	0.00	0.04	0.04	0.11	.08
Left cardiac lobe	0.00	0.00	0.21	0.13	.11
Left apical lobe	0.00	0.00	0.00	0.25	.11
Total lung avg.[d]	0.01	0.00	0.17	0.16	.09

[a]Each value is the mean of the number of surviving piglets.

[b]Trial endpoint at 23 d.

[c]CTN=control with neomycin-medicated milk replacer, LIN=lincomycin 22mg/kg body wt, LAO=long-acting oxytetracycline 100mg/injection, L+L=combination of LIN and LAO.

[d]Total lung avg. = Summation of lobe lung lesion means for each treatment/4.

Discussion

Trial 1. The usage of high levels of long-acting oxytetracycline was shown to be deleterious to the artificially reared piglet. Treatment with 200 mg of LAO per injection restricted growth compared to the 100 mg dosage. The LALO appeared to act as a growth promotant, whereas, the LAHI restricted growth compared to the CTRL. This is best illustrated by figures 1 and 2 which show the quadratic effect of LAO dosage treatment.

The upper dosage level, LAHI, is apparently too high for the newborn piglets to utilize. The LAHI piglets gained almost 20 less g per day and weighed .44 kg less than the CTRL at the end of the trial indicating a change in metabolism within the animal due to treatment.

The LALO had the highest means for performance though they were not different from the control. The LALO piglets gained 17 g more per day and weighed .31 kg more than the CTRL at the end of the trial. Several early studies with the tetracyclines (Braude et al., 1953; Braude et al., 1955; Fuller et al., 1960) found them very effective in stimulating growth and improving feed efficiency when given at 22 ppm in the feed to growing-finishing pigs. The use of LALO agrees with these results that a lower level of LAO can influence growth performance of the pig.

Both dosage levels of LAO had no effect on the average scour score. No changes in fecal bacterial counts were observed in several trials (Fuller et al., 1960; Gabuzda et al., 1958; Kikuchi et al., 1973) due to tetracycline treatment which would be indicative of a change in the gastrointestinal flora.

Piglets treated with lincomycin scoured more than untreated or LAO treated animals. Several reports have shown that one of the major side effects of

lincomycin treatment in human patients is diarrhea (Holloway et al., 1963, Harnecker et al., 1963; McGehee et al., 1968; Finegold et al., 1966; Gorgach et al., 1969; Bartlett et al., 1972). The data in this trial agree with the findings that lincomycin can increase the incidence of diarrhea in the test subject. DeGeeter et al. (1976) observed less diarrhea in lincomycin treated animals than with the non-medicated control pigs.

The increase in scours did not have adverse effects on performance because the LIHI piglets had a superior feed efficiency and somewhat better gain than the CTRL. This is contrary to common belief that a scouring pig is less efficient and slower growing.

Analysis of blood composition can be advantageous to measure the state of an animal's metabolism. Large deviations from accepted blood values would indicate a change in metabolism due to treatment, disease or stress. Weaning a piglet on the first day increases an already stressful experience in the early stages of life.

The first bleeding, on d 3, displays increased values of leukocytes with values ranging from 10.9 - 16.6 x 10^3. Normal leukocyte counts are generally in the range of 10 - 12 x 10^3 (Swenson et al., 1958). Three treatments - CTRL, LIHI and LALO - have rather high leukocyte counts possibly due to the stress of early weaning. The LILO and LAHI both have leukocyte counts which are within the range of a normal nursing piglet but they are depressed compared to the other treatments (figure 4). This depression is reflected in the neutrophils (figure 5) which are quantitatively reduced the most of leukocytes. The percentage of neutrophils to lymphocytes in the total leukocyte count is reported to be 70:20 in the day old pig and 50:40 in the week old pig (Swenson et al., 1958). In this trial, the CTRL, LIHI and LALO all have percentages close to 65:30, whereas, the LILO and LAHI are 52:37 and 44:43, respectively. The metabolism of the piglets in LAHI and LILO did definitely change, but what caused that change is unknown.

In the second bleeding, the leukocyte counts (figure 4) are evening out more as seen by the LILO piglets increasing and the CTRL lowering their respective counts. This is reflected well by the drop in neutrophils (figure 5). Hemoglobin and hemaocrit were the only parameters that were affected. Both of these parameters for LAHI are within the range of being normal and did not depress growth. The percentage comparison for neutrophils:lymphocytes is 46:47 as an average for all treatments, which is normal of a 17 d pig.

Comparison of the macroscopic lung lesions showed that the incidence of MP was low. More lesions appeared on the right side of the lung in the cardiac and apical lobes as is expected for MP (Betts, 1952; Pattison, 1956). The LALO and CTRL had the highest incidence of MP lesions, with the other treatments having comparable averages. The variation in these means is large with some pigs scoring the maximum and another scoring the minimum for a lobe, which can correspond to the true mean not being accurately represented. Although there were treatment effects for the right apical and cardiac lobes, the litter has a larger effect on incidence of MP lung lesions.

Selecting a dosage rate for Trial 2 was determined from the performance and blood data. Considering the depression in growth performance and the lower blood parameters, LALO was chosen over LAHI to be the dosage level for the remaining trials. The LIHI was chosen over LILO because there was a slight improvement in growth performance and the LIHI did not depress the blood parameters in the first bleeding. No adverse effects were observed by use of the higher dosage of lincomycin.

Trial 2. The gastrointestinal flora was evaluated in 19 piglets from 4 litters. Injecting the animals with LAO alone increased the number of coliforms and lactobacillus in the cardiac region of the stomach. The differences in the stomach

were not carried through the remaining portion of the gastrointestinal tract (GIT).

Lincomycin plus LAO increased the lactobacillus count in the duodenum but depressed the number of coliforms in the colon compared to the CTO. A lower number of coliforms then was seen in the feces but no reduction in the incidence of scours was observed. The LIN did not change bacterial counts in any portion of the GIT. Gorgach et al. (1955) utilized normal and ileostomized human patients to evaluate the influence of lincomycin on the intestinal microflora. The antimicrobial was given in doses of 4 g daily for 7 d. This level of administration gave significant reductions in anaerobes, streptococci and coliforms. The LIN did not show this response possibly due to dosage level.

The lincomycin piglets had the best growth performance of all treatments raised in the artificial rearing cages. The LIN outperformed CTO, CTN and LAO in average daily gain and had the best feed efficiency. As in Trial 1, the LIN had the highest incidence of scours, however, it did not effect the growth parameters. Harnecker et al. (1963) and Holloway et al. (1963) demonstrated that lincomycin had good activity against gram positive organisms. It is possible that elimination of gram positive organisms could allow for the proliferation of gram negative organisms within the GIT. Apparently this has a beneficial effect since LIN had the best growth with the highest incidence of scours. The improved growth performance agrees with several studies (DeGeeter et al., 1980; Dewilde and Vanhemelrijck, 1980; Pollmann et al., 1980).

The increased improvement in growth performance can also be seen in L+L. The addition of LIN to LAO tends to show some benefit over the LAO by itself. The relationship of LIN to incidence of scours can be seen in this treatment.

The injection of PHA intradermally into the flank has been shown to determine the immune status of the pig (Blecha et al., 1983). No change in the immune status was observed among the treatments. The CTN showed the highest

skin fold difference relating to a greater immune level.

Isolation of piglets, upon removal from the artificial rearing cages, did not effect performance. Exposure of the piglets to the disease endemic in the herd was considered to be a way to reduce growth performance. At the completion of the nursery phase, no location difference was found. The data were pooled across location and no differences were found due to treatment.

No gross lesion differences were observed for treatment or location. Very few pigs had any gross lesions although a slight amount of MP was detected for all pigs on the microscopic plates. Lung lesions showed a significant effect of litter which is similar to the conclusions in Trial 1.

Several studies (Wilson et al., 1970; Kjar, 1977; DeGeeter, 1979) have shown a positive effect of lincomycin on resolving MP in pigs. Neri et al. (1980) and Kunesh (1981) saw no change in coughing due to lincomycin treatment of MP. Treatment with the tetracyclines has shown that a prophylactic effect is possible (Betts and Beveridge, 1955; Bornfors and Lannek, 1955; Lannek and Bornfors, 1956; Goodwin and Whittlestone, 1960), or therapeutic action (Mare and Switzer, 1966; Eggert et al., 1980; Jensen et al., 1967). Many reports have observed the possibility that the agent inducing MP cannot be eliminated by antimicrobial therapy (Slotkin et al., 1967; Larin et al., 1967; Huhn, 1971; Smith et al., 1967). The data from this trial suggest that antimicrobials did not effect MP severity, but growth was improved by the addition of lincomycin.

Trial 3. The infecting of piglets in this trial with MP was an attempt to introduce a virulent culture into the animals and then determine the effect of the antimicrobial treatment on the infection. The control piglets were purposefully reared in a separate room to remove any chance of reinfection by the CTN. The performance data was therefore confounded because of environmental change.

The piglets reared at the Swine Research Farm got E.coli scours several days after the trial began and 12 out of 30 died. The performance data is similar for all treatments but this only accounts for the surviving piglets.

The piglets were sacrificed on d 23 and no treatment differences were found for gross lesions or microscopic lesions. The culture of MP either did not infect the piglets or they resisted the infection and showed no evidence of MP.

Summary

Three experiments were conducted to examine the influence two antimicrobial agents had on growth, blood parameters, the intestinal flora population and the therapeutic action on mycoplasma pneumonia. In Experiment 1, 55 piglets from 11 litters were assigned to one of five treatments to determine a dosage rate for manipulating the microflora of the artificially reared piglet. Two levels of lincomycin (L) were 11 mg (LILO) and 22 mg (LIHI) injection per kg of body weight, and two levels of long-acting oxytetracycline (LAO) were 100 mg (LALO) and 200 mg (LAHI) per injection compared to a control (CTRL). Lincomycin was administered intramuscularly for 3 days after birth and from day 14 to 16 of the trial. The LAO was administered on day 1 and 14. Piglets were weaned from the dams at 12 to 24 hours postpartum and fed to appetite a milk replacer medicated with neomycin (275 ppm). Piglets were artificially reared in individual cages in an environmentally controlled room for 21 days. Growth and feed efficiency, scour score, leukocyte and erythrocyte counts were evaluated. Piglets were sacrificed and lobes of the lung were scored for severity of mycoplasma pneumonia (MP) lesions. The LAHI reduced ($P<.05$) growth, feed efficiency and blood parameters. The LIHI had a slight improvement ($P>.05$) in growth, feed efficiency, and leukocyte count over LILO. These data suggest that the dosage rate of LAO is 100 mg per injection and L is 22 mg per kg of body weight for improvement in performance of artificially reared piglets.

In Experiment 2, the effect of antimicrobial therapy on the intestinal flora was evaluated in 20 piglets from 4 litters which were assigned to one of five treatments. The five treatments were: (1) control with non-medicated milk replacer (CTO); (2) control with neomycin medicated milk replacer (CTN); (3) lincomycin at

22 mg injection per kg of body weight (LIN); (4) long-acting oxytetracycline at 100 mg per injection (LAO); (5) and a combination of LIN and LAO (L+L). Pigs were reared and the same parameters were evaluated as in experiment 1. Coliform (EC) and lactobacillus (LB) counts from five portions of the gastrointestinal tract (GIT) and feces were enumerated. Cell-mediated immunity was evaluated by injecting phytohemagglutinin (PHA) intradermally in the flank. The LAO increased ($P<.05$) EC and LB in the stomach. The L+L increased ($P<.05$) LB in the duodenum and depressed ($P<.05$) EC in the colon. Forty additional piglets from five litters were assigned to treatments 2-5 to evaluate the effect of isolation after antimicrobial therapy. The piglets were randomly allotted to either the research herd nursery or an isolation room. At the end of the seven-week nursery period the pigs were evaluated for average daily gain (ADG), feed efficiency (F:G), scour score (SS) and lungs were scored for MP lesions. The ADG, F:G and SS were: .11, 1.10, 1.29; .13, 0.91, 1.63; .11, 0.98, 1.40; .12, 0.96, 1.51; for CTN, LIN, LAO and L+L, respectively. The LIN increased ($P<.05$) SS and growth of the piglet while in artificially rearing cages. There was no location effect on performance and all treatments performed similarly ($P>.05$) during the nursery phase. No lung lesion differences ($P>.05$) were observed among treatments. These data suggest that antimicrobials did not effect MP severity but growth was improved and GIT bacterial populations were altered.

In Experiment 3, forty piglets from ten litters were assigned by litter to one of four treatments to evaluate the influence of a challenge of MP after antimicrobial therapy. Piglets were reared artificially and parameters evaluated were the same except no bleeding data were collected. The four treatments were the same as treatment 2-5 in Experiment 2. All piglets were exposed at day 3 to a virulent culture of MP inoculated intranasally. Control piglets were reared in a separate room to prevent continuous exposure of MP to the other treatments.

Piglets were sacrificed on day 21 of the experiment and lobes of the lung were evaluated for MP lesions. Piglets reared in the artificial rearing room at the University Swine Farm contracted E.coli scours and 12 out of 30 died amongst the treatments. Although the animals that lived performed similarly to the control piglets which were isolated, the environmental difference is confounded. No differences ($P>.05$) were observed for any lobe or as an average of the total lung among treatments. The culture of MP either did not infect the piglets or they resisted the infection and showed no evidence of MP.

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APPENDIX

Table 1. Analysis of Variance of Growth Performance and Scours in Trial 1.

		Mean Squares[a]				
Source of Variance	df	Initial Weight	Final Weight	Average Daily Gain	Feed:Gain	Average Scour Score
Total	52					
Litter	10	.64**	3.41*	1310.49	.08*	.48*
Treatment	4	.03	3.82*	1868.59*	.12*	1.43**
Residual	38	.03	1.48	658.74	.03	.21

[a] Represents ** P<.001, * P<.05.

Table 2. Analysis of Variance of Lung Lesions in Trial 1.

Source of Variance	d.f.	Mean Squares				
		Right Cardiac	Right Apical	Left Cardiac	Left Apical	Total Lung Average
Total	52					
Litter	10	.68	1.12*	.03	.12	.24
Treatment	4	.86	1.09	.03	.06	.29
Residual	38	.44	.53	.04	.06	.14

* P<.05.

Table 3. Analysis of Variance of Blood Parameters in Trial 1 - Bleeding 1.

		Mean Squares					
Source of Variance	d.f.	WBC[a]	RBC	HGB	HCT	POLY	BAND
Total	54						
Litter	10	12.96	1.05*	4.11**	37.89**	9959933	231599
Treatment	4	73.05**	.78	4.53*	31.42*	68560104*	50739
Residual	40	12.18	.36	1.07	9.53	9380107	175486

** P<.001, * P<.05.

[a]WBC=Leukocytes, RBC=Erythrocytes, HGB=Hemoglobin, HCT=Hematocrit, POLY=Neutrophils, BAND=Band Cells.

Table 4. Analysis of Variance of Blood Parameters in Trial 1 - Bleeding 1.

		Mean Squares					
Source of Variance	d.f.	LYMPH[a]	MONO	EOS	BASO	MYEL	META
Total	54						
Litter	10	930381	247778	32195*	2914	59.07	4994
Treatment	4	930895	645825*	21304	3842	59.08	4667
Residual	40	1892488	197387	13020	2534	59.07	8522

* $P<.05$.

[a]LYMPH=Lymphocytes, MONO=Monocytes, EOS=Eosinophils, BASO=Basophils, MYEL =Myelocytes, META=Metamyelocytes.

Table 5. Analysis of Variance of Blood Parameters in Trial 1 - Bleeding 2.

		Mean Squares					
Source of Variance	d.f.	WBC[a]	RBC	HGB	HCT	POLY	BAND
Total	50						
Litter	10	31.82	.59*	1.99*	20.99*	14911284	81012
Treatment	4	18.31	.17	2.64*	11.53	14632730	60511
Residual	36	24.11	.26	.67	6.00	18445957	66894

* $P<.05$.

[a]WBC=Leukocytes, RBC=Erythrocytes, HGB=Hemoglobin, HCT=Hematocrit, POLY=Neutrophils, BAND=Band Cells.

Table 6. Analysis of Variance of Blood Parameters in Trial 1 - Bleeding 2.

		Mean Squares					
Source of Variance	d.f.	LYMPH[a]	MONO	EOS	BASO	MYEL	META
Total	50						
Litter	10	8020287	183055	14184	1679	163.4	8082
Treatment	4	988803	240412	10453	1516	182.9	8252
Residual	36	6734885	131362	21875	1314	180.2	9506

[a]LYMPH=Lymphocytes, MONO=Monocytes, EOS=Eosinophils, BASO=Basophils, MYEL=Myelocytes, META=Metamyelocytes.

Table 7. Least Squares Analysis of Variance of Growth Performance and Scours in Trial 2.

Source of Variance	d.f.	Mean Squares: Initial Weight	Final Weight	Average Daily Gain	Feed:Gain	Average Scour Score
Total	56					
Litter	8	3.04**	10.89**	1292.9*	.14	.18
Treatment	4	.38	4.78	1290.5	.02	.29*
Residual	44	.23	2.16	556.6	.08	.09

**P<.001, *P<.05.

Table 8. Least Squares Analysis of Variance of Cell-Mediated Immunity in Trial 2.

Source of Variance	df	Mean Squares
Total	57	
Litter	8	1.55
Treatment	4	1.10
Residual	45	1.06

Table 9. Analysis of Variance of Microscopic Lung Lesions in Trial 2.

Source of Variance	df	Mean Squares
Total	39	
Litter	4	.63*
Treatment	3	.17
Residual	32	.20

*P<.05.

Table 10. Least Squares Analysis of Variance of Blood Parameters in Trial 2.

		Mean Squares			
Source of Variance	df	Leukocytes	Erythrocytes	Neutrophils	Lymphocytes
Total	57				
Litter	8	16.40	2.71*	5679794	3096340
Treatment	4	43.86*	.44	7532175	7041222
Residual	45	11.10	.76	3065797	4747043

*P<.05.

Table 11. Least Square Analysis of Variance of Blood Parameters in Trial 2.

		Mean Squares			
Source of Variance	df	Bandophils	Eosinophils	Monocytes	Basophils
Total	57				
Litter	8	437596	23485	17223	3389
Treatment	4	492619	87100*	70135	3657
Residual	45	208783	30184	39686	2325

*P<.05.

Table 12. Least Squares Analysis of Variance of Coliform Counts in Trial 2.

		Mean Squares					
Source of Variance	df	Stomach	Duodenum	Ileum	Cecum	Colon	Feces
Total	18						
Litter	3	3.19	3.49	5.63	2.01	1.10	2.79
Treatment	4	19.74*	7.41	4.67	4.47	.90	4.49
Residual	11	3.31	8.57	11.30	7.65	.39	11.90

*P<.05.

Table 13. Least Square Analysis of Variance of Lactobacillus Counts in Trial 2.

		Mean Squares					
Source of Variance	df	Stomach	Duodenum	Ileum	Cecum	Colon	Feces
Total	18						
Litter	3	2.92*	.82	.25	9.38	1.39*	.09
Treatment	4	1.05	.99	.83	2.22	.11	.35
Residual	11	.67	.55	1.40	6.02	.36	.67

*P<.05.

Table 14. Least Square Analysis of Variance of Nursery Performance in Trial 2.

		Mean Squares			
Source of Variance	df	Weaning Weight	Final Weight	Average Daily Gain	Feed:Gain
Total	37				
Litter	4	7.76*	148.51*	.06*	.001
Treatment	3	2.99	6.05	.01	.03**
Location	1	.21	.33	.001	.001
Residual	29	2.04	52.10	.02	.001

**P<.001, *P<.05.

Table 15. Least Square Analysis of Variance of Growth Performance in Trial 3.

		Mean Squares			
Source of Variance	df	Initial Weight	Final Weight	Average Daily Gain	Feed:Gain
Total	26				
Litter	9	.41**	4.60	1154.6	.05
Treatment	3	.05	3.02	1150.3	.11
Residual	14	.01	2.47	966.3	.04

**P<.001.

Table 16. Least Square Analysis of Variance of Lung Lesions in Trial 3.

Source of Variance	df	Mean Squares				
		Right Cardiac	Right Apical	Left Cardiac	Left Apical	Total Lung Average
Total	26					
Litter	9	.33	.05	.06	.09	.09
Treatment	3	.18	.02	.11	.08	.05
Residual	14	.15	.03	.07	.07	.04

MICROFLORA MANIPULATION OF ARTIFICIALLY REARED PIGLETS

by

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Three experiments were conducted to examine the influence two antimicrobial agents had on growth, blood parameters, the intestinal flora population and the therapeutic action on mycoplasma pneumonia. In Experiment 1, 55 piglets from 11 litters were assigned to one of five treatments to determine a dosage rate for manipulating the microflora of the artificially reared piglet. Two levels of lincomycin (L) were 11 mg (LILO) and 22 mg (LIHI) injection per kg of body weight, and two levels of long-acting oxytetracycline (LAO) were 100 mg (LALO) and 200 mg (LAHI) per injection compared to a control (CTRL). Lincomycin was administered intramuscularly for 3 days after birth and from day 14 to 16 of the trial. The LAO was administered on day 1 and 14. Piglets were weaned from the dams at 12 to 24 hours postpartum and fed to appetite a milk replacer medicated with neomycin (275 ppm). Piglets were artificially reared in individual cages in an environmentally controlled room for 21 days. Growth and feed efficiency, scour score, leukocyte and erythrocyte counts were evaluated. Piglets were sacrificed and lobes of the lung were scored for severity of mycoplasma pneumonia (MP) lesions. The LAHI reduced ($P<.05$) growth, feed efficiency and blood parameters. The LIHI had a slight improvement ($P>.05$) in growth, feed efficiency, and leukocyte count over LILO. These data suggest that the dosage rate of LAO is 100 mg per injection and L is 22 mg per kg of body weight for improvement in performance of artificially reared piglets.

In Experiment 2, the effect of antimicrobial therapy on the intestinal flora was evaluated in 20 piglets from 4 litters which were assigned to one of five treatments. The five treatments were: (1) control with non-medicated milk replacer (CTO); (2) control with neomycin medicated milk replacer (CTN); (3) lincomycin at 22 mg injection per kg of body weight (LIN); (4) long-acting oxytetracycline at 100 mg per injection (LAO); (5) and a combination of LIN and LAO (L+L). Pigs were

reared and the same parameters were evaluated as in experiment 1. Coliform (EC) and lactobacillus (LB) counts from five portions of the gastrointestinal tract (GIT) and feces were enumerated. Cell-mediated immunity was evaluated by injecting phytohemagglutinin (PHA) intradermally in the flank. The LAO increased ($P<.05$) EC and LB in the stomach. The L+L increased ($P<.05$) LB in the duodenum and depressed ($P<.05$) EC in the colon. Forty additional piglets from five litters were assigned to treatments 2-5 to evaluate the effect of isolation after antimicrobial therapy. The piglets were randomly allotted to either the research herd nursery or an isolation room. At the end of the seven-week nursery period the pigs were evaluated for average daily gain (ADG), feed efficiency (F:G), scour score (SS) and lungs were scored for MP lesions. The ADG, F:G and SS were: .11, 1.10, 1.29; .13, 0.91, 1.63; .11, 0.98, 1.40; .12, 0.96, 1.51; for CTN, LIN, LAO and L+L, respectively. The LIN increased ($P<.05$) SS and growth of the piglet while in artificially rearing cages. There was no location effect on performance and all treatments performed similarly ($P>.05$) during the nursery phase. No lung lesion differences ($P>.05$) were observed among treatments. These data suggest that antimicrobials did not effect MP severity but growth was improved and GIT bacterial populations were altered.

In Experiment 3, forty piglets from ten litters were assigned by litter to one of four treatments to evaluate the influence of a challenge of MP after antimicrobial therapy. Piglets were reared artificially and parameters evaluated were the same except no bleeding data were collected. The four treatments were the same as treatment 2-5 in Experiment 2. All piglets were exposed at day 3 to a virulent culture of MP inoculated intranasally. Control piglets were reared in a separate room to prevent continuous exposure of MP to the other treatments. Piglets were sacrificed on day 21 of the experiment and lobes of the lung were evaluated for MP lesions. Piglets reared in the artificial rearing room at the

University Swine Farm contracted E.coli scours and 12 out of 30 died amongst the treatments. Although the animals that lived performed similarly to the control piglets which were isolated, the environmental difference is confounded. No differences ($P>.05$) were observed for any lobe or as an average of the total lung among treatments. The culture of MP either did not infect the piglets or they resisted the infection and showed no evidence of MP.