AN ABSTRACT OF THE THESIS OF

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Title: EFFECT OF FEEDING CONCENTRATES OF LACTOBACILLUS LACTIS ON FECAL BACTERIAL FLORA, SCOURING, AND WEIGHT GAIN IN SWINE

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A frozen concentrate (>10^9 cfu/ml) of a human strain of Lactobacillus lactis (strain MLC) was fed to swine. Bottle feeding resulted in reduced fecal coliform counts and incidence of scouring in nursing pigs. In one group of pigs treated 54 days, the Lactobacillus to coliform ratio was 1280:1; in the control group the ratio was 2:1. The continued suppression of coliforms by the MLC Lactobacillus strain after treatment was discontinued was observed for 30 days.

One hundred twenty-five pigs and their dams were fed concentrates of Lactobacillus lactis MLC through the drinking water system using a commercial water proportioner. The pigs were managed by normal husbandry practices which included a ration containing antibiotics. A significant reduction in coliform counts in the treated pigs was seen by the third week of treatment; and after thirteen weeks of

treatment the coliform counts were reduced by 96%. A significant reduction in coliform counts was also seen in the control pigs by the fifth week of the experiment. The coliform counts in the treated pigs, however, were significantly lower than those of the controls by the third week and thereafter during the experiment. Lactobacillus counts did not increase significantly higher in the treated pigs than in the controls; however, the Lactobacillus to coliform ratio changed from 1:1 to 30:1, and the percentage of coliforms in the total aerobic flora dropped from 21% to 1%. Incidence of scouring was less in the treated pigs than in the control pigs, and there were indications of improvement in weight gain performance in the treated pigs.

A similar experiment was conducted in which antibiotics were withheld from the ration. Reduction in coliform counts was significant in treated pigs by the third week of the experiment and reached 99% by seven weeks treatment. There was no significant reduction in coliform counts in the control pigs during the experiment, and Lactobacillus counts increased significantly higher in the treated pigs than in the controls. A shift in the balance of fecal bacteria was seen in the treated pigs with a change in the Lactobacillus to coliform ratio from 1:1 to nearly 300:1, and a reduction in the percentage of coliforms in the total aerobic flora from 37% to less than 1%. Scouring was not reduced in the treated pigs; weight gain in both the treated and control groups was similar and substandard.

The influence of feeding the Lactobacillus lactis MLC

concentrate to sows was studied. The Lactobacillus MLC concentrate had no effect on the fecal or vaginal flora of the sows, and the fecal flora developed similarly in pigs from treated and control sows.

The pattern of development of the fecal Lactobacillus and coliform flora in newborn pigs during the first 48 hours after birth was studied. Lactobacilli were detected in the feces of newborn pigs as early as four hours after birth and coliforms were detected by eight hours. Coliforms generally surpassed the lactobacilli in number during the first day and remained near ten times greater in number up to 48 hours. By six weeks age, however, the coliforms had decreased in number and the lactobacilli had increased in number to become the dominant flora. A positive correlation between low coliform counts and high Lactobacillus counts during the first 48 hours with high weaning weights was detected, and regression of weaning weight on early coliform and Lactobacillus counts in the pigs studied was found to be significant.

The ecology of autochthonous colonizing bacteria and the nature of the host-symbiote relationship which exists between an animal species and its colonizing lactobacilli are discussed. The potential of the use of Lactobacillus organisms in preventive treatment of intestinal diseases and the importance of these studies to the swine producer are pointed out. It is suggested that lactobacilli native to the animal species with which they are used may be required in order to realize the full potential of Lactobacillus therapy.

Effect of Feeding Concentrates of *Lactobacillus lactis* on Fecal Bacterial Flora, Scouring, and Weight Gain in Swine

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INTRODUCTION

Death of baby pigs due to scouring (diarrhea) is widely reported to be a major cause of economic loss in the swine industry. In a recent poll, pork producers listed mastitis-metritis-agalactia complex (MMA), transmissible gastroenteritis (TGE), and baby pig scours as their most important swine health problems (Leman, 1970).

Baby pigs are usually born into a highly contaminated environment. Scouring is most commonly due to colibacillosis caused by enteropathogenic serotypes of *Escherichia coli* which are usually found in the pig's surroundings. Pigs are born without antibody protection and they receive their only protection for the first four weeks from antibodies received in the mother's colostrum. By three weeks of age, pigs begin eating solid food and the amount of maternal antibody received drops off sharply. Therefore, during the third and fourth weeks of age pigs suffer an immunity gap in a very hostile microbial environment.

Pigs are especially prone to colibacillosis during three periods--the first week of age, the third and fourth weeks of age, and after weaning. Scouring which occurs during the first week is especially

prevalent in pigs removed from the sow and reared artificially. During this period the colibacillosis is most commonly manifest as acute enteritis, and mortality rate is high. Scouring occurring during the third and fourth weeks and after weaning causes less mortality but affects weight gain performance greatly.

Theobald Escherich isolated and described an organism from the feces of a newborn baby in 1885. He believed that the organism (now called Escherichia coli) was a harmless saprophyte. In 1889, Laurelle furnished the first suggestion that E. coli may be pathogenic; and Jensen in 1891 associated E. coli with "kalberruhr," a calf diarrhea that had been recognized in Denmark for about 100 years. Today the association of E. coli with enteric disease in swine is also recognized.

The presence of enteric disease problems in pigs is well documented. There are many reports which establish that death loss from diarrhea in pigs is quite high, and that E. coli has an important role in these losses. England (1974) estimated that 90% of the health problems of the Oregon State University swine herd, which is raised under a Specific Pathogen Free (SPF) type management system, are due to colibacillosis.

It is evident, then, that the control of colibacillosis ranks very high as a priority consideration by pork producers. Although many approaches to controlling the infection and maintaining the pigs have

been partially successful, a definitive answer to the problem of colibacillosis does not yet exist.

Chemotherapy is the most popular and probably the most effective means of control at hand today. Many antibiotics have been proclaimed as the best answer to the problem. Most researchers agree that oral medication is more effective than parenteral administration to the pigs. Consequently (due also to the effectiveness of oral antibiotics in increasing growth rate and the efficiency of food utilization) the routine use of antibiotics in feed rations in the United States is very widespread; more than two and a half million tons are used per year in the United States (Hays, 1969).

The practice of feeding low levels of antimicrobial drugs with the ration to swine to increase weight gain and help prevent scours has also resulted in widespread antibiotic resistance, not only in E. coli but in many other bacteria pathogenic for humans as well.

Antibiotics, then, are not only losing their effectiveness, but, are also creating possible human health hazards. Sandine et al. (1972) have reviewed the significance of drug resistant E. coli as a hazard to human health. They have cited a serious increase in the incidence of fatal infantile gastroenteritis due to highly resistant enteropathogenic strains of E. coli.

The drug resistance and infectivity of these organisms is mediated by resistance transfer factors (RTF). (RTF are genetic

extrachromosomal episomes which transfer multiple drug resistance to other bacterial cells during physical contact.) The use of antibiotics favors the development of bacteria carrying RTF; and there are now a growing number of studies which demonstrate how RTF of animal origin can be transmitted to man.

In addition to the increasing incidence of disease due to enteropathogenic strains of E. coli, other diseases such as bacillary dysentery and salmonellosis, which in the past have been successfully treated with antibiotics, are becoming more serious today due to RTF acquired from E. coli.

Due to the potential of risk to public health by the widespread and indiscriminate use of antibiotics in animal feeds, many researchers are turning their attention to preventive measures such as vaccination, the use of copper sulfate as a feed additive, the establishment of SPF herds, better management conditions, and the feeding of normal intestinal bacteria capable of inhibiting harmful strains of E. coli.

It is well known that the intestinal microflora play important roles in the health of the individual, and that a balance exists among the various species of which the normal intestinal bacteria are comprised. The scouring which occurs in young pigs infected with E. coli is accompanied by a shift in the relative numbers in the species which normally inhabit the intestinal tract. There are many factors

which can affect the balance and thereby predispose the individual to shifts in the population of bacteria.

Scouring usually occurs at times which are stressful to the pigs. In addition to a balance in the population of bacteria in the intestinal tract, there also exists a balance between the resistance of the host and the pathogenicity of the microorganisms present. The types of E. coli present may range through a spectrum from true harmless saprophytes to opportunists to true pathogens. If the host's resistance becomes lowered during periods of stress, the balance shifts toward greater pathogenicity of the opportunists--which are normally held in check. In addition, other pathogenic species such as Vibrio and Salmonella, which are not normal inhabitants, may take the opportunity to establish during periods in which the balance in the population of intestinal bacteria, or in the host's resistance, is upset by stress, antibiotics, and many other factors.

The above has led to the present study in which concentrates of Lactobacillus organisms have been fed to pigs in an attempt to establish and stabilize a desirable intestinal flora. It is known that lactobacilli exist in an equilibrium state in the intestine with the coliform flora.

Lactobacilli are members of the lactic acid bacteria (Family Lactobacteriaceae) which have been praised for their health-giving properties for many years. They are widely distributed in nature

and, since they produce lactic acid as the principal end product of their carbohydrate fermentation, they have been useful to man for thousands of years as a food preservative. Because of their long association with man in his diet they have evolved as important members of the indigenous intestinal flora. They exist in balance with the other intestinal and vaginal microorganisms and have assumed a protective role.

Man has recently begun to recognize the importance of these bacteria (especially the Lactobacillus and Bifidobacterium genera) in human health, and there is little doubt now that colonization by lactic acid bacteria is a major factor in minimizing enteric disease in the newborn (Muralidhara, 1974).

This knowledge has been put to use in the livestock industry by some producers (especially in Europe) and many others have expressed interest. There has also been a recent increase in the popularity of fermented milk products--especially in yoghurt containing Lactobacillus acidophilus, and in acidophilus milk which is used in conjunction with antibiotic therapy.

The basic premise of this study, then, is that by feeding large numbers of viable lactobacilli, it might be possible to depress the numbers of E. coli present, and/or stabilize the ratios of numbers of

intestinal lactobacilli to coliforms in a manner ultimately resulting in a lower incidence of scouring and greater weight gain performance in the swine herd.

REVIEW OF LITERATURE

Enteric Colibacillosis of Swine

There are several disease syndromes which may be placed under the broad general heading of colibacillosis. They have been characterized in a number of recent review articles (Leman and Lehr, 1970; Barnum, 1971; Sojka, 1972a and 1972b; Kohler, 1972; and others). They are diarrheal disease of newborn pigs (neonatal colibacillosis), coliform enteritis of weaned pigs (weanling colibacillary diarrhea), edema disease (enterotoxemia), and syndromes resulting from bacteremia and septicemia in newborn pigs. Of these, the first two are the most common and are important to pork producers. Neonatal colibacillosis, the most prevalent of these syndromes, is seen as diarrhea, usually without morphological evidence of enteritis (Smith and Jones, 1963), and resembles cholera in man (Smith and Jones, 1963; Moon et al., 1966a; Nielsen et al., 1968; Gyles, 1968; Gyles and Barnum, 1969). The last of these syndromes is not further discussed in this review since it is not an enteric manifestation of colibacillosis.

Clinical Picture

Many workers have described similar sets of clinical characteristics in neonatal colibacillosis (Barnum, 1971; Underdahl and

Melbus, 1971; Dillard, 1964; and others). In general, pigs which become afflicted with neonatal colibacillosis are normal at birth and develop diarrhea during the first few hours or days of life. Their coats become rough and dull and the pigs appear emaciated. They are dehydrated and frequently drink water from the dam's trough. They display generalized weakness and loss of weight, listless appearance, and unsteady gait. Affected pigs may vomit, and most continue to nurse until they become depressed prior to death. The perineum becomes pasted with fluid or semisolid feces which are watery and white to yellow in color. The base of the tail and the labia frequently are inflammed and the tail may slough.

The disease pattern on a farm may be sporadic or enzootic (Barnum et al., 1967). Characteristically, not all litters farrowed during an enzootic of colibacillosis--and sometimes not all pigs in any one litter--are affected. Most often though, all the pigs in a litter are affected (Dillard, 1964).

Mortality rate is high. Underdahl and Melbus (1971) found that mortality is near 70% when pigs are infected before the third day after birth, but less than 40% when infected after two weeks. Occasionally a whole litter may die; more often a few survive and make complete recovery (Stevens, 1963a).

Stevens (1963a) has referred to coliform enteritis of weaned pigs as "catarrhal enteritis." Weaned pigs are usually affected

within one or two weeks after weaning. Often large, thriving pigs collapse and die after a short period of diarrhea (Sojka, 1972a). The clinical picture resembles that of neonatal colibacillosis, but mortality is low and sometimes only one or two pigs in a litter are affected (Stevens, 1963a). The usual clinical manifestations are fever, diarrhea, dehydration, and emaciation. There may be erythema and purplish discoloration of the underline and legs of severely affected pigs (Barnum et al., 1967). Post-mortem examination usually reveals generalized catarrhal enteritis (Stevens, 1963a).

Edema disease is an acute disease of young pigs which occurs commonly about one week after dietary changes (especially weaning) (Sojka, 1972a). It has been noted to occur under other conditions as well, such as shipping or vaccination in pigs as young as four days old (Barnum et al., 1967). Various aspects of this condition have been reviewed by Sojka (1965, 1972a, 1972b), Barnum et al. (1967), Shimmelpfennig (1970), Nielsen and Clugston (1971). Typically, the larger more rapidly growing pigs are affected while other remain normal. Affected pigs are usually not seen to be sick but are merely found dead; when seen, they show muscular incoordination, partial paralysis, and blindness, and are occasionally found in lateral recumbency, paddling, or trembling. A hoarse squeal as the result of laryngeal edema may occur. The eyelids may be thickened by edema which may extend over the frontal bones. Most affected pigs die

within 24 hours, but some recover and a few display clinical signs for a few days.

Bacteriological Findings

Escherichia coli, which for a long time has been generally considered to be a normal inhabitant of the intestinal tract has also been seen to be associated with pathological conditions in farm animals and man. A number of research workers have examined the causes of coliform-associated pig mortality, and, in nearly every instance, hemolytic strains of E. coli have figured prominently in death caused by enteritis and scouring. Smith and Jones (1963) reported that their bacteriological findings were consistent; certain E. coli serotypes which were often hemolytic proliferated in the small intestine of pigs with neonatal coli diarrhea. The E. coli were confined to the intestinal tract and bacteremia was not a usual feature of the disease, even though it occurred in terminal stages at times. Kenworthy and Crabb (1963) noted that the hemolytic E. coli appeared in the jejunum and ileum of baby pigs at the onset of diarrhea and gastroenteritis. Other bacteria did not increase when this occurred. The intestinal flora associated with early weaned pigs was studied by Chopra et al. (1963a). They found the greatest increase in E. coli (coliforms) when diarrhea occurred. Sojka (1972a) reported on a bacteriological examination of over 6000 dead pigs, carried out by the Veterinary

Investigation Service in England and Wales, which showed that 36% of the deaths could be attributed to E. coli and only 1.3% to salmonellae. Most deaths occurred in young pigs.

Bacteriological findings in weanling colibacillary diarrhea resemble those of neonatal coli diarrhea according to Stevens (1963a). Serotypes of E. coli in profuse pure culture, similar to those associated with neonatal colibacillosis, can be recovered from the intestines of pigs with weanling coliform enteritis (Roberts and Vallely, 1959). Richards and Fraser (1960) isolated hemolytic E. coli from the gastrointestinal tracts of all of the 30 pigs which died of acute enteritis post-weaning and also from rectal swabs of three non-fatal cases.

In edema disease, abundant hemolytic E. coli (often pure culture) of certain serotypes are isolated from the large and small intestine, and from the mesenteric lymph nodes, but there is no invasion of other tissues (Stevens, 1963a; Sojka, 1972b). Representatives of serotypes found in pigs with edema disease were also found by Richards and Fraser (1960) in the strains of hemolytic E. coli isolated from cases of enteritis. According to Sojka (1972b), hemolytic strains isolated from cases of edema disease belong to relatively few serological groups, some of which are generally non-enteropathogenic while others are usually enteropathogenic. Due to the effects of enterotoxins produced by these strains, diarrhea usually

occurs in edema disease associated with the latter. Erskine, Sojka, and Lloyd (1957) and workers listed by Sojka (1972b) have reproduced a "condition indistinguishable from field outbreaks of bowell edema" by the intravenous innoculation into susceptible pigs of extracts of E. coli isolated from cases of edema disease.

Sojka (1965) reviewed the development of reliable serological methods for differentiation of strains of E. coli. Many workers have demonstrated the association between certain serological types and disease conditions in man and animals. Even though there are a vast number of E. coli serotypes, relatively few are associated with man and animals, and with a few exceptions, they appear to be host-specific (Sojka, 1972a).

Dillard (1964) pointed out that colibacillosis is a major cause of economic loss in the swine industry. Even well-managed herds, according to McErlean (1960), often are involved, and the contents of the small intestine usually provide overwhelming evidence that E. coli is the chief cause of piglet death and impairment of herd performance.

Predisposing Factors

Intensive swine production methods greatly concentrate swine community populations through confinement rearing in limited space. Close association, as pointed out by Dillard (1964), aids in spreading

pathogenic agents in such an environment. Barnum (1971) stressed the importance of clean quarters and frequent disinfection of the environment, especially near farrowing time, in reducing the exposure of the sow and her litter to enteropathogenic E. coli. Dillard (1964) has listed a number of conditions as factors which tend to make the pig more susceptible to colibacillosis. Among these are inadequate bedding, significant variations in environmental conditions, nutrition of the dam, agalactia and mastitis of the dam, brucellosis in the herd, leptospirosis in sows, continued inbreeding, or any other condition which may result in weak pigs in the litter.

Variations in the amount of immunity acquired by the pig via the colostrum during the first 24 hours has been cited by Stevens (1963a) as reasons why not all pigs in a litter are infected at times, and why there seems to be great variation in the outcome of infection between one pig, or one litter, and another. Such factors as agalactia in the sow, or pigs being too weak at birth to suck would influence the amount of colostrum received. The timing of infection in relation to taking colostrum might also be important. Saunders et al. (1960) thought that some sows might have more antibody in their colostrum than others depending on their previous contact with pathogenic serotypes of E. coli; and he noted that the incidence of colibacillosis is higher in litters of gilts than in those of sows.

Leece (1969) demonstrated the role of the highly contaminated

environment into which baby pigs are born as an important predisposing factor for enteric colibacillosis in pigs. To do this he caught pigs at the moment of birth and removed them to individual isolated cages with automatic feeding devices. These colostrum-deprived pigs by two weeks of age outgained naturally suckled pigs, and death losses were near zero as opposed to a 20% to 30% loss in pigs reared conventionally. Coalson and Leece (1973) found that pigs which nursed 12 hours and 36 hours respectively were equal in performance when farrowed in a relatively sanitary environment, but when farrowed in a less desirable environment a "catastrophic diarrhea" developed in pigs nursing 12 hours while littermates nursing 36 hours remained mainly asymptomatic. Thus, artificial rearing of pigs in an environment contaminated with other swine does seem possible provided the pigs are farrowed in a relatively sanitary environment and the pigs are allowed to nurse for 12 hours.

A change in environment and in diet may be quite significant in the post-weaning syndrome. An immunity gap also occurs at this time when passive immunity provided by the sow suddenly stops before the pig's active immunity is fully developed. These factors combined with a low iron level at this time have an additive effect in predisposing the weaned pig to infection with enteropathogenic E. coli (Stevens, 1963a).

Pathogenesis

Enteropathogenic *E. coli*

The term enteropathogenic *E. coli* (EEC) was introduced by Neter *et al.* (1955) to distinguish enteric disease causing strains of *E. coli* from those strains associated with other diseases. EEC were recognized by Adam (1923) and today are known to cause severe and highly fatal infantile gastroenteritis in hospitals (Tomic-Karovic and Fanjek, 1962; South, 1971; Gorbach and Khurana, 1972). A problem of some magnitude with disease due to EEC strains also exists in laboratory animals (Schiff *et al.*, 1972); EEC strains were isolated from mice, rats, hamsters, gerbils, guinea pigs, agouti, beagle dogs, and rhesus monkeys. Only 25% of these showed disease symptoms.

The role played by hemolytic *E. coli* in colibacillosis in pigs has been pointed out above. Recently Glantz and Kradel (1971) noted colibacillosis in swine in the United States caused by the Abbotstown strain of *E. coli*. Researchers in England and Canada reported that this serotype produces a powerful enterotoxin.

The exact role of hemolysins in the production of the disease is not known for sure. Hemolytic strains of *E. coli* have been generally implicated in colibacillosis in swine, but the causal relationship has not been demonstrated. Smith (1963) showed that most strains of

E. coli that cause diarrhea in pigs produce hemolysin; however, some non-pathogenic strains inhabiting the alimentary tract of man and animals also have this property. Kenworthy and Crabb (1963) found that when scouring occurred in newborn pigs, hemolytic strains of E. coli increased by at least 99%. It was shown by Smith and Halls (1967a) that the production of alpha-hemolysin by E. coli is controlled by a transmissible plasmid; however, alpha-hemolysin may not be a virulence determinant since the diarrhea producing capacity of EEC has been shown to be independent of the presence of this plasmid (Smith and Lingood, 1971).

The enteropathogenic potential, according to Moon and Whipp (1971), is made up of two components--first, colonization in the jejunum in large numbers, and second, stimulation of the movement of water and electrolytes across an intact intestinal epithelium into the lumen. Similarly, Punyashthiti and Finkelstein (1971) suggested that for an enterotoxic E. coli to express its enteropathogenicity in the small bowel, at least three conditions must be fulfilled: (1) the accidentally introduced strain must be able to establish itself (colonize) in the upper small intestine of the host, (2) the strain must be able to produce enterotoxin, and (3) the host must be reactive and responsive to this enterotoxin. Work by Smith and Halls (1968b), Barnum (1971), Sojka (1972), Muralidhara (1974), and others supports this concept. Bertschinger et al. (1972) has recently indicated that

human EEC can be divided into two groups--one that penetrates the intestinal epithelium and does not produce enterotoxin, and a second that remains in the lumen (colonizes) and produces enterotoxin.

An enterotoxin produced by EEC colonizing the small intestine, then, seems to be the primary cause of diarrhea in pigs with colibacillosis. Studies by Smith and Halls (1967a and 1967b), Kohler (1968), Nielsen and Sautter (1968), Gyles and Barnum (1969), Smith and Gyles (1969 and 1970), Kohler (1971), and Sojka (1972) on ligated sections of pig intestines have supported this.

Work by Porter and Kenworthy (1969), however, demonstrates that the production of toxic diamines in the small intestine by decarboxylation of the amino acids arginine, ornithine, and lysine by E. coli may be an important cause of diarrhea in pigs at weaning time.

The nature of EEC enterotoxin has been studied by Smith and Halls (1968a and 1968b), Kohler (1968), Gyles and Barnum (1969), Smith and Gyles (1970), Kohler (1971), and Jacks et al. (1973). Two forms of the toxin are known. One is heat labile (LT), and the other is heat stable (ST) (Taylor and Bethlehem, 1966; Smith and Gyles, 1970; Gyles, 1971 and 1972). The LT type enterotoxin is antigenic and is neutralized by antiserum, while the ST type is not. EEC strains possess the latter type. Those having the K88 antigen produce both types of enterotoxin. Both forms of enterotoxin are controlled by transmissible plasmids (Gyles, 1972). The LT type

enterotoxin produced by Vibrio cholerae and procine EEC are similar--both produce similar responses in ligated segments of small intestine of pigs and rabbits (Gyles and Barnum, 1969; Smith and Gyles, 1970).

Smith and Halls (1968b) discovered a genetic factor, designated Ent that is probably a plasmid. This factor was responsible for enterotoxin production in six of 51 EEC strains from pigs, and was transmissible to other strains of E. coli, to Salmonella typhimurium, and to S. choleraesuis by conjugation in mixed culture. Smith and Gyles (1970) reported that both LT and ST preparations from porcine EEC strains possessing Ent produced diarrhea in experimental pigs, whereas similar preparations from strains without Ent were mostly harmless.

Mechanisms of Diarrhea Production

The mechanisms by which EEC produce diarrhea are speculative, but they appear to be associated with enterotoxin. EEC colonize the anterior small intestine where they produce an enterotoxin which in the presence of intact epithelium causes massive outpouring of fluid from the tissue into the intestinal lumen and thereby cause diarrhea and dehydration (Moon, 1969; Sojka, 1972). Kohler and Cross (1969), and Smith and Halls (1967a) have shown that diarrhea is consistently produced when bacteria-free filtrates from EEC are

administered. The apparent response is that of an uncontrollable loss of isotonic fluids. Young pigs have lost as much as 18% of their initial body weight within six hours after oral administration of LT enterotoxin (Kohler, 1972). This results in diarrhea and dehydration. The accumulation of fluid in the intestine is due either to reduced flux of fluid from the intestinal lumen into the blood or by increased secretion of fluid from the blood into the lumen, or a combination of both.

Barnum et al. (1967) listed the following theories to explain the altered fluid flux:

1. Capillary permeability is altered, allowing the escape of colloids and other osmotically active particles such as sodium into the intestinal lumen.

2. Absorption of osmotically active particles from the intestinal lumen is blocked.

3. Osmotically active metabolites are produced.

4. E. coli split intestinal macromolecules into many smaller osmotically active particles.

5. The absorptive surface area of the intestine becomes altered and cannot efficiently absorb digested particles. The intestinal wall behaves as a semipermeable membrane, allowing osmosis.

These hypotheses depend on the increase in osmotically active particles within the intestinal lumen which cause water to be held or secreted into the lumen to maintain correct osmolality.

Nielsen et al. (1968), however, feels that the accumulation of

abnormal, osmotically active particles is not likely since the fluid that accumulates in ligated loop experiments is nearly iso-osmotic and has an ionic composition similar to plasma.

In any case it appears that the fluid losses are not a result of a classic inflammatory response with discharge of mucus from the goblet cells (Kohler, 1972). Nielsen et al. (1968) has shown that the fluid that accumulates in the intestinal lumen during colibacillosis has a low protein content and is therefore not typical of an inflammatory exudate. The epithelium of the intestinal mucosa remains intact; no gross microscopic or macroscopic changes are observed in affected individuals (Taylor, 1961). There is no penetration by bacteria into other parts of the body (Sojka, 1972).

Nielsen et al. (1968) suggested that movement of fluid into the intestine and the resulting diarrhea could be caused by increased membrane permeability as the result of enterotoxin action on vascular permeability, the development of an abnormal osmotic gradient as a result of enterotoxin action, or by increased active transport and secretion of water into the lumen.

Electron microscopic studies by Kenworthy et al. (1967) support evidence that colibacillosis causes an increase in membrane permeability, while studies by Fordtran et al. (1967) and Carpenter et al. (1968) provided evidence for active solute transport or secretion into the intestine.

It has recently been suggested by Al-Awqati et al. (1972) and Evans et al. (1972) that the fluid accumulation produced by EEC enterotoxin might be associated with increased adenyl-cyclase activity. This is also considered to be the case with cholera enterotoxin (Carpenter, 1971; Sharpe and Hynie, 1971; Kimberg, et al., 1971). The enterotoxin activates intestinal adenyl cyclase in the lateral border of the mucosal cells, and the resulting increase in intracellular cyclic AMP (adenosine 3':5' monophosphate) is a signal that produces changes in ion transport which can explain the loss of fluid in the intact animal.

Many investigators have suggested that the pathogenesis of human cholera, caused by Vibrio cholerae is similar to enteric colibacillisis. Both produce an enterotoxin distinctly different from the endotoxin of the cell wall of gram negative bacteria (Smith and Halls, 1967a; Gyles and Barnum, 1969; Leman and Lehr, 1970; Moon and Whipp, 1971). In fact, Gyles and Barnum (1969), and Smith and Sack (1973) have shown that the enterotoxins from EEC and V. cholerae were antigenically related. In the first instance enterotoxin of V. cholerae was neutralized by E. coli P307 OK antiserum; and, in the second, cholera antitoxin from immune sera neutralized E. coli toxin in rabbit ileal loops, but the reverse was not true in this study.

Role of Endotoxins

Death from enteric colibacillosis is not usually associated with endotoxic shock (Barnum et al., 1967) but probably results from prolonged dehydration and electrolyte imbalances which cause cardiac failure. Hemoconcentration and acidosis (as a result of the loss of large amounts of water, sodium, bicarbonate, and chloride), and uremia are common at the terminal stages of the disease (Barnum et al., 1967).

E. coli endotoxin, however, may be involved in enteritis in pigs. Shreeve and Thomlinson (1972) suggested that sudden deaths, a feature of neonatal E. coli diarrhea, might be explained by the lethal effect of endotoxin acting before the enterotoxin induced diarrhea. E. coli endotoxin also appears to be involved in hemorrhagic enteritis, a disease characterized by sudden deaths and inflammation in the large intestine which occurs in pigs in the post-weaning period (Stevens, 1963b).

Thomlinson (1963 and 1969), Stevens (1963b) and others have suggested that hemorrhagic enteritis is due to an anaphylactic reaction. Pigs have a varying degree of hypersensitivity to certain E. coli serotypes present in small numbers in the intestine. If these E. coli begin to multiply rapidly, a sudden absorption of E. coli polysaccharide endotoxin from the intestine takes place producing an

anaphylactic reaction in previously sensitized animals. Whether this reaction produces hemmorhagic enteritis or edema disease depends on the degree of hypersensitivity of the animal and on the amount of polysaccharide absorbed.

Others, however, consider that edema disease is caused by absorption of a specific edema causing endotoxin called "neurotoxin" (Schimmelpfennig, 1970) or "EDP" (Edema Disease Principle) (Nielsen and Clugston, 1971). When absorbed, it causes injury to vasculature and affects vascular permeability.

Role of Intestinal Microflora Balance

The protective effect of a normal, well balanced intestinal microflora has been documented in a recent research review by Sandine et al. (1972) and by Muralidhara (1974). It has been shown by many workers cited by these authors that the intestinal bacteria play a vital role in human and animal health and are a highly significant factor in the resistance of human beings and animals to enteric infections. Disturbances in the natural balance may, then, lead to the proliferation of members capable of producing enteric disease.

Kenworthy and Crabb (1963) reported that while the intestinal tract is sterile at birth, within 24 hours lactobacilli, gram positive cocci, Clostridium perfringens, and E. coli appear; gram positive cocci and Bacteroides sp. appear later. They found that when

scouring occurred, hemolytic strains of E. coli increased by at least 99% and that stress brought on by weaning contributed to the shifting balance of organisms.

The intestinal flora associated with enteritis of early weaned pigs was studied by Chopra et al. (1963a). They found, similarly, that the greatest increase in coliforms occurred during diarrhea. They also indicated that there was a balance between lactobacilli and coliform bacteria in non-scouring pigs which became greatly altered when diarrhea was present. Hill and Kenworthy (1969) implied that high levels of hemolytic E. coli may be tolerated in the presence of established and continuously proliferating lactobacilli.

Other workers have also emphasized the importance of a proper microbial balance in the intestinal tract to disease resistance in pigs (Kershaw et al., 1966; Cole et al., 1968). This has naturally led to studies in which certain bacteria, especially lactobacilli, have been fed to scouring pigs infected with EEC in attempts to restore the proper balance of bacteria. This area will be pursued later in this review.

There is also evidence for antagonism among the E. coli types inhabiting the intestinal tract. According to Dillard (1964) pathogenic serotypes of E. coli usually exist as a small proportion of the bacteria comprising the intestinal flora, however, they may approach a dominant status when conditions unfavorable to previously dominant

bacteria are present. For this reason, finding pure or nearly pure cultures of pathogenic strains of E. coli is a characteristic clinical manifestation of EEC infection.

In a study on the ecology of E. coli in the alimentary tract of swine, Craven (1969) concluded that the intestinal tract of young pigs usually contains a single, or very few strains of E. coli which dominate at any one time but whose replacement can be rapid and complete. Barnum (1971) found that not all litter mates have similar coliflora either, and there can occur abrupt and complete changes in litter coliflora as well as that in the individual. He noted that the coliflora of sows differed from that of the progeny also, in that there was a high proportion of non-typable strains, and a greater number of types at any one time; and there was no succession of dominant types. The distribution of types in the environment paralleled their occurrence in pigs.

Since an EEC may be a temporary dominating strain, factors responsible for domination could be of assistance in control. The possibility that colicins were a factor in domination was noted by Craven (1969), since he had observed that dominant E. coli types were colicin producers. (Colicins are bactericidal substances in the Enterobacteraceae which are active upon other related bacteria with the degree of specificity of bacteriophage, but without the capacity to reproduce themselves. Colicin sensitivity is related to

antigenic structure (Barnum et al., 1967). Branche et al. (1963) felt that the ability of some strains to maintain themselves was associated with colicin production, since bacterial antagonism in the urinary tract of rats had been demonstrated to be mediated by colicin production (Braude and Siemienski, 1968). They demonstrated bacterial antagonism between procine isolates of E. coli in gnotobiotic pigs when a colicin producer dominated a colicin sensitive strain. However, when a colicin resistant mutant of the sensitive strain was used in the same system, a similar pattern of replacement was evident. This and other evidence, as when two non-colicin producers were used, indicated that a mechanism other than colicin production was responsible for replacement.

Colonization by EEC

There are many reports on the pathogenicity of E. coli. Takeuchi (1967) classified pathogenic intestinal bacteria into three groups according to their site of action in the intestines. They are:

1. pathogens that invade the intestinal mucosal layer,
2. pathogens that attach themselves to the intestinal epithelium but are not invasive,
3. pathogens that neither penetrate nor attach themselves to the intestinal epithelium but still produce symptoms.

Muralidhara (1974) cited considerable evidence that EEC colonize the mucosal layer and villi of the small intestine in large

numbers; whereas, non-EEC are non-colonizing. In 1973 he studied frozen thin sections from the small intestines of scouring pigs and was able to classify EEC in Takeuchi's second group. The colonizing EEC were seen attached in large numbers to the free border of the villus epithelium and between adjoining villi. They were not grouped as invasive since they were seen only occasionally within the epithelium cells. In addition to being located on villi, EEC were also observed at the apical region of intestinal crypts, but were rarely observed within the basal region of the crypts. It was postulated that this was due to a greater exposure if the EEC to the villi than the crypts.

Staley et al. (1969) found that in starved newborn piglets, EEC are associated with the exfoliation of microvilli prior to the attachment of bacteria to the apical plasma membrane; however, in a contradictory report by Drees and Waxler (1970), degeneration of microvilli was rarely observed.

In 1971, Mouwen (1971) studied the villous architecture in the small intestines of scouring and normal three week old pigs using stereomicroscopy. He described nine different morphological types of villi and found differences in the types of villi found between normal and scouring pigs. Changes were seen in all regions of the intestines. He found that the severity of mucosal changes in the jejunum increased distally and that the mucosa in the duodenum was

more severely altered than in the jejunum. Least serious change was seen in the ileum.

Strains of EEC isolated from diseased pigs frequently possess K88 antigen (Orskov et al., 1964; Orskov and Orskov, 1966). Its production is controlled by a transmissible plasmid that can be spontaneously lost (Orskov and Orskov, 1966; Smith and Halls, 1968b). In 1967, Stirm et al. (1967a and 1967b) suggested that the K88 antigen may be an important factor in colonization of the anterior small intestine by some porcine EEC strains since it forms a substantial layer of fine filaments (pili) complementary to fimbrae on the surface of the cell conferring great adhesive properties to the organism. Gyles (1972) also suggested that pili may be plasmid mediated virulence determinants. Smith and Lingood (1971) found that when the K88 plasmid was removed from an EEC strain, its diarrhea producing capacity was lost, but was again restored when a K88 plasmid was introduced. These workers suggested that K88 enables the organism to proliferate in the anterior small intestine; the Ent plasmids appear to play no part in this proliferation--only in the diarrhea that follows. Jones and Rutter (1972) studied the role of K88 antigen of E. coli in neonatal diarrhea in pigs by comparing a K88-positive strain with three K88-negative strains derived from the K88-positive strain. They found that K88-positive bacteria adherred to the mucosa of the small intestine, whereas K88-negative bacteria

did not attach and were distributed throughout the lumen. Smith and Lingood (1971) felt that since not all porcine EEC strains possess the K88 antigen, the production of mucinase might also be important in colonization by EEC on the villous mucopolysaccharide layer of the pig small intestine. However, Arbuckle (1970) demonstrated that there was no difference between the mucinase production of the EEC strains and of non-EEC strains isolated from healthy pigs.

Control of Colibacillosis in Pigs

The control of colibacillosis in young pigs now ranks as one of the most important problems facing the pork producer today. Although many approaches to controlling the infection and maintaining the pigs have been partially successful, a definitive answer to the problem of colibacillosis does not yet exist.

Antibiotic Therapy and Antibiotic Resistance--Public Health Considerations

The use of antibiotics for the prevention and the treatment of scours in pigs is now very popular as evidenced by the large scale and highly competitive advertising of products by the drug industry, and by the wide variety of antibiotic products now available for use with pigs.

In 1949, Henderson and McKay (1949) successfully treated

white scours in calves by oral dosage with streptomycin. The spectacular results led to treatment through supplemented feed in the early 1950's when antibiotics became less expensive and stable preparations were available (Barnum, 1973). Many antibiotics have since been proclaimed for their effectiveness by many researchers (Beckett et al., 1961; Cloyd, 1962; Chopra et al., 1963b; Stevens, 1963a; Hughes, 1963; Kohler and Bohl, 1964; Kenworthy and Crabb, 1965; Ringarp and Wemmert, 1965; Mongeau and Larivee, 1965; Collear and Smith, 1966; and others).

The widespread use of antibiotics in feed rations in the United States is due also to their effectiveness in increasing growth rate and feed efficiency (Jukes, 1971, 1972a, 1972b; Nebraska Swine Report, 1971).

There now exists a very high rate of multiple antibiotic resistance by E. coli and many other pathogenic bacteria as well. This problem has been considered in depth by Smith (1967) and Barnum (1973), and by other workers (Smith and Crabb, 1957; Sojka and Carnaghan, 1961; H. W. Smith, 1968 and 1969; D. H. Smith, 1969); and it now appears that antibiotics are of marginal merit due to problems of drug resistance.

Barnum (1973) summarized the most important aspects of these problems of drug resistance in pathogenic bacteria at a 1972 symposium sponsored by the Health Protection Branch of the

Department of National Health and Welfare at Ottowa, Canada, as the following:

1. The most serious problem of multiple resistance is the inability to provide adequate antimicrobial therapy in animals or man to infections by such organisms.

2. Organisms carrying plasmids that control antibiotic resistance are more likely to carry plasmids associated with pathogenic properties. Smith and Halls (1968b) have shown that the production of enterotoxin by E. coli strains is plasmid-controlled.

3. Since the resistance patterns of enteric organisms is varied, laboratory testing of all isolates is a prerequisite before specific antibiotic therapy can be initiated.

4. The multiple drug resistance of enteric organisms in many animal populations is so extensive that there is little possibility of an early change to a sensitive flora.

5. With the present practice of antibiotic supplemented feeds and antibiotic therapy, a continuous selective pressure is exerted that favors the continuance or increase of resistant strains.

At first thought to be due to spontaneous mutation and selection, the development of resistant strains is now concluded to be due to infective drug resistance (R factors) whereby multiple drug resistance is transferred from a resistant to a sensitive cell by contact (Akiba et al., 1960; Watanabe, 1963, 1967 and 1971; Rownd, 1967; Meynell et al., 1968; Anderson, 1968; Mitsuhashi, 1969 and 1971; Smith, 1971; Rownd et al., 1971. That this occurs in vivo between members of the enterobacteria (shigellae, salmonellae, E. coli, Klebsiella, Proteus, and related organisms) inhabiting the intestinal tract of man

and animals is now well documented and recognized as a major hazard to public health (D. H. Smith, 1969). Barnum (1973) summarized the mechanism of action as follows:

> The genes responsible for infectious drug resistance consist of (a) the genetic determinants that code for factors which facilitate the transfer, and (b) those that code for resistance, one for each antibiotic. These genes are located extrachromosomally, and are known as plasmids. The two factors, resistance determinants (R determinants) and transfer factors (RTF), can exist independently. The sex component factor (RTF) enables the cell to act as a genetic donor, and also induces the cell to produce specific cell appendages known as sex pili. These are pili which appear in the electron microscope as fine filaments, and are vital in sexual conjugation as they unite the donor and recipient cell. In some manner not completely understood, the R determinants and the RTF are transferred very quickly. Plasmid multiplication in the recipient cell happens more quickly than cell division, so that spreading of the determinants of drug resistance occurs in the sensitive population.

A number of reports have agreed that the percentage of multiple resistant strains that have transferrable drug resistance has increased in recent years (Tanka et al., 1969; Moorehouse, 1971; Mitsuhashi, 1971; Walton, 1971); and the evidence is "overwhelming that an alarming percentage of the enteric flora of man and animals possess transferrable drug resistance. This reservoir has and will influence the treatment and management of bacterial diseases due to gram negative enteric organisms" (Barnum, 1973).

The growing concern over the magnitude of this problem has led to the organization in 1967 by the National Academy of Sciences--National Research Council of a symposium on the use of drugs in

animal feeds at which over 40 papers were presented giving evidence suggesting that infectious multiple drug resistance is an important public health concern (National Academy of Sciences, 1969); and, in a conference held by the New York Academy of Sciences in 1970 (New York Academy of Sciences, 1971) many papers provided additional cause for the growing public health concern over infectious multiple drug resistance.

As an indication of the growing threat to human health, a study carried out by Moorehouse and McKay (1968) in a large children's hospital in Dublin, Ireland, showed that 15 out of 22 infants excreted infectious drug resistant strains of E. coli when admitted to the hospital, and all 22 children shed the multiple antibiotic resistant strains before leaving. In addition, these authors found that antibiotic resistant enterobacteria carrying R factors were isolated from 81 of 100 healthy urban infants and there was no correlation between previous drug therapy and the resistant flora.

At a symposium at the 73rd Annual American Society of Microbiology meetings, Dr. Gorbach (Gorbach and Pierce, 1973) stated that at any one time there are as many as 200 million people in the world suffering from enteric infections, the majority of which are caused by enteropathogenic E. coli. This becomes more significant in light of many recent reports linking infectious drug resistant organisms of animal origin to the production of human disease. In

1968, Anderson (1968) reported the animal origin of Salmonella strains with R factors in the human population; and in 1969, Moore-house et al. (1969) isolated E. coli carrying R factors from both cooked and uncooked sausage which reportedly evolved in the intes-tines of pigs. In 1970, Walton (1970) surveyed each of 400 pork and beef carcasses and found that the majority yielded drug resistant strains of E. coli, 40% of which were able to transfer resistance. Similarly, Kelly et al. (1971) isolated E. coli strains from the muscle and kidney from 25 to 26 calves after slaughter, 96 of which were resistant to four or more drugs. All strains isolated possessed transferrable resistance.

In addition to the transfer of drug resistant pathogens from animals to man, Anderson (1968) has reported that drug resistant non-pathogens of animals may reach and colonize the environment of man for a sufficient length of time for the transfer of resistance to the human host flora. Furthermore, clinical evidence was obtained by Aserkoff and Bennett (1969) that salmonellae gained from contami-nated turkey gained R factors from the antibiotic resistant enteric flora of persons consuming the infected meat.

The data is insufficient, however, to prove conclusively that foods of animal origin are a source of infectious drug resistance in humans. For example, Guinee et al. (1970) in a study designed to test the supposition that livestock are a reservoir of multiple

resistant bacteria found that the percentage of resistant E. coli was larger in a group of vegetarians and babies studied than in a group of meat eating individuals. On the other hand, Linton et al. (1972) found that the incidence of drug resistant E. coli was 63% in those whose occupation involved close contact with farm animals, compared to 29% in other populations.

Several recent reports have provided evidence for a link between the use of antibiotics as feed additives in livestock and the presence of R factors in human contacts. Tschape et al. (1973) found that transferrable tetracycline resistance in E. coli found in 50% of 1000 healthy children in kindergarten in Germany was due to tetracyclines used as feed additives in animal production. Wells and Jones (1973) found that pigs which received antibiotics in the feed carried more multiply resistant R factor bearing coliforms than pigs which had not been given antibiotics, and that human contacts of the antibiotic treated pigs had a higher incidence of antibiotic resistant coliforms with R factors than human contacts of pigs which had not been given antibiotics. They suggested the following possible course of events:

Antibiotics
↓
Animal Gut Flora
↓
Antibiotic Resistant Coliforms With R Factors in Animal Guts
↓

Ingestion of Animal Products/Human Contact

↓

Antibiotic Resistant Coliforms With R Factors in Human Gut

↓

Transfer of R Factors By Conjugation

↓

Antibiotic-Resistant Human Coliforms (Reservoir of R Factors)

↓

Conjugation With Antibiotic Sensitive Salmonella/Shigella

↓

Antibiotic Resistant Human Pathogens

Research by Latimer (1974) on R factor transfer among bacteria isolated from the environment in a companion project to the present study at Oregon State University has indicated that R factors from R^+ isolates obtained from pigs are transferrable in vitro to a variety of bacteria from different environmental sources (some of which are considered opportunistic human pathogens). It was suggested that these resistant organisms might play a role in in vivo dispersion of R factors through animals and animal handlers into areas where the presence of such R factors would constitute a serious hazard to human health.

Barnum (1973) pointed out that it must be recognized that man can be exposed to resistant E. coli in ways other than through meat; nevertheless, one must not assume that food could not be a potent source of bacteria carrying transferrable multiple resistance. The contamination of a relatively few individuals with such strains, and the resulting change in flora could affect a much wider population through subsequent human-human contact.

Barnum (1973) has summarized the public health hazards from antibiotics in farm animals. They can arise from:

(a) tissue residues of antibiotics in milk and meat

(b) tissue residues of the metabolites of antibiotics

(c) transfer of multiple resistant organisms

These hazards for man are:

1. sensitization and anaphylaxis
2. direct toxicity of drug or its metabolite
3. disease caused by resistant bacteria of animal origin
4. drug resistant bacteria of agricultural origin, transferring their ability to resist antibiotics to human pathogens

The increase in magnitude of the animal and human disease problem caused by antibiotic resistant bacteria has led scientists to recommend discriminant use of drugs both as animal feed supplements and in disease therapy (Findland, 1969; D. H. Smith, 1969; H. W. Smith, 1969), though at least one author (Kampelmacher, 1969) has expressed the opinion that the increase in numbers of pathogenic drug resistant bacteria has not seriously hampered disease therapy in man. In England, the addition of antibiotics to feed for cattle and swine is now prohibited (Swan, 1969) as a result of the increase in drug resistant salmonellae which gained R factors from E. coli. These salmonellae have caused deaths in humans. Recent announcements from the Food and Drug Administration (FDA

Antibiotic Task Force Report, 1972) indicate that the United States is moving in this direction also.

Pfizer, Inc. has recently begun marketing a new chemotherapeutic agent, 3-methyl-(2-quinoxaline)-carbazate N^1N^4 dioxide (carbadox), under the trade name Mecadox. Although the mode of action has not yet been determined, it has been shown to be effective in controlling scours in young Australian pigs, and there was a dose related response in growth rate and feed conversion efficiency. It is claimed that it has value as a feed additive in intensive animal production because it is not an antibiotic and is not used therapeutically in human medicine (Holder and Sinclair, 1972).

From the foregoing, then, it is quite evident that it is becoming imperative to search out alternatives to the use of antibiotics for the prevention and treatment of enteric diseases in livestock, both because they no longer have the effectiveness they once had and because of the potential threat to human health their use poses.

Management

The prevention of colibacillosis requires, above all else, good management practices. The eradication of EEC strains is impractical due to their ubiquitous distribution; however, any measures designed to reduce the concentration of EEC in the environment are desirable. The goal of husbandry practices in preventing or

controlling colibacillosis is to prevent exposure of the animal to pathogenic strains of E. coli, or to produce an animal with a high degree of resistance to colibacillosis (Barnum et al., 1967; Arbuckle, 1968). Barnum (1971) listed three major points by which the control of colibacillosis can be accomplished: (1) reduction of pathogenic strains in the environment to minimize the domination of the coliflora by such strains, (2) reduction of active proliferation of the organisms in the upper intestinal tract, and (3) resistance of the host to clinical disease either through immune mechanisms in the neutralization of toxic factors (enterotoxin) or by genetically non-reactive animals.

Moon (1969) has stated that continuous farrowing in the presence of EEC may make the control of enteric colibacillosis impossible with existing methods. Measures, such as thorough cleaning and disinfection followed by a period of vacancy of the farrowing quarters between successive farrowings have been reported to be effective in preventing baby pigs from being born into a highly contaminated environment (Barnum, 1971; Van Marke, 1972); however, these measures are reported to be laborious to implement (England et al., 1972).

The operation of closed herds as a method of strictly controlling EEC in the environment by preventing introduction of E. coli strains foreign to the herd was recommended by Barnum et al. (1967). In a presentation detailing alternatives to the use of antibiotics in swine

herd management, D. C. England (England et al., 1972) pointed out the success the SPF (Specific Pathogen Free) approach has had in eliminating specific target diseases (atrophic rhinitis, virus pig pneumonia, and transmissible gastroenteritis). He feels that widespread adoption of an SPF-like system by a major proportion of swine producers can help to reduce production losses from the most serious diseases (including colibacillosis) for which antibiotics are now used.

Nutrition has been considered by many to be an important factor in the prevention of colibacillosis. It has been shown that vitamin A deficiency in the dam and iron deficiency anemia in the young pig may be predisposing factors in colibacillosis (Barnum et al., 1967). Losos (1964) reported that a highly nutritious diet is more likely to cause the development of a dominant hemolytic E. coli population than a diet low in nutritional value. It has also been reported that conventional and gnotobiotic pigs fed a diet with a base of whole cows milk are highly susceptible to bacterial infection (Kenworthy, 1968).

Thomlinson (1969) noted that gastric pH of pigs is important in reducing numbers of hemolytic E. coli moving into the small intestine; and he found that when pigs are fed finely ground meal, the pH does not become high enough in the center of the feed mass before it passes into the small intestine. When pigs were restricted to

coarsely ground feed the numbers of E. coli moving into the intestine were reduced.

Resistance and Immunity

Resistance of the host to colibacillosis is acquired passively through the ingestion of maternal colostrum containing antibodies against E. coli (Barnum, 1971). The role of pig colostrum in the prevention of colibacillosis, and the nature of its protective effect has been reviewed by Barnum (1971) and Muralidhara (1974). Immunoglobulins in the colostrum constitute around 64% of the whey protein, and are comprised of IgG (79.7%), IgA (14.5%), and IgM (6.27%) (Porter, 1969a). It was demonstrated by Porter (1969b) that the colostral antibodies to E. coli are associated with IgA. He found that these antibodies could not be detected in the serum of the sow or of the pig, but that other IgA antibodies not associated with antibody to E. coli were absorbed from the colostrum. This suggested the production of IgA antibody in the mammary gland of the sow that remains in the gut and acts locally.

Kohler (1967) and Arbuckle (1968) both found that specific serum antibody level derived either actively or passively does not protect the pig from clinical enteric disease produced by the pathogen, and does not inhibit proliferation of the organism within the gastrointestinal tract; however, Miniats et al. (1970) demonstrated that oral

administration of specific antibody to gnotobiotic pigs protected them successfully during the period of treatment. These findings confirmed the original observation of Owen et al. (1961) that orally administered serum from normal sows was more valuable for prevention of naturally occurring diarrhea than was parenterally innoculated serum.

In a recent experiment Coalson and Leece (1973) allowed one group of pigs to nurse for 12 hours after birth and another group to nurse for 36 hours. Both groups were farrowed under less than sanitary conditions. The group which was allowed to nurse for the longer period remained essentially asymptomatic compared with the other group which suffered severe diarrhea. Both groups had equal circulating immunoglobulin levels, but the intestinal epithelium in the pigs nursing for 36 hours was exposed to immunoglobulin longer than the pigs nursing 12 hours. This led these researchers to propose that the pathogenesis and pathology of the disease is mainly in the intestine. This requires that moderation of the infection must occur in the intestine by continuous bathing of the intestine by inhibiting immunoglobulin, and that it is independent of circulating immunoglobulin.

This, and other evidence cited by Muralidhara (1974) tends to confirm the role of colostral IgA as a mediator of local intestinal immunity. Thus, consumption of a significant amount of colostrum

shortly after birth is an important factor in the prevention of neonatal colibacillosis. Barnum (1971) has cited a dramatic fall in the total protein content of colostral whey within 24 hours after birth with half of this fall occurring within four to six hours. Since the majority of pigs commence suckling within a few minutes after birth, and since pigs born early move from teat to teat taking colostrum that is let down at each birth, Bourne (1969) concluded that pigs born early in the farrowing process are likely to have physiological and immunological advantage over littermates. Therefore, a management practice of removing each pig as it is born from the sow and returning the entire litter may have practical advantage.

In view of the protective value of colostral antibody, it seems logical to protect newborn animals against colibacillosis by vaccinating dams with E. coli bacterins, or to orally administer hyperimmune serum (since colostral antibodies are derived from the blood) directly to the newborn. Various workers have demonstrated that colostrum from sows immunized by either intramammary or parenteral administration of specific E. coli antigens is protective against specific E. coli strains when fed to pigs; whereas, colostrum from unimmunized sows is not protective (Rejnek et al., 1968; Wilson, 1972). The action of the immunoglobulins seems to be that of enterotoxin neutralization (Gyles and Barnum, 1969; Wilson, 1972) since there is not an accompanying reduction in coliform counts (Miniats, 1970). This

was supported in work by Muralidhara (1974) which demonstrated that oral doses of immune serum had no effect in preventing colonization by E. coli in the small intestine.

Rutter and Anderson (1972) studied the effect of vaccinating the dam on piglet mortality and found that although there was a reduction in the mortality of pigs from vaccinated compared with non-vaccinated dams, the reduction was not significant when the variation between litters was taken into account.

Recently the Unilever Research Center in England has announced the development of an effective oral vaccine which it intends to market in leading pig producing countries. The oral vaccine is reported to be effective only while it is being fed (Pig International, 1973).

There are factors in colostrum and milk in addition to immunoglobulins which may also play an important role. Masson and Heremans (1971) have shown the presence of large quantities of iron-binding protein in milk of which the greater proportion was lactoferrin, though small amounts of transferrin were also present. Bullen et al. (1972) have shown that lactoferrin in combination with specific antibody to E. coli is responsible for a bacteriostatic effect, and they have suggested that iron-binding proteins of milk play an important role in resistance to E. coli infection, particularly in the small intestine. Several other workers have demonstrated the

bacteriostatic effect of iron-binding proteins (Masson and Heremans, 1966; Oram and Reiter, 1968).

Lactobacillus Therapy of Intestinal Disease

The importance of a balanced intestinal microflora to the health of the young pig has already been pointed out. Desirable members of the intestinal flora are not well defined but numerous studies dating from the early 1900's to the present indicate that lactic acid bacteria, especially those of Lactobacillus and Bifidobacterium genera, are important. The precise nature of their beneficial effect, direct or indirect, remains to be proven, though studies recently carried out by Mata et al. (1972) leave little doubt that intestinal colonization by lactic acid bacteria is a major factor in minimizing enteric disease among newborn infants. Attention was drawn to the fact that the proper balance of organisms in the intestines of infants is important to their post-natal adjustment as early as 1899 when Tissier (1899) noted the presence of characteristic Y-shaped (bifidus) lactic acid bacteria in the feces of breast-fed babies to the exclusion of coliforms and other bacteria.

Attempts to alter or maintain a proper microbial balance in the intestinal tract to improve health date back to early work by Metchnikoff (1903, 1907, 1908) at the Pasteur Institute. He noted that in some European countries where people consumed large amounts of

yogurt containing lactobacilli, the incidence of intestinal disorders was comparatively low. Since that time, and after clarification provided by Rahe (1915) that L. acidophilus rather than L. bulgaricus implanted in the intestinal tract, lactobacilli have been used extensively in therapy. The older literature regarding its therapeutic value has been documented in one bibliography of abstracts (Frost and Hankinson, 1931) and three textbooks (Kopeloff, 1926; Rettger and Cheplin, 1921; Rettger et al., 1935).

K. S. Muralidhara has worked together with this author on different aspects of the same research effort (effect of feeding lactobacilli to swine on intestinal colonization by E. coli), and he has included in his Ph.D. thesis (Muralidhara, 1974) a complete review of the role of the indigenous intestinal bacterial flora in host resistance to enteric infection, and of the history and effectiveness of previous use of lactobacilli in intestinal disease therapy. In addition, Sandine et al. (1972) (the same research group), in their review of the use of lactic acid bacteria in food and health, also discussed the role played by lactic acid bacteria in human and animal health. For this reason a detailed review in this same area will not be presented here, and the reader is referred to these other works. Important findings of Muralidhara (1974) will be included in the discussion of this thesis.

MATERIALS AND METHODS

Frozen concentrate of Lactobacillus organisms was used experimentally in three ways to ascertain its influence on changes in the numbers and ratios of fecal lactobacilli and coliforms, incidence of scouring, and weight gains in suckling and weaned pigs. The three methods of use were (1) hand-fed in specified amounts to individual pigs, (2) through the drinking water to dams and their litters, and (3) by feeding through the water supply to dams during lactation prior to breeding, through gestation, to farrowing, and to both dams and their offspring during lactation subsequent to farrowing.

Lactobacillus Organisms Used

The Lactobacillus MLC concentrates used in this study were obtained from Microlife Technics, Sarasota, Florida. Mr. Stewart M. Farr of Microlife Technics originally isolated the bacterium from the human intestinal tract. Muralidhara (1974) characterized the organism using the tests indicated in the report of the Taxonomic Sub-Committee on Lactobacilli and Closely Related Organisms (Anonymous, 1968), as possessing all the typical characteristics of the genus Lactobacillus. Based on fermentation characteristics, antigenic analysis, guanine + cytosine content, and DNA-DNA hybridization experiments, he subsequently identified Lactobacillus MLC as

Lactobacillus lactis. The organism was found to be sensitive to sodium taurocholate in spite of its original habitat being the human intestine, and even though it was found to colonize the intestinal tract of pigs. It was also found to have little similarity with L. acidophilus.

Management of the Oregon State University Swine Herd

The Oregon State University swine herd is operated under an SPF (specific pathogen free) type program, and is maintained under carefully controlled conditions, but the usual incidences of scouring caused by E. coli are observed. Animals receive an oral antibiotic ("Day One") at birth containing neomycin and iron; creep feed from 10 days to 60 pounds weight includes ASP 250 made up of chlortetracycline, sulfamethazine, and penicillin; then a growth ration, also containing ASP 250 is fed up to 150 pounds weight; and, finally, the pigs are fed a finish ration with all antibiotics removed up to 215 to 225 pounds. The average daily weight gain averages about 1.7 to 2.0 pounds per day from 60 to 200 pounds under these conditions. Oral doses of water soluble tylosin are used to treat scouring when it is observed.

The entire herd is maintained in confinement on partially slotted floors. All animals used in the herd are born and reared in the herd, with only a rare exception (one animal introduced physically in

the last 10 years). The microbiological status is thus expected to be more stable than with animals in contact with soil, and in a herd to which new animals from other sources are frequently added.

Sampling and Enumerating Procedures

The following procedures were adhered to in all experiments of this study. Fecal samples were collected with a sterile acetate swab, taking care to prevent contamination from the perineal region of the pigs and sows sampled. Bacterial counts were made according to the method described by Smith and Crabb (1961). Within 30 minutes of collection, a 1:10 dilution (W/V) of the fecal sample in sterile distilled water was made and mixed well using a vortex mixer to yield a homogeneous suspension. From very young pigs it was occasionally impossible to obtain a large enough sample to make a 1:10 dilution practical and the dilution (W/V) was increased to 1:50 or 1:100.

Vaginal mucus samples from sows were collected using a sterile plastic speculum (3/4' diameter x 13" length) through which was inserted a 4" x 4" sterile cotton gauze pad with attached string which had been previously weighed. The gauze pad was allowed to remain in contact with the cervical area for one minute and then was retrieved through the speculum using the attached string. Prior to sampling, the perineal area of the sow was scrubbed with an iodine disinfectant solution and then rinsed off with sterile water to prevent

contamination of the sample with disinfectant. Bacterial counts were then made according to the methods described by Smith and Crabb (1961) for fecal counts with the modification that a 1:100 or 1:500 (W/V) dilution of the mucus sample in sterile distilled water was made.

The principal bacteria identified and counted were Escherichia coli and lactobacilli. E. coli counts were estimated from the number of typical lactose-fermenting colonies (coliforms) on MacConkey's Agar incubated at 37°C for 24 hours. Typical colonies were further characterized using the various biochemical tests described in Bergey's Manual of Determinative Bacteriology (1969).

Lactobacillus organisms counted were based on characteristic colonies found on Rogosa's agar (Rogosa et al., 1951) incubated in the presence of 95% nitrogen and 5% carbon dioxide at 37°C for 48 hours. To achieve this N_2-CO_2 atmosphere, Torbal steel cylinders (Model AJ-2), or in the case of a large number of samples, an anaerobic incubator, containing the innoculated plates were evacuated three times using a vacuum pump to 25 lbs Hg with the gas mixture delivered from a pressure tank. Typical colonies were characterized using the biochemical tests provided in the API test pack system (Analytab Products, Inc., 516 Mineola Ave., Carle Place, N. Y.). Usually only one colony type was seen; occasionally, however, different colony types were seen which also were determined to belong

to the genus Lactobacillus.

In addition, total aerobic counts were obtained using brain heart infusion agar with 5% sheep blood incubated aerobically at 37°C for 48 hours; total anaerobic counts were obtained on the same medium incubated by the same method as was used for Lactobacillus counts.

Fecal Bacterial Flora in Normal and Scouring Pigs

As a preliminary to this investigation, the fecal bacterial flora of normal pigs of different ages selected at random from the Oregon State University Swine Herd was studied with respect to numbers of coliforms, lactobacilli, streptococci, Bacteroides, and clostridia and reported by Muralidhara (1974). This material will also be reported in this dissertation due to its relation to the work conducted in this investigation.

As described in the review of literature, the balance among intestinal bacteria is important in maintaining normal health. For unknown reasons stress conditions cause changes in this balance. Since it is also known that swine are prone to scour at stages in their life when stress occurs, it was considered pertinent to study changes in relative numbers of desirable (lactobacilli) and undesirable (EEC or coliform) bacteria during periods of scouring and stress.

Bottle Feeding Experiments

A litter of nine pigs was divided into two groups and a fecal sample was taken from each pig before putting it on test. The plating procedure was the same as described earlier. Attention was given to *E. coli*, *Lactobacillus*, total aerobic and total anaerobic counts. One group of four pigs from this litter was fed the usual ration while the ration of the other five pigs was supplemented with the concentrate of MLC *Lactobacillus*. The feeding schedule for the concentrate was as follows:

10 to 15 ml per pig per day from birth to weaning

30 to 40 ml per pig per day after weaning

Concentrate feeding was started when the pigs were three days old. Fecal samples were taken every day for the first week, every other day from the second week until weaning, and every third day thereafter. The sampling and plating were done as described earlier.

A second experiment was undertaken with a different feeding schedule. A litter of nine pigs was used as above with the daily feeding schedule as follows:

0 to 15 days of age	10 ml per pig
16 to 30 days of age	15 ml per pig
31 to 8 weeks of age	30 ml per pig
Over 8 weeks of age	50 ml per pig

Feeding Lactobacilli Through the Drinking Water System

After studying the influence of the Lactobacillus MLC concentrate on pigs fed individually, a series of three experiments were undertaken to determine the feasibility of feeding animals on a large scale and to obtain a larger, more complete body of data to which statistical analyses could be applied. This was accomplished by feeding the concentrate through the drinking water system to a large number of pigs and their dams.

Water Proportioner Set-Up

The water supply of the pens used to house the Lactobacillus treated pigs was fitted with a commercially available water proportioner commonly used to meter antibiotics through the water. This equipment metered the Lactobacillus concentrate into the water at a fixed rate (1 oz. per gallon) as the water was consumed by pigs. The concentrate (with the addition of 10% lactose) to be distributed into the water system was stored in a four-liter reservoir in a refrigerator located adjacent to the proportioner which dispersed the concentrate into the water. Fresh bacterial concentrate and lactose were added daily except more often when increased water consumption necessitated additional supply. Attempts were made to have a constant supply of concentrate available with a minimal wastage from the

daily change.

Before beginning the herd feeding experiments, the number of organisms which individual pigs would consume each day with normal water intake was approximated by determining the dilution factor of the concentrate in the water system and the approximate amount of water individual pigs consumed each day. The Lactobacillus MLC concentrate contained a minimum of 50×10^9 viable cells/ml; 900 ml were added to 2980 ml of water containing about 10% lactose which provided a dilution factor of 900/3880 or 0.23. Therefore there were $(50 \times 10^9)(0.23)$ or 11.5×10^9 cells/ml in the water proportioner reservoir. Since the proportioner delivered one ounce (30 ml) per gallon (3880 ml) a second dilution factor of 30/3910 or 0.008 resulted. Therefore, there were (11.5×0.008) or 0.092×10^9 cells/ml in the water lines. This assured that pigs drank water containing at least ten million cells/ml, since it already was established that the cells were quite stable with regard to viability. If pigs drink an average of one liter of water per day, they would then receive 92×10^9 viable organisms per day.

As a further check the numbers of viable organisms coming through the water taps in the pigs pens were determined over a ten-hour period from the time fresh concentrate was added to the reservoir by plating dilutions of water samples at the drinking source before the water proportioner system was used in any experiments.

At various time intervals after the addition of fresh concentrate to the reservoir throughout the first experiment, samples of water at the taps in random pens were taken. At the same time samples of concentrate in the reservoir were also taken. These were subsequently plated and tested for pH.

Experiment No. 1

In the first experiment 24 pens of pigs (12 treated through the water system with Lactobacillus MLC concentrate and 12 untreated) were studied. The pigs were brought into the water treated and control pens during the third week of age. The pigs were managed by the established husbandry practices at the Oregon State University Swine Center. This included a ration supplemented with antibiotics (ASP 250). Both the treated and control pigs received the same ration. Prior to the beginning of treatment, fecal samples were taken from each litter of the Lactobacillus MLC treated pigs and a comparable sample was taken from the control pigs as well. Samples were taken from three randomly selected pigs from each pen weekly through the 15th week of age for the water treated pigs and through the 11th week of age for the control pigs. Plate counts of Lactobacillus, coliform, total aerobic and total anaerobic bacteria were made as described earlier. Daily records of scouring and weight gain performance records were kept.

Experiment No. 2

After the completion of the first experiment it was decided that it would be beneficial to conduct a similar experiment, this time without the use of antibiotics in the ration and with the addition of a control group of pigs hand fed concentrates of Lactobacillus MLC. Twenty-four litters of pigs were used for this experiment. Twelve litters and their dams were treated with Lactobacillus MLC as before through the drinking water system, and the remaining twelve litters served as controls. From the control litters two pigs were selected at random from each litter (24 pigs) to be bottle fed concentrates of Lactobacillus MLC daily. The daily feeding schedule was the same as described for the second bottle feeding experiment. From the water treated and control groups two pigs were selected at random from each litter to be sampled (24 treated pigs and 24 control pigs) before the start of the experiment and thereafter the same two pigs were sampled at each sampling period. The 24 bottle fed pigs were also sampled at each sampling period. Sampling and plate counts for Lactobacillus, coliform, total aerobic and total anaerobic bacteria were conducted as described earlier. The following sampling schedule was followed:

Sample 1 . . . . . At Start (Two weeks age)

Sample 2 . . . . . Week 3 (Five weeks age)

Sample 3 . . . . . Week 4 (Six weeks age--Pre-wean)

Sample 4 . . . . . Week 5 (Seven weeks age--Post-wean)

Sample 5 . . . . . Week 7 (Nine weeks age)

Each pig was weighed as it was sampled in order to record the average daily gain. Daily scouring records for each pig litter were kept. The pigs were managed by the usual husbandry practices of the Oregon State University Swine Center except that a ration containing no antibiotics was fed. Pigs were treated with water soluble antibiotics for scouring only if it became very severe or very widespread.

Experiment No. 3

The third experiment was conducted for two reasons. It was desirable to determine whether feeding the Lactobacillus MLC concentrate to sows for an extended period prior to farrowing would have any effect on the subsequent development of the fecal flora of pigs in their litters; and this experiment also gave us the opportunity to observe the development of the fecal coliform and Lactobacillus flora in newborn pigs for the first 48 hours.

Forty sows were initially used for this experiment (20 water treated and 20 control). However, due to a variety of reasons several sows were removed from the experiment so that 18 water treated and 12 control sows actually finished this experiment. Beginning during the early lactation period, the treated sows received the Lactobacillus MLC water treatment as described earlier. Treatment

of the sows was continued through gestation and the next farrowing and subsequent lactation. Fecal samples were taken and plated for Lactobacillus, coliform, total aerobic, and total anaerobic counts by the methods described earlier.

The literature review of K. S. Muralidhara (1974) cites evidence that the development of the fecal bacterial flora in human infants may be related in some way to the vaginal bacterial flora of the mother. Therefore, in addition to fecal bacterial counts, the vaginal flora of the treated and the control sows was also sampled and an attempt was made to enumerate the coliforms and the lactobacilli as well as total aerobic and total anaerobic flora. Vaginal samples were taken and plated as described earlier. The following sampling scheme was followed:

Sample 1 . . . . . Early Lactation

Sample 2 . . . . . Pre-Weaning

Sample 3 . . . . . Estrus (Before Breeding)

Sample 4 . . . . . 30 Days Gestation

Sample 5 . . . . . 60 Days Gestation

Sample 6 . . . . . 90 Days Gestation

Sample 7 . . . . . 112 Days Gestation (Pre-Farrowing)

Lactobacillus MLC water treatment was continued to the treated dams and their litters through the subsequent lactation.

Fecal samples were taken at birth from two pigs (usually the

first two pigs born) from the litters of each treated and control sow. The same two pigs from each litter were then sampled according to the following schedule:

Sample 1 . . . . . 4 Hours After Birth

Sample 2 . . . . . 8 Hours After Birth

Sample 3 . . . . . 12 Hours After Birth

Sample 4 . . . . . 16 Hours After Birth

Sample 5 . . . . . 24 Hours After Birth

Sample 6 . . . . . 32 Hours After Birth

Sample 7 . . . . . 40 Hours After Birth

Sample 8 . . . . . 48 Hours After Birth

Sample 9 . . . . . 6 Weeks Age (Weaning)

Since it was known that the first few samplings under this schedule should reflect very few or no bacterial counts, it was possible to determine to what extent unavoidable fecal contamination occurring during sampling would result. Therefore, a pig was caught in a sterile pan at birth and sacrificed. Cultures were taken from the contents of the large intestine, small intestine, and rectum using aseptic surgical technique, as well as from the exterior of the pig and from vaginal fluid from the sow. Plate counts were determined for numbers of Lactobacillus and coliforms present.

Pigs from both the treated and control litters were observed daily for scouring until weaning, and weaning weights were taken.

Again, routine management procedures at the Oregon State University Swine Center were observed with both the sows and their litters except that antibiotics were withheld from the ration. Antibiotics were again used to treat scours only in extreme cases.

Statistical Analysis

The data obtained from the three Lactobacillus MLC water feeding experiments were analysed using standard statistical methods and procedures as described by Steel and Torrie (1960) with the aid of the computer at the Oregon State University Computer Science Center. Significance of difference was determined using Table A.3, "Values of t" of Steel and Torrie (1960). The probability of the values obtained being due to chance alone was tested and is presented at 95% and 99% confidence limits. Significance of correlation coefficients obtained was determined using Table A.13, "Significant Values of r and R" of Steel and Torrie (1960), and significance of regression coefficients obtained was determined using Table A.6, "Values of F" of Steel and Torrie (1960) again at 95% and 99% confidence limits.

Geometric means of bacterial counts were determined and log transformations of all counts were utilized for all statistical analyses in order to normalize the data in an observable range and observe the variances on the same relative scale. This was necessitated since the valid application of tests of significance in the analysis

of variance requires that experimental errors be independently and normally distributed with a common variance. The values obtained in these experiments covered an extremely wide range and error effects which were multiplicative on their original scale were made additive on the logarithmic scale.

The significance of difference between geometric mean counts of treated and control pigs at each sampling period, and the significance of difference of geometric mean counts within the treatments (treated and control) was determined for each experiment for Lactobacillus, coliform, total aerobic, and total anaerobic counts.

In Experiment No. 2, correlation and regression coefficients were determined within treatments for the pre- and post-weaning sample geometric means on the initial sample geometric means of Lactobacillus and coliform counts. Also calculated within treatments were correlation and regression coefficients of average daily gain with or on geometric mean Lactobacillus and coliform counts for each sampling period. These were also calculated for average daily gain for all sampling periods with or on the average of the geometric mean Lactobacillus and coliform counts over all sampling periods.

In Experiment No. 3, correlation and regression coefficients were determined for geometric means of Lactobacillus and coliform counts with or on the Lactobacillus and coliform geometric means of samples three to nine combined. Also determined were the

correlation and regression coefficients of weaning weights on the geometric mean Lactobacillus and coliform counts of samples three to nine combined for each pig, and of weaning weight on weaning sample geometric mean counts for lactobacilli and coliforms.

RESULTS

Fecal Bacterial Flora in Normal and Scouring Pigs

Table 1 shows the numbers of different genera of bacteria recovered from fecal samples taken at random from pigs of different ages in the Oregon State University Swine Herd. The data generally confirm the findings of Smith and Crabb (1961) on the numbers and types of bacteria found in pig feces. From Table 2 it may be seen that 32×10^9 cfu/gm of feces for the total (arithmetic sum) anaerobic count on selective media compares favorably with 67×10^9/gm for the total anaerobic count found on brain heart infusion blood agar. However, the aerobic counts on selective media (12×10^8 cfu/gm) were about one log lower than the total aerobic count on blood agar. This indicated that the selective media were inhibiting some coliforms and/or staphylococci, or that other bacteria such as Gram-negative cocci were numerically important in the aerobically incubated samples.

Changes which take place in the fecal bacterial flora in pigs with scouring are seen in Table 3 and in Figure 1. From these results it is evident that during scouring the numbers of lactobacilli remained the same. This pattern of increase in coliforms with scouring was observed in the majority of scouring pigs examined. In the case of healthy non-scouring pigs, the Lactobacillus counts

Table 1. Normal fecal bacterial flora in pigs at various ages.

Age	cfu/gm of Feces					
	Coliforms	Lactobacilli	Streptococci	Staphylococci	Bacteroides	Clostridia
1st week	500,000,000	800,000,000	400,000,000	20,000	400,000,000	200,000
2nd week	1,000,000,000	1,000,000,000	60,000,000	5,000,000	1,600,000,000	3,000,000
Weaned (6 weeks)	600,000,000	3,000,000,000	90,000,000	100,000	740,000,000	3,000,000
Adult	300,000,000	2,000,000,000	2,000,000	100,000	140,000,000	700,000

Table 2. Bacterial flora of pig feces determined on selective media.

Organism	Cfu/gm	
	Selective Media	Blood Agar
Coliforms	1,000,000,000	
Staphylococcus aureus	220,000,000	
Sum of the aerobic counts[a]	1,200,000,000	
Total aerobic counts		59,000,000,000
Bacteroides sp.	1,100,000,000	
Clostridium sp.	14,000,000	
Lactobacillus sp.	26,000,000,000	
Streptococcus sp.	5,000,000,000	
Sum of anaerobic counts	32,000,000,000	
Total anaerobic counts		67,000,000,000

[a]Arithmetic sum to two significant figures.

Table 3. Changes in the fecal coliform and Lactobacillus counts at the time of scouring in eight suckling pigs.

Fecal Consistency	Cfu/gm[a]	
	Coliforms	Lactobacilli
Normal	200,000,000	20,000,000,000
Normal	400,000,000	20,000,000,000
Normal	80,000,000	20,000,000,000
Normal	200,000,000	20,000,000,000
Scouring	19,000,000,000	20,000,000,000
Scouring	22,000,000,000	18,000,000,000
Soft	14,000,000,000	20,000,000,000
Soft	6,000,000,000	15,000,000,000

[a]Data represent bacterial counts from fecal samples taken at three-day intervals.

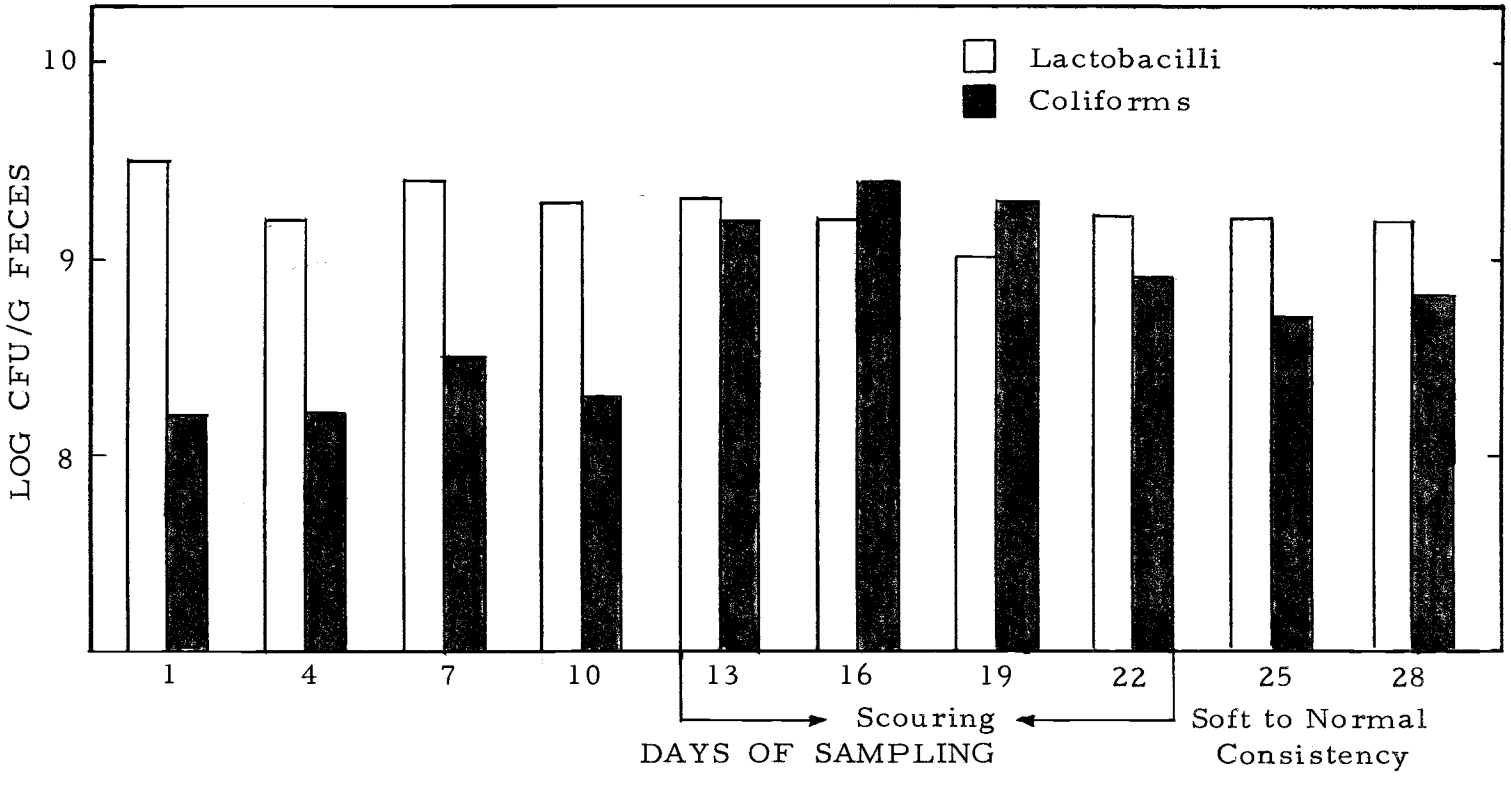


Figure 1. Fecal Lactobacillus and coliform counts in pigs from birth to four weeks of age.

were usually slightly higher in number than the coliform counts (Figure 1 and Table 3).

Bottle Feeding Experiments

Results of the first experiment are shown in Figures 2 and 3. It can be seen that there was not much reduction in coliform counts up to the weaning period (8 weeks); also, at the time of weaning the ratio of Lactobacillus to coliforms was almost 1:1. However, when an increased amount (30 ml) of the concentrate was fed, the next sampling (two days later) showed a sudden decrease in the coliform counts (Table 4). This indicated that the quantity of concentrate given early in the experiment was insufficient. After six months of treatment, a 95% decrease in the coliform counts was found in comparison to the control animals, but little reduction in the severity of scouring was noted.

Results of the next feeding experiment with a different feeding schedule appear in Figures 4 and 5. Here a gradual decrease (Figure 5) in the coliform counts can be seen; also, there was no rise in coliform counts either at the second or third week of age nor after weaning. After 90 days of Lactobacillus MLC feeding there was almost a 99.9% decrease in coliform counts. The E. coli counts of control pigs (Figure 4) were about 700,000,000 per gram (average data of four pigs), while the counts from pigs fed concentrates of

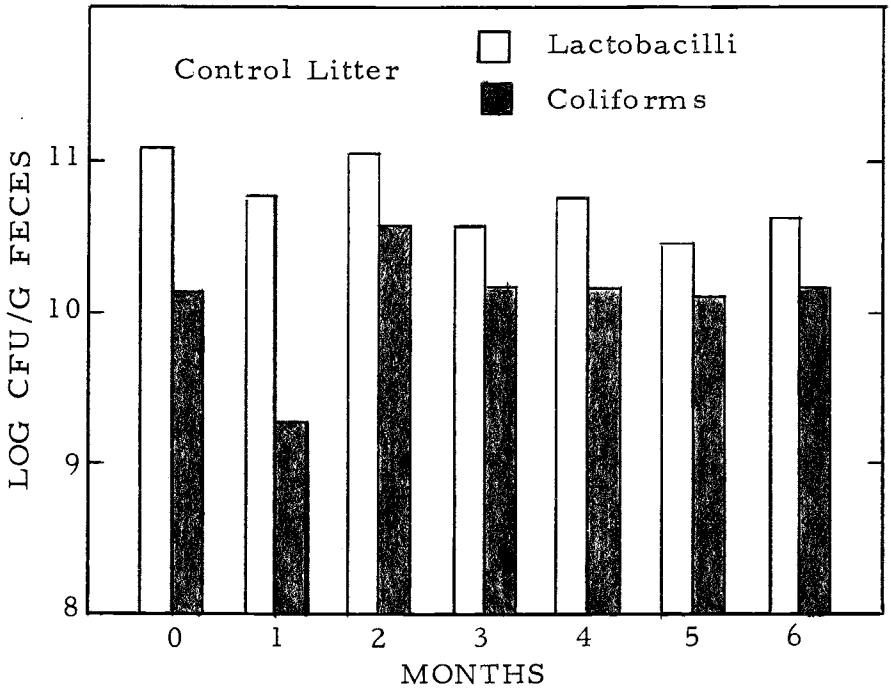


Figure 2. Fecal Lactobacillus and coliform counts in control pigs.

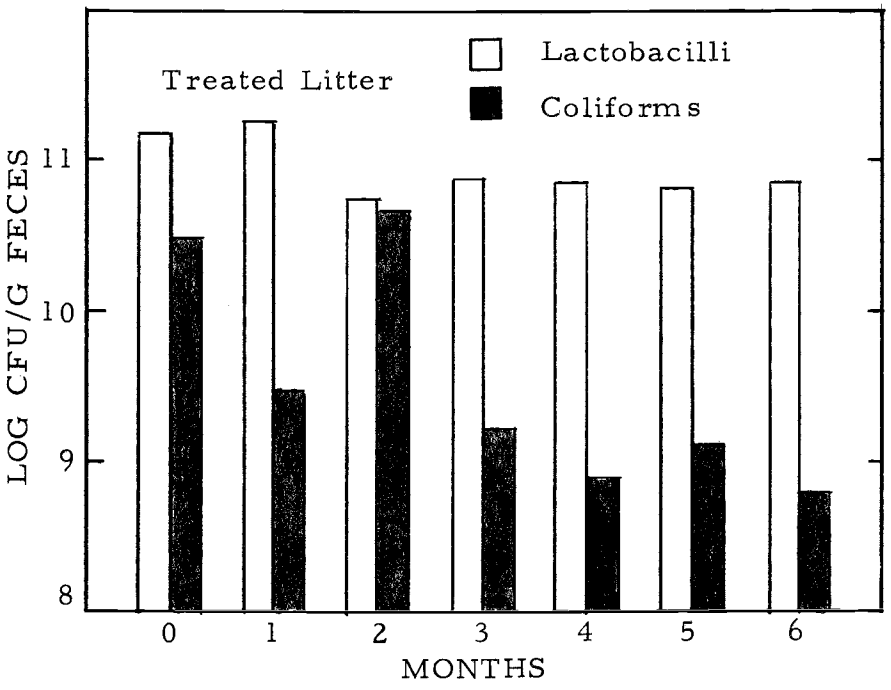


Figure 3. Fecal Lactobacillus and coliform counts in pigs fed concentrates of Lactobacillus MLC.

Table 4. Data showing a decrease in coliform counts following an increase from 15 ml to 30 ml of Lactobacillus MLC concentrate fed per pig per day.

	Coliform Counts[a,b]	Lactobacillus Counts[a]
	600,000,000	20,000,000,000
Before increasing the dosage of MLC concentrate	1,800,000,000	15,000,000,000
	1,100,000,000	10,000,000,000
	10,000,000	20,000,000,000
Two days after increasing the dosage of MLC concentrate to 30 ml	11,000,000	20,000,000,000
	10,000,000	20,000,000,000

[a]Counts represent average of four pigs.

[b]Counts obtained from fecal samples taken from test pigs once every two days.

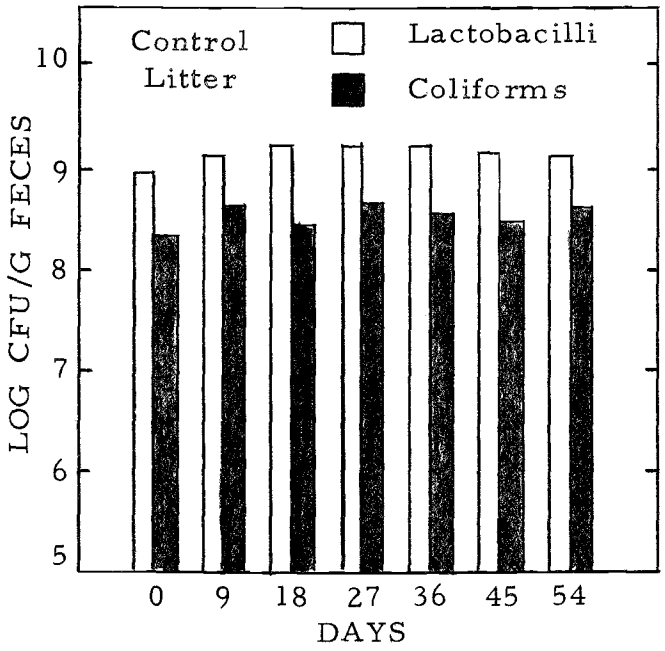


Figure 4. Fecal Lactobacillus and coliform counts in pigs from the control group.

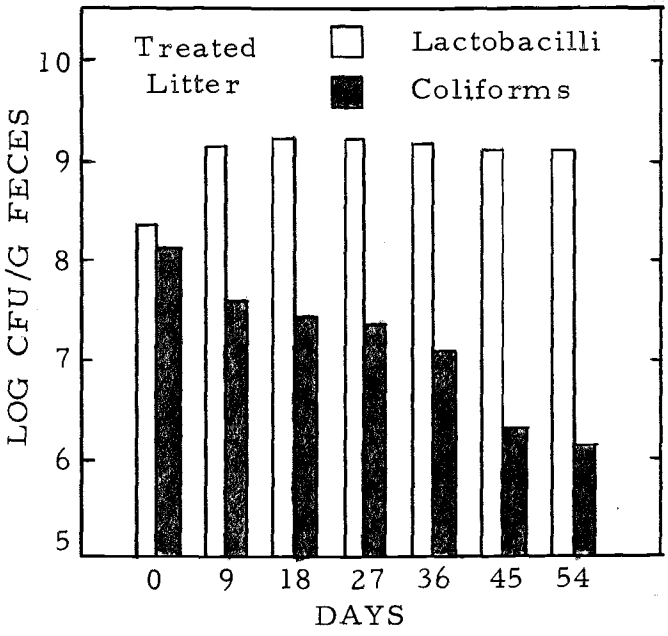


Figure 5. Fecal Lactobacillus and coliform counts in pigs fed concentrates of Lactobacillus MLC.

MLC Lactobacillus for 54 days were only 600,000 per gram (average of five pigs). Also, it was observed that the incidence, duration, and severity of scouring was very low in treated animals. Following removal of the pigs from the Lactobacillus diet, the continuing effect of the organisms in suppressing coliform organisms was evident. This may be seen in Figures 6 and 7. Even 30 days after the last feeding (Figure 7) the coliform counts were still at the same level as observed at the time of feeding.

Feeding Lactobacilli through the Drinking Water System

Following these studies, the feasibility of feeding lactobacilli to a large number of pigs in a herd via the drinking water system was studied in three experiments. Since this was to be attempted using an antibiotic water proportioner to meter the Lactobacillus MLC concentrate into the drinking water, the system was first tested with respect to viability of the organisms reaching the pigs at the water taps in the pens. The results are presented in Table 5. Results of counts of viable organisms in the drinking water and in the concentrate reservoir, and the corresponding pH of samples collected on various days throughout the first water feeding experiment at various time intervals are presented in Tables 6 and 7. These results indicated that the Lactobacillus MLC organisms survived for at least 36 hours in the system without significant loss in viability, and that

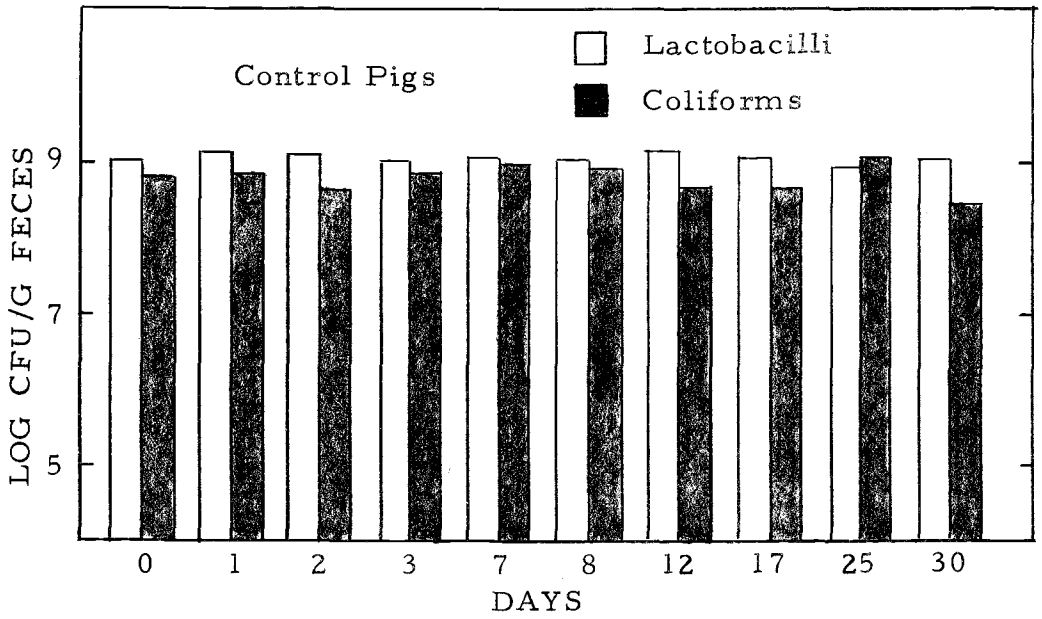


Figure 6. Fecal Lactobacillus and coliform counts from control pigs.

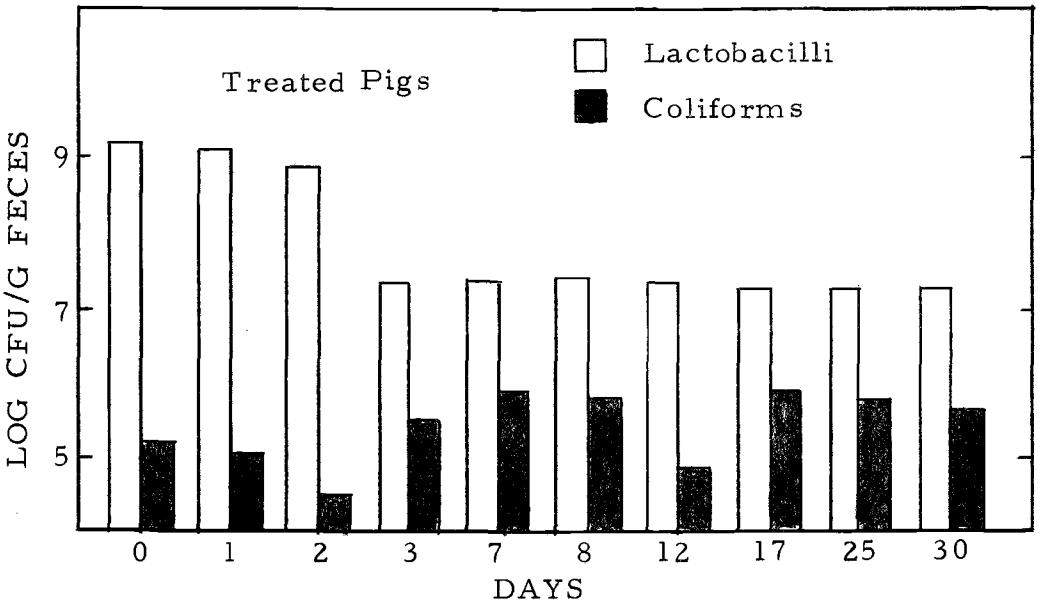


Figure 7. Fecal Lactobacillus and coliform counts after the feeding of concentrates of Lactobacillus MLC was discontinued for 30 days.

Table 5. Viable counts (cfu/ml) of MLC Lactobacilli at the water taps in the pens for a ten-hour period after fresh concentrate was added to the reservoir of the water distribution system.

Time (hours)	Cfu/ml H_2O x 10^8
0	1.5
4	1.8
7	1.5
10	1.4

Table 6. Viable counts (cfu/ml) of MLC Lactobacilli and pH from water samples collected at the drinking source[a] at various time intervals from the addition of fresh concentrate to the reservoir of the water distribution system.

Time (hours)	Cfu/ml $H_2O \times 10^8$	pH
$\frac{1}{2}$	3.8	6.4
$\frac{1}{2}$	1.8	6.3
$\frac{1}{2}$	2.1	6.1
$\frac{1}{2}$	1.7	6.2
$\frac{1}{2}$	0.7	6.2
1	1.7	6.2
3	0.4	6.0
3	0.4	5.8
4	0.9	5.9
12	4.6	5.8
18	1.2	6.4
24	3.7	5.6
24	2.1	5.3
24	1.1	5.4
36	1.9	4.6

[a]Samples collected from randomly chosen pens on random days throughout water feeding Experiment No. 1.

Table 7. Viable counts (cfu/ml) of MLC Lactobacilli and pH from samples of MLC Lactobacillus concentrate taken from the reservoir of the water distribution system[a] at various time intervals from the addition of fresh concentrate.

Time (hours)	Cfu/ml $H_2O \times 10^8$	pH
$\frac{1}{2}$	8.1	4.9
$\frac{1}{2}$	7.9	5.2
3	2.9	4.9
3	2.4	5.0
4	1.9	5.2
14	5.9	4.9
18	4.5	5.1
24	4.7	5.1
24	4.1	5.1
36	6.4	4.9
48	7.2	5.2
>72	<0.1	4.7

[a]Samples collected on random days throughout water feeding Experiment No. 1.

the counts were similar throughout the experiment. There was a decrease in pH of the water from 6.4 at one half hour to 4.6 at 36 hours even though there was no decrease in pH of the concentrate in the reservoir.

Experiment No. 1

The changes in fecal lactobacilli, coliforms, and total aerobic and anaerobic counts are shown in Table 8 and Figures 8, 9, and 10. Results are expressed as geometric mean of plate count data from fecal samples grouped by week of age of the animals on test. The age of weaning was not noted here because the weaning age varied from litter to litter in this experiment. There was a significant decrease in coliform count by the third week of treatment in the water treated pigs, whereas there was not for the control pigs. The control pigs did have a significant decrease in coliform count, however, by the seventh week of age. This change may be due in part to increased consumption of the ration containing an antibiotic; it is, however, consistent with findings by Moon and Whipp (1970) that pigs gain a natural resistance to EEC with age. It is important to recognize in the present experiment that a significant reduction was seen at an earlier age in the treated pigs than in the control pigs. A significant difference between coliform counts in treated pigs and those of control pigs was also seen (Table 8) by the third week of treatment.

Table 8. (Experiment No. 1) Fecal Lactobacillus, coliform, total aerobic, and total anaerobic geometric mean counts (cfu/g feces x 10^8) and L/C ratios of geometric means in control and Lactobacillus MLC treated pigs.[a]

Sample Number	Week of Age	Lactobacillus W[b]	Lactobacillus C[c]	Coliforms W	Coliforms C	Aerobic W	Aerobic C	Anaerobic W	Anaerobic C	L/C W	L/C C
1	3rd	2.52	3.25	4.25	4.36	20.29	18.60	30.76	26.00	0.59	0.74
2	4th	4.71	2.93	2.93	4.70	13.05	12.63	17.94	23.36	1.61	0.62
3	5th	4.60	2.72	**0.777‡	2.20	10.00	**7.44	*16.22	**8.00	5.92	1.24
4	6th	4.27	5.53	**0.512‡‡	4.43	*7.79	*8.47	*10.90	*9.76	8.34	1.25
5	7th	*7.40	**11.76	**0.391‡‡	**1.32	**7.40	**6.41	**9.42	*10.66	18.92	8.94
6	8th	**10.23	**12.18	**0.117‡‡	**0.776	*9.55	**4.79	*14.54	*11.15	87.01	15.71
7	9th	**10.36	*6.55	**0.236	**0.228	**7.66	6.34	*15.57	18.07	43.90	28.67
8	10th	**16.45	*9.80	**0.264	**0.270	*8.37	4.52	14.90	10.63	62.30	36.27
9	11th	**14.81	*10.15	**0.491‡‡	*1.99	8.77	15.32	15.15	*12.22	30.17	5.09
10	12th	**14.04	--	**0.362	--	12.29	--	18.03	--	38.78	--
11	13th	**13.51	--	**0.327	--	**8.92	--	*14.30	--	41.35	--
12	14th	**13.78	--	*0.398	--	16.38	--	17.52	--	34.64	--
13	15th	*7.04	--	**0.180	--	17.41	--	15.63	--	39.13	--

[a] Significance of difference denoted by *(P<.05) and **(P<.01) within treatments from first sample, and by ‡(P<.05) and ‡‡(P< 01) between treated and control groups.

[b] 12 pens of pigs fed MLC Lactobacillus through the drinking water system; 3 pigs from each pen sampled at random from each pen weekly.

[c] 12 pens of control pigs; 3 pigs from each pen sampled at random from each pen weekly.

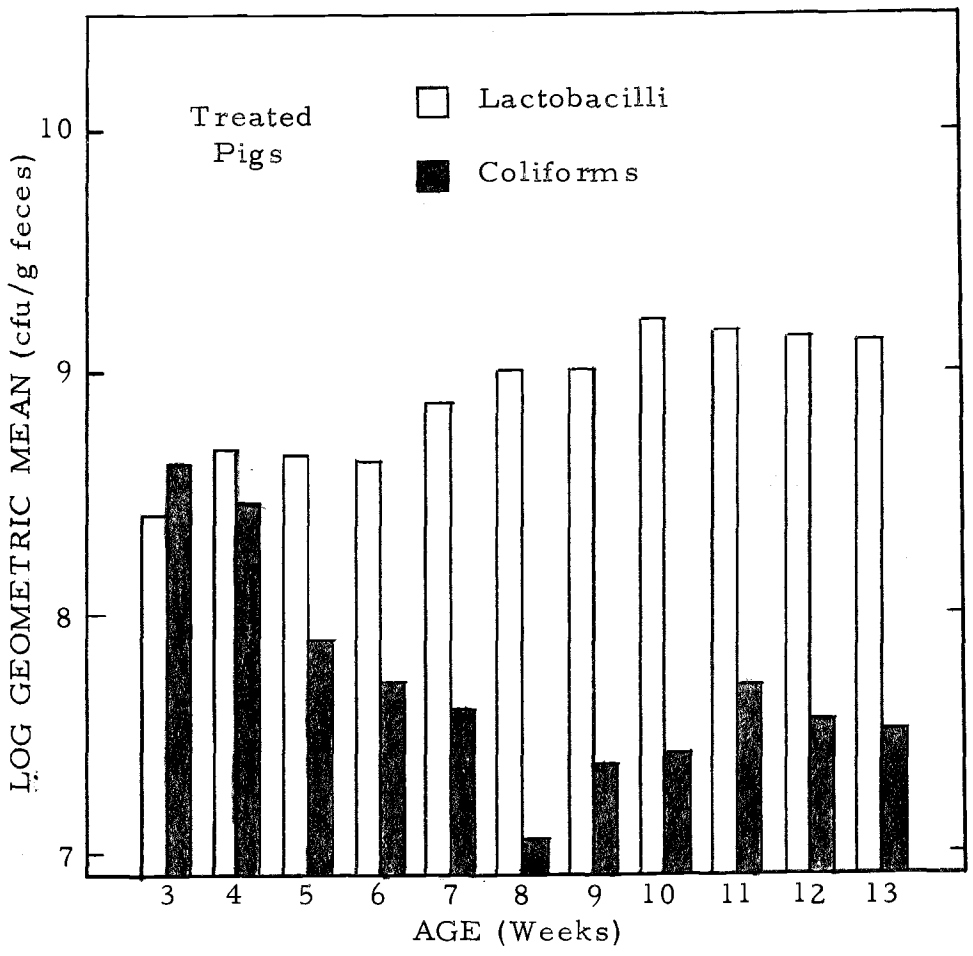


Figure 8. Relative changes in fecal Lactobacillus and coliform geometric mean counts for Lactobacillus MLC water-treated pigs in Experiment No. 1.

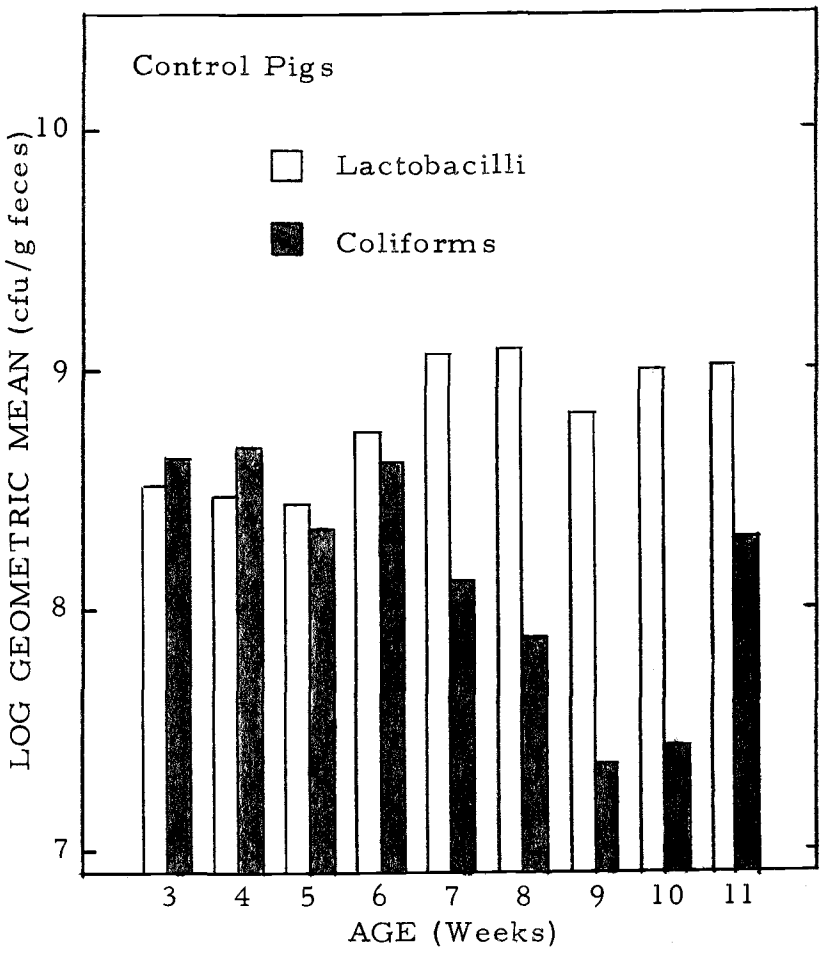


Figure 9. Relative changes in fecal Lactobacillus and coliform geometric mean counts for control pigs in Experiment No. 1.

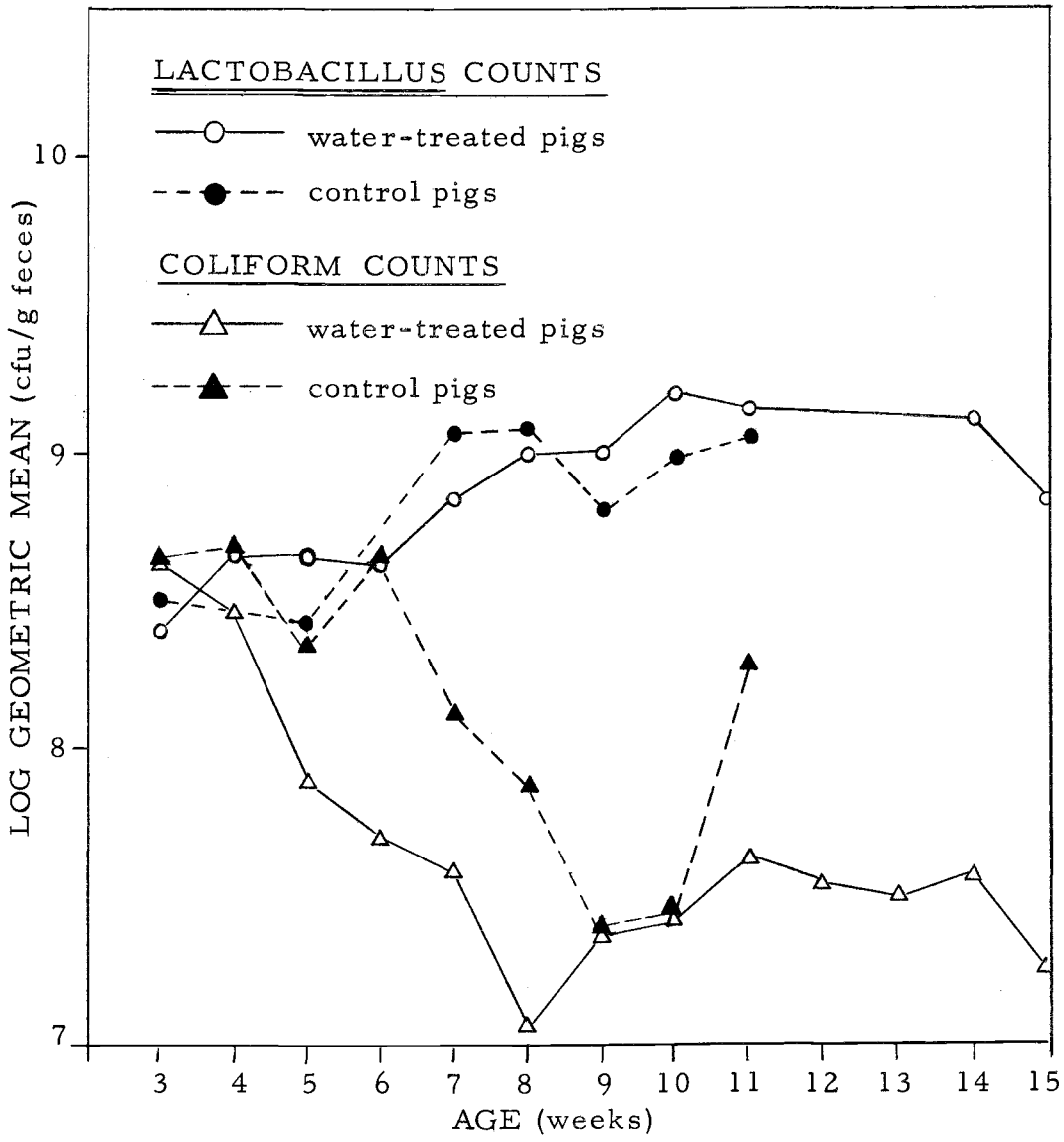


Figure 10. Geometric means of Lactobacillus and coliform counts in Lactobacillus MLC water-treated and control pigs in Experiment No. 1.

This persisted through the sixth week of the experiment. During the seventh and eighth week, the differences were not significant; however, by the ninth week the differences were again significant. This lack of significant differences might be explained by sampling errors inasmuch as differences were again significant at the ninth week. Other logical reasons aren't readily available. By the ninth week of treatment (eleventh week of age) there was an 88.45% reduction in coliform count of the treated pigs compared to a 54.36% reduction in the control pigs; and by the thirteenth week of treatment there was a 95.76% reduction in coliform count in the treated pigs. (Corresponding data for control pigs was not available). These results tend to confirm results of earlier hand feeding experiments.

A significant increase in Lactobacillus counts was seen in both the treated and control pigs; however, confirming what had been noted in earlier experiments, the differences between Lactobacillus counts in treated pigs and those of control pigs were not significant at any time during the experiment. Neither was there any significant difference between treated and control pigs as far as total aerobic counts were concerned at any time during the experiment. The reason for this is not known. Nevertheless, because of the reduction in coliform counts, the Lactobacillus to coliform ratio changed from near 1:1 to 30:1 by the ninth week of treatment in the treated pigs, whereas, the Lactobacillus to coliform ratio in the control pigs was

5:1 during the ninth week of treatment (Table 8 and Figure 11). Similarly, a steady reduction in the percentage of coliforms in the total aerobic count from 21% to 1% in the treated pigs is shown in Table 9, whereas no trend in change of percentage was seen in the control animals. This demonstrates a shift in the balance of the intestinal microflora.

Table 10 summarizes the incidence of scouring in Experiment No. 1. The treated pigs in this case showed a favorable response to the Lactobacillus MLC water treatment with a somewhat lower scouring incidence than the control pigs.

The growth performance data for Experiment No. 1 (Table 11) are of somewhat questionable reliability since they are derived from pigs of non-uniform age, size, and duration of treatment. Inasmuch as primary emphasis was on the microflora changes, less than precise attention was devoted to performance per se. The data given in Table 11 are included here because if they do represent a real effect, they are indicative of a beneficial response and were thus instrumental in designing future experiments.

Experiment No. 2

Bacteriological results similar to those seen in the previous bottle feeding experiments and in the first water feeding experiment were encountered in this experiment. This experiment was conducted

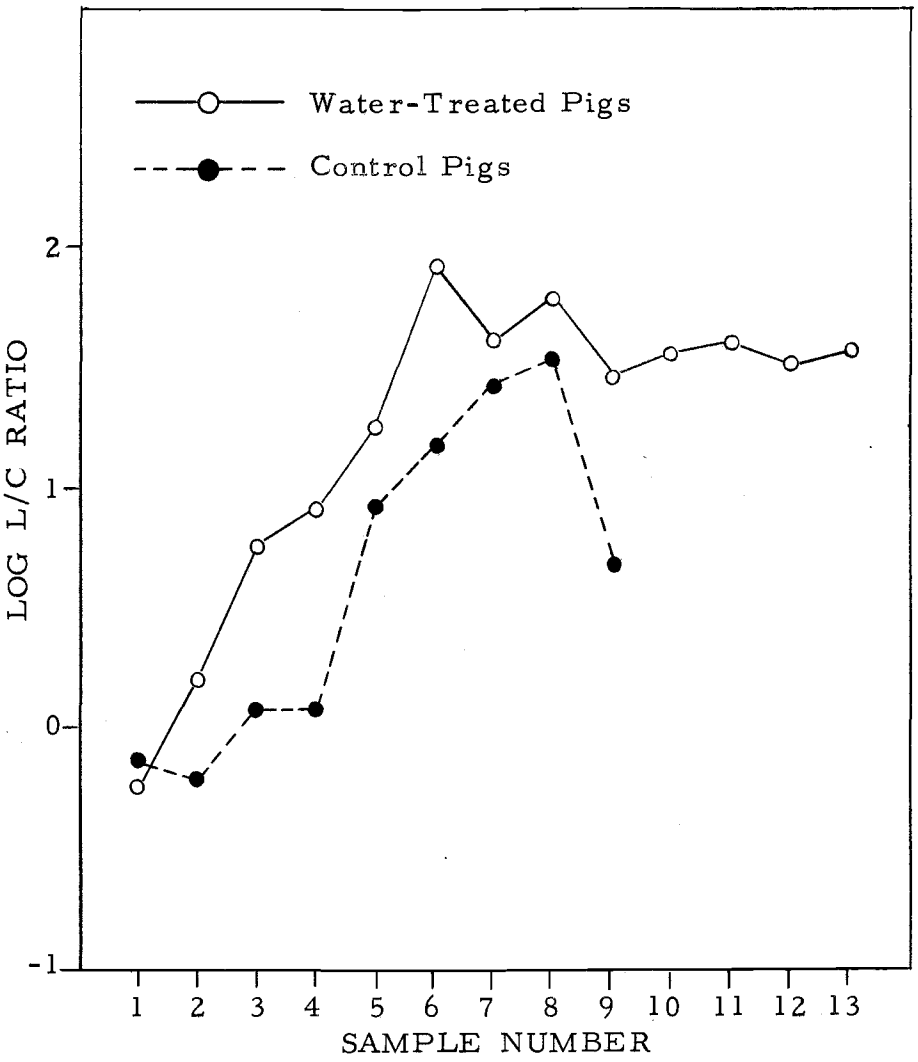


Figure 11. L/C ratios of geometric means in Lactobacillus MLC water-treated and control pigs in Experiment No. 1.

Table 9. Lactobacillus and coliform percentages of total fecal bacterial counts[a] of pigs in Experiment No. 1.

Sample Number	Week of Age	Lactobacilli as % of Anaerobes		Coliforms as % of Aerobes	
		W[b]	C[c]	W	C
1	3rd	8.19	12.49	20.97	23.46
2	4th	26.24	12.53	22.42	37.23
3	5th	28.37	34.03	7.77	29.55
4	6th	39.18	56.72	6.57	52.31
5	7th	78.58	110.41	5.29	20.52
6	8th	70.33	109.31	1.23	16.19
7	9th	66.56	36.24	3.08	3.60
8	10th	110.41	92.13	3.16	5.97
9	11th	97.73	83.03	5.60	13.02
10	12th	77.88	--	2.95	--
11	13th	94.46	--	3.66	--
12	14th	78.66	--	2.43	--
13	15th	45.02	--	1.03	--

[a] Percentages of geometric mean counts
[b] Water-treated
[c] Control

Table 10. Incidence of scouring by pen groups in Experiment No. 1.[a]

	Water-Treated	Control
No. Pens	21	29
No. Pens Severe Scouring (Treated with Antibiotic)	2 (9.5%)	10 (34%)
No. Pens Moderate Scouring (Not Treated)	2 (9.5%)	16 (55%)
No. Pens Slight Scouring	13 (61%)	35 (121%)

[a]Any scouring by one or more pigs in a group constitutes an incidence; recurrences were counted when a time interval greater than one week separated last occurrence.

Table 11. Growth performance of pigs in Experiment No. 1.

Suckling Pigs	Pigs Treated Two Weeks[c]		Pigs Treated Four Weeks[d]	
	W[a]	C[b]	W	C
No. Pigs	26	67	49	13
Initial Weight (lb)	9.8	10.5	11.2	10.7
Weaning Weight (lb)	16.7	18.0	26.9	23.4
Average Daily Gain (lb)	0.37	0.49	0.61	0.49

Weaned Pigs	Pigs Treated Four Weeks[e]		Pigs Treated Six Weeks[f]		Pigs Treated Eight Weeks[g]	
	W	C	W	C	W	C
No. Pigs	28	31	32	21	25	18
Initial Weight (lb)	31.2	19.6	18.4	15.8	22.0	21.0
Final Weight (lb)	59.8	35.1	53.8	45.5	78.5	82.8
Average Daily Gain (lb)	0.95	0.53	0.80	0.69	1.03	1.01

[a] Water-Treated
[b] Control
[c] 16.0 days treated, 15.2 days control
[d] 26.2 days treated, 26.5 days control
[e] 29.9 days treated, 29.1 days control
[f] 43.7 days treated, 42.9 days control
[g] 52.0 days treated, 62.8 days control

without the use of antibiotics in the ration. Pigs received a standard ration containing antibiotics in previous experiments. The changes in fecal lactobacilli, coliforms, and total aerobic and anaerobic counts are shown in Table 12 and Figures 12, 13, 14, and 15. It is interesting to note that a similar pattern of reduction in coliform count in treated pigs was encountered when antibiotics were withheld from the ration as was seen when pigs received antibiotics in the ration. It is also worthy to note, in this experiment, that there was no difference between the results from bottle fed pigs and pigs treated with Lactobacillus MLC through the drinking water. This time, however, there was no significant reduction in coliform count in the control pigs up to nine weeks age. The difference in this respect between Experiment No. 1 and Experiment No. 2 may be due to the absence of antibiotics in the ration in the latter.

Significant reductions in coliform count in the water treated pigs were seen by the first sample taken after treatment was begun (third week of treatment), and in the hand treated pigs by the fourth week of treatment. Also, for both the water treated and hand treated pigs, coliform counts differed significantly (lower) from coliform counts in the control pigs by the fourth week of treatment. These differences remained significant until the experiment's end (seven weeks treatment). It is felt that since this second water treatment experiment was conducted with much greater control over herd and

Table 12. (Experiment No. 2) Fecal Lactobacillus, coliform, total aerobic, and total anaerobic geometric mean counts (cfu/g feces x 10^8) and L/C ratios of geometric means in control and Lactobacillus MLC treated pigs.[a]

Sample Number	Week on Expt.	Lactobacillus			Coliforms			L/C		
		W[b]	B[c]	C[d]	W	B	C	W	B	C
1	0	3.71	2.90	5.04	3.99	2.69	1.99	0.93	1.07	2.53
2	3	5.12	4.46	5.29	**0.861	1.35	2.78	5.94	3.31	1.90
3	4	3.09	4.02	4.54	**0.364‡	**0.258‡	0.931	8.49	15.56	4.87
4[e]	5	4.70	**14.56	6.48	**0.159‡	**0.554	0.928	29.46	26.26	6.98
5	7	*13.00	*10.50	4.92	**0.0435‡‡	**0.0443‡‡	1.09	298.87	236.99	4.53

Sample Number	Week on Expt.	Aerobic			Anaerobic		
		W	B	C	W	B	C
1	0	14.82	15.03	9.93	25.03	23.24	15.55
2	3	7.83	6.30	9.49	12.47	*9.28	16.86
3	4	**2.69‡	**4.88	7.04	**7.40	**8.36	10.08
4[e]	5	**3.39‡	8.52	9.65	**6.63‡	13.94	17.19
5	7	**5.77	10.24	6.79	14.90	19.03	13.75

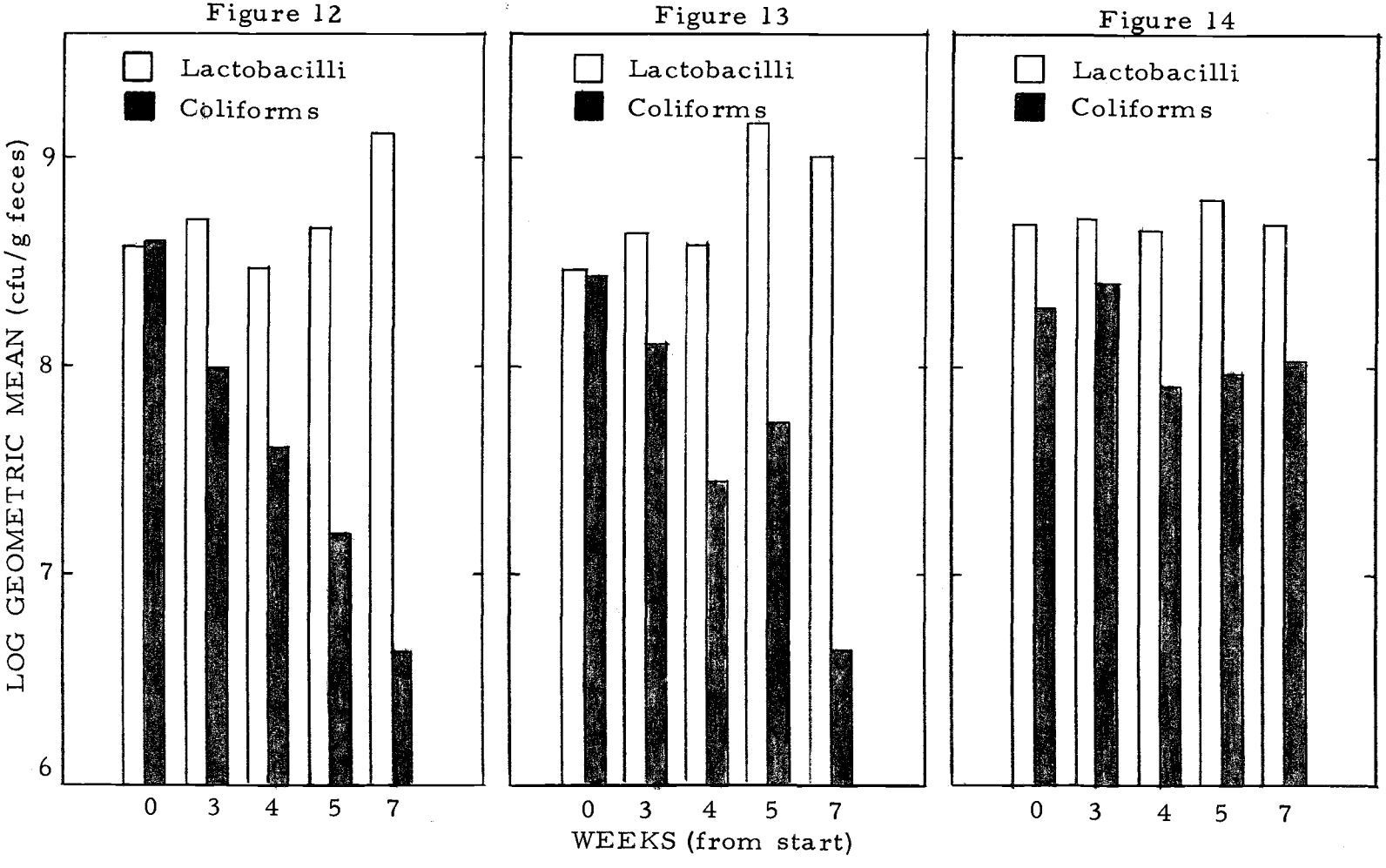
[a] Significance of difference denoted by *(P<.05) and **(P<.01) within treatments from first sample and by ‡(P<.05) and ‡‡(P<.01) between treated and control groups.

[b] 12 litters of pigs fed MLC Lactobacillus through the drinking water system; 2 pigs from each litter sampled.

[c] 24 pigs (2 from each control litter) bottle fed MLC Lactobacillus and sampled.

[d] 12 control litters; 2 control pigs from each litter sampled.

[e] Sample after weaning.



Figures 12, 13, 14. Relative changes in fecal Lactobacillus and coliform geometric mean counts for Lactobacillus MLC water-treated pigs (Fig. 12), Lactobacillus MLC bottle-fed pigs (Fig. 13), and control pigs (Fig. 14) in Experiment No. 2.

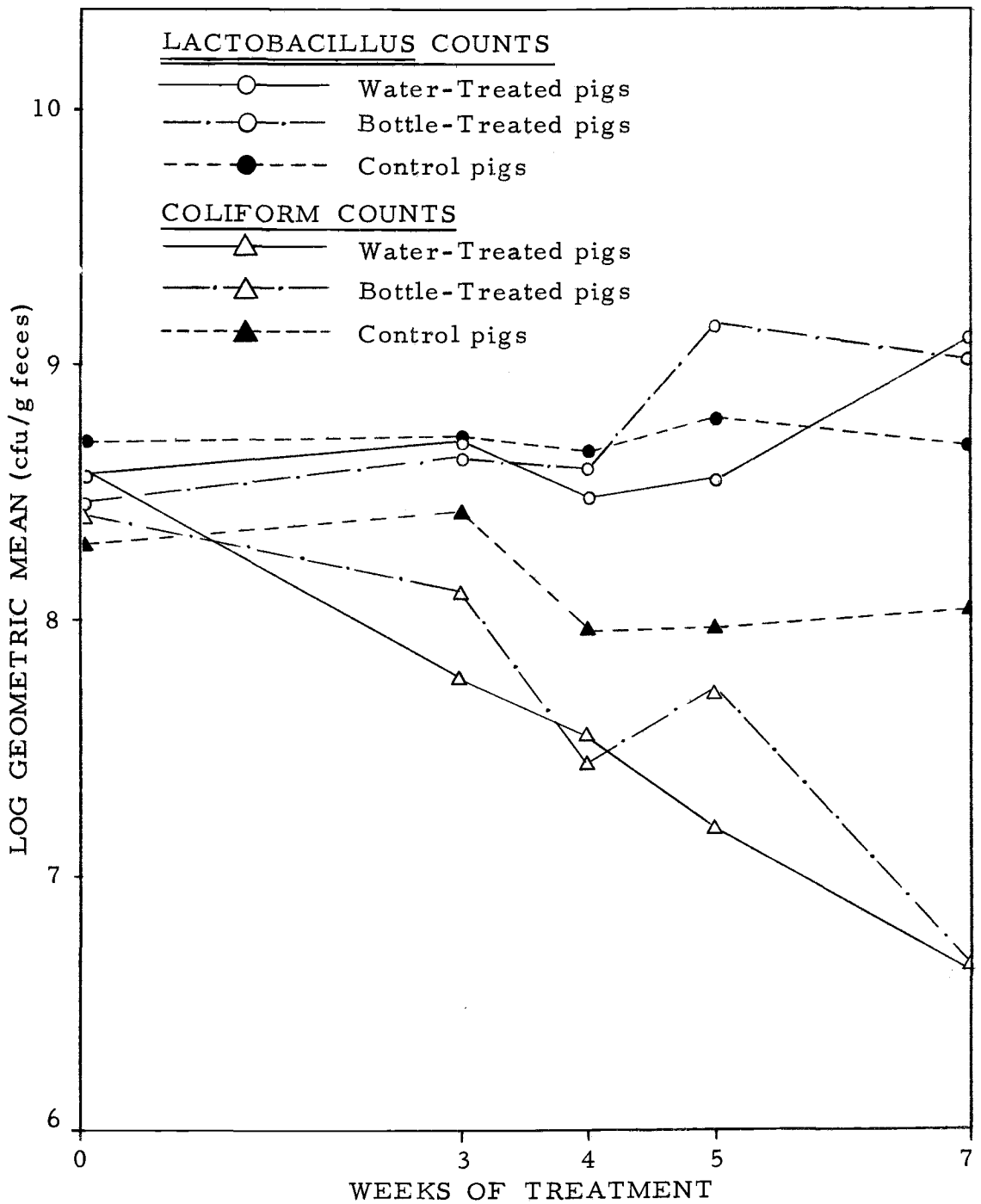


Figure 15. Geometric means of Lactobacillus and coliform counts for Lactobacillus MLC water-treated, bottle-treated, and control pigs in Experiment No. 2.

sampling conditions, the lack of significant differences during the seventh and eighth week of Experiment No. 1 may have been due to experimental error since a similar situation was not seen in this experiment. By the seventh week of treatment (ninth week of age) the decrease in coliform count in the water treated and hand treated pigs had exceeded the decrease seen in the water treated pigs in Experiment No. 1 by nearly ten times. The coliform counts in the water treated and hand treated pigs decreased by 98.9% and 98.4% respectively, while there was no decrease in the control pigs. In this experiment there was a significant increase in Lactobacillus count by seven weeks of treatment in both the water and hand treated pigs, whereas, in the control pigs there was none. Again, this difference from Experiment No. 1 may be due to the absence of antibiotics in the ration in this experiment and their presence in the previous experiment.

In Experiment No. 2 the shift in the balance of the intestinal microflora was clear. Table 12 and Figure 16 show a steady and continuous increase in the Lactobacillus to coliform ratio from 1:1 (approximately) to 299:1 in the water treated pigs and to 237:1 in the hand treated pigs compared to no change at all in the control pigs. A change (Table 13) in the coliform percentage of the total aerobic fecal flora from 27% to less than 1% in the water treated pigs and from 18% to less than 1% in the hand treated pigs compared with

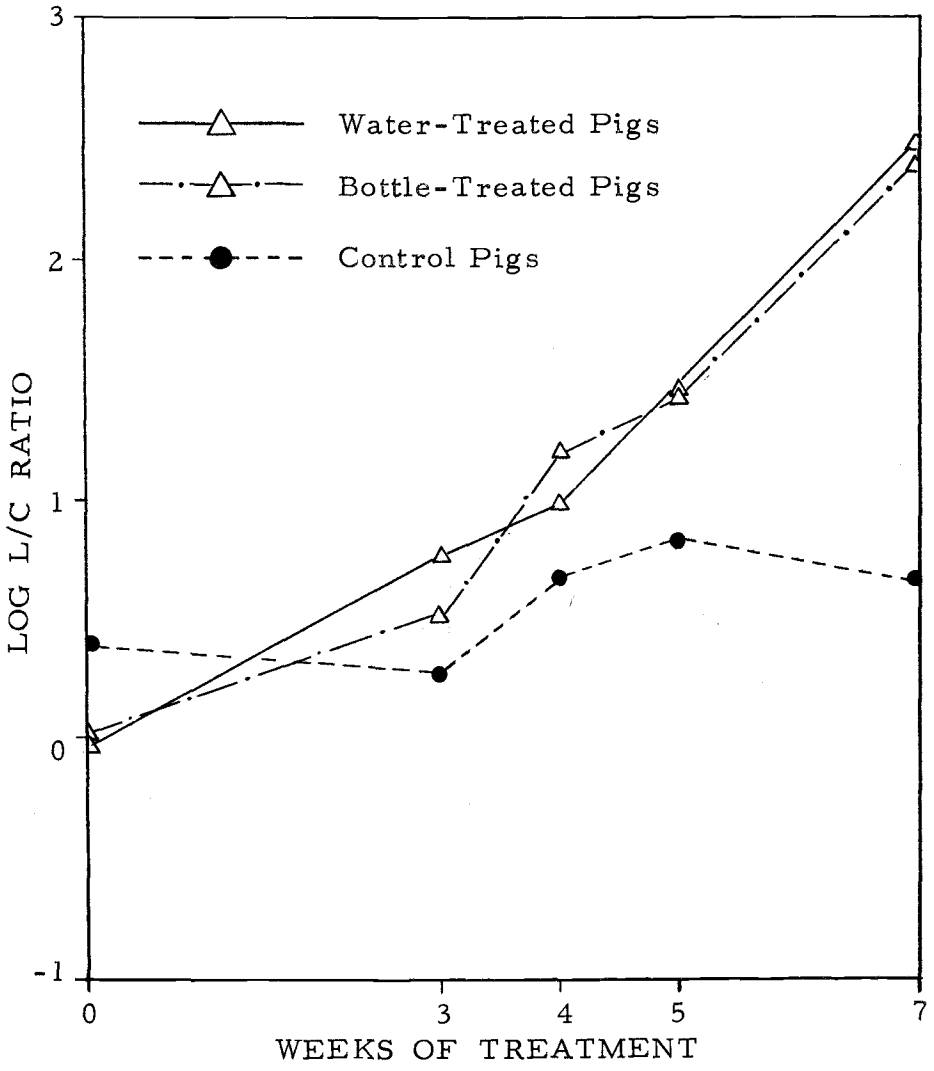


Figure 16. L/C ratios of geometric means in Lactobacillus MLC water-treated, bottle-treated, and control pigs in Experiment No. 2.

Table 13. Lactobacillus and coliform percentages of total fecal bacterial counts[a] of pigs in Experiment No. 2.

Sample Number	Week on Expt.	Lactobacilli as % of Anaerobes			Coliforms as % of Aerobes		
		W[b]	B[c]	C[d]	W	B	C
1	0	14.84	12.46	32.40	26.93	17.92	20.07
2	3	41.07	48.00	31.38	11.00	21.37	29.29
3	4	41.77	48.14	45.02	13.56	5.30	13.23
4[e]	5	70.82	104.39	37.68	4.70	6.51	9.61
5	7	87.28	55.16	35.81	0.75	0.43	16.01

[a] Percentages of geometric mean counts

[b] Water-treated

[c] Bottle-treated

[d] Control

[e] Sample after weaning

little change in the percentage in the control pigs also demonstrates the marked shift in balance which took place in the treated pigs.

The data from Experiment No. 1 were analysed for correlations between Lactobacillus and coliform counts of initial samples and samples taken on weeks five and seven of the experiment. No significant correlation of the initial Lactobacillus and coliform counts was found with later Lactobacillus and coliform counts in the pigs studied.

A summary of scouring data for Experiment No. 2 appears in Table 14. It is evident that despite the marked bacteriological effect of the Lactobacillus MLC concentrate on the treated pigs, the scouring incidence for both treated and control animals was essentially the same.

Weight gain data for Experiment No. 2 are presented in Tables 15 and 16. Again it is evident that no changes in performance occurred as a result of the Lactobacillus MLC feeding. In fact, both the treated and the control pigs performed substantially poorer than normal for the Oregon State University Swine Herd. Correlation and regression analyses between average daily gains and weaning Lactobacillus and coliform counts, and Lactobacillus to coliform ratio failed to reveal any significant correlations between average daily gains and weaning Lactobacillus or coliform counts or their ratios for either the control or the treated pigs studied.

Table 14. Summary of scouring incidence in Experiment No. 2.

	Water Treated	Hand Treated	Control
No. Pigs	102	24	78
No. Litters	12	12	12
No. Pigs Scouring 1st Week	6	5	8
No. Pigs Scouring Pre-Wean[a]	24	14	16
% Pigs Scouring Pre-Wean	23.5	58.0	20.5
No. Litters Scouring Pre-Wean[b]	8	--	7
No. Pigs Scouring Post-Wean	28	14	24
% Pigs Scouring Post-Wean	27.5	58.0	30.8
No. Litters Scouring Post-Wean	6	--	4

[a]One control pig died of scouring pre-wean.

[b]Number of hand treated litters not given since the hand treated pigs are from control litters.

Table 15. Average daily gains (mean ADG in pounds per day) of the pigs sampled in Experiment No. 2.

Time Period	Water-Treated	Bottle-Treated	Control
Birth to Start	0.35	0.37	0.39
Week 1 to 3	0.34	0.37	0.35
Week 3 to 4	0.39	0.56	0.34
Birth to Week 4[a]	0.37	0.39	0.37
Week 4 to 5	0.21	0.23	0.17
Week 5 to 7	0.48	0.40	0.36
Week 4 to 7	0.39	0.36	0.31
Birth to Week 7[b]	0.37	0.35	0.35

[a]Weaned after sample week 4. Weaning weights--Water-treated 16.1 lb, bottle-treated 16.5 lb, control 16.3 lb.

[b]Final Weights--Water-treated 23.7 lb, bottle-treated 23.7 lb, control 23.3 lb.

Table 16. Growth performance of all pigs in Experiment No. 2.

	Water-Treated	Bottle-Treated	Control
Suckling Pigs			
No. Pigs	85	24	79
Age at Weaning (days)	39	39	39
Weaning Weight (lb)	16	17	16
Weaned Pigs			
No. Pigs	48	22	44
Final Age (days)	100	100	100
Final Weight (lb)	55	59	59
Average Daily Gain	0.61	0.64	0.66

Experiment No. 3

Table 17 shows the fecal geometric mean counts obtained from sows treated with concentrates of Lactobacillus MLC through the drinking water system for a period of 20 weeks, and from control sows for the same period. It is evident that no changes took place in the fecal Lactobacillus or coliform counts, or in their ratios in the treated sows. There were no significant differences seen between counts obtained from treated and control sows. It should be noted that the sows tested had rather low fecal coliform counts at the start of the experiment in comparison with counts from younger untreated pigs from previous experiments (nearly 10 times lower counts). It is also to be noted that fecal coliform percentage of total aerobic counts (Table 18) was much lower in the sows tested than in younger untreated pigs from previous experiments. There were no differences between the coliform percentages of treated and control sows, and they were on the order of the same percentages seen in the younger pigs from previous experiments after prolonged treatment with the Lactobacillus MLC concentrate. The Lactobacillus percentage of anaerobes (Table 18) was similarly higher than that seen in younger pigs also.

Vaginal Lactobacillus, coliform, and total bacterial geometric mean counts from the same sows are presented in Table 19. No effect of the Lactobacillus MLC water treatment was seen on the

Table 17. (Experiment No. 3) Fecal Lactobacillus, coliform, total aerobic, and total anaerobic geometric mean counts (cfu/g feces x 10^8) and L/C ratios of geometric means in control and Lactobacillus MLC treated sows.[a]

Sample	Lactobacillus		Coliforms		Aerobic		Anaerobic		L/C	
	W[b]	C[c]	W	C	W	C	W	C	W	C
Lactation	4.06	2.74	0.274	0.691	3.14	3.99	4.51	5.20	14.85	3.97
Pre-wean	2.64	4.14	*0.073	*0.133	5.97	7.27	6.18	9.81	36.13	31.12
Estrus	3.14	8.32	0.187	0.290	8.79	9.74	*18.25	12.55	17.24	66.23
30-days gestation	**21.67	**16.78	0.414	0.408	**22.72	**23.11	**31.35	**35.03	52.40	41.10
60-days gestation	**21.76	**25.97	0.418	*0.143	**18.18	9.08	**35.34	**31.47	52.04	181.82
90-days gestation	9.29	**17.18	0.636	0.309	**16.98	*13.63	*22.99	**36.16	14.61	55.42
112-days gestation	18.52	15.71	0.169	0.352	10.06	12.09	14.31	15.71	109.84	44.66

[a] Significance of difference denoted by *(P<.05) and ** (P<.01) within treatments from first sample, and by ǂ(P<.05) and ǂǂ(P<.01) between treated and control groups (no significance between treatments was seen).

[b] 20 sows fed MLC Lactobacillus through the drinking water system.

[c] 20 control sows.

Table 18. Lactobacillus and coliform percentages of total fecal bacterial counts[a] of sows in Experiment No. 3 at various stages of the reproductive cycle.

Sample	Lactobacilli as % of Anaerobes		Coliforms as % of Aerobes	
	W[b]	C[c]	W	C
Lactation	90.12	52.78	8.71	17.33
Pre-wean	42.66	42.19	1.22	1.83
Estrus	17.24	66.23	2.13	2.98
30-days gestation	69.14	47.90	1.82	1.77
60-days gestation	61.57	82.53	2.30	1.57
90-days gestation	40.41	47.33	3.74	2.27
112-days gestation	129.43	100.00	1.68	2.91

[a] Percentages of geometric mean counts.

[b] Water-Treated

[c] Control

vaginal bacterial counts in the sows tested. Lactobacillus and coliform percentages of the vaginal counts from the sows are presented in Table 20.

Before sampling baby pigs farrowed by the sows in this experiment, a baby pig which had been caught in a sterile pan at birth was sacrificed and the intestinal contents and exterior of the pig were sampled. The results are presented in Table 21. All portions of the intestinal tract proved to be sterile, however, both the vaginal fluid from the sow and the exterior of the pig yielded coliform and Lactobacillus counts in the range of 1×10^4 cfu/g fluid. It seemed appropriate, then, to consider counts of 1×10^4 or less, when sampling pigs at birth and for the first 48 hours, to be due to contamination. Such counts were subsequently disregarded. It was decided that this residual amount of contamination would not affect the accuracy of counts 1×10^5 or higher significantly.

The development for the first 48 hours of the Lactobacillus and coliform flora and their ratios, and the weaning Lactobacillus and coliform flora of pigs farrowed by the Lactobacillus MLC water treated and control sows in this experiment is presented in Table 22. There were no significant differences between coliform counts of pigs from treated and control litters during the first 48 hours. Significant differences between Lactobacillus counts from treated and control litters were, however, detected in the 16 hour and 48 hour samples

Table 19. (Experiment No. 3) Vaginal Lactobacillus, coliform, total aerobic, and total anaerobic geometric mean counts (cfu/g x 10^5) and L/C ratios of geometric means in control and Lactobacillus MLC treated sows.[a]

Sample	Lactobacillus		Coliforms		Aerobic		Anaerobic		L/C	
	W[a]	C[b]	W	C	W	C	W	C	W	C
Lactation	1.83	0.974	0.642	0.109	72.23	1.52	78.01	3.77	2.84	8.92
Pre-wean	4.01	3.55	0.194	0.173	31.91	*24.31	38.47	**34.91	20.68	20.19
Estrus	0.462	0.756	0.133	0.118	**10.67	12.35	*11.03	18.56	3.47	6.43
30-days gestation	*0.097	0.354	**0.024	0.034	24.41	13.18	9.74	*179.08	4.07	10.48
60-days gestation	**0.072	1.25	*0.090	0.070	**6.46	14.38	*8.86	28.50	0.87	17.92
90-days gestation	0.253	2.25	**0.021	0.027	**8.34	*15.12	12.59	10.18	12.23	83.51
112-days gestation	0.633	2.42	*0.104	0.145	17.83	9.45	26.95	16.62	6.10	16.63

[a] Significance of difference denoted by *(P<.05) and **(P<.01) within treatments from first sample, and by ǂ(P<.05) and ǂǂ(P<.01) between treated and control groups (no significance between treatments was seen).

[b] 20 sows fed MLC Lactobacillus through the drinking water system.

[c] 20 control sows.

Table 20. Lactobacillus and coliform percentages of total vaginal bacterial counts[a] of sows in Experiment No. 3 at various stages of the reproductive cycle.

Sample	Lactobacilli as % of Anaerobes		Coliforms as % of Aerobes	
	W[b]	C[c]	W	C
Lactation	2.34	25.87	0.89	7.18
Pre-wean	10.41	10.16	0.55	0.71
Estrus	4.18	4.07	1.25	0.95
30-days gestation	1.00	0.20	0.09	0.26
60-days gestation	0.87	4.38	1.38	0.48
90-days gestation	2.01	22.09	0.25	0.18
112-days gestation	2.35	14.54	0.58	1.54

[a]Percentages of geometric mean counts

[b]Water-treated.

[c]Control

Table 21. Lactobacillus and coliform counts (cfu/g feces or fluid) from contents of three portions of the intestines[a] and from the exterior of a pig sacrificed at birth,[b] and from vaginal fluid from the sow at birth.

	LI	SI	R	E	VF
Lactobacillus cfu	NG	NG	NG	1.2×10^4	1×10^4
Coliform cfu	NG	NG	NG	1.1×10^4	2.4×10^4

[a]Large intestine (LI), small intestine (SI), rectum (R), exterior (E), vaginal fluid discharge from the sow at birth (VF).

[b]Pig was caught in a sterile container at birth; samples were taken using aseptic surgical technique.

Table 22. (Experiment No. 3) Development of fecal Lactobacillus and coliform flora (geometric mean cfu/g feces x 10^8) and L/C ratios of geometric means for the first 48 hours after birth in pig litters from control and Lactobacillus MLC treated sows, and fecal counts at weaning.[a]

Hours After Birth	Lactobacillus		Coliforms		L/C	
	W[a]	C[b]	W	C	W	C
0	0	0	0	0	--	--
4	0.00085	0.00035	0.00009	0.00007	8.31	4.75
8	0.0124	0.00395	0.00575	0.0477	2.15	0.08
12	0.273	0.0818	2.02	3.10	0.14	0.03
16	0.647‡‡	0.0691	4.74	5.67	0.14	0.01
24	1.87	1.03	9.62	10.70	0.19	0.10
32	1.50	0.583	14.67	7.16	0.10	0.08
40	1.25	0.939	12.18	18.27	0.10	0.05
48	3.79‡	0.736	16.90	17.07	0.22	0.04
Weaning	8.93‡	3.86	0.143‡‡	1.38	62.24	2.79

[a]Significance of difference denoted by ‡ ($P<.05$) and by ‡‡ ($P<.01$) between treated and control groups.

[b]20 litters from MLC Lactobacillus water treated sows; treatment was continued to the pigs until weaning; 2 pigs from each litter sampled.

[c]20 litters from control sows; 2 control pigs sampled from each litter.

after birth but not in the rest of the 0 - 48 hour samples. The Lactobacillus counts did appear to be lower in the control pigs than in those from treated sows even though the differences were not found to be significant. The Lactobacillus to coliform ratios (Table 22 and Figure 17) also appeared to be greater for the first 48 hours in the pigs from treated sows.

The pattern of development of Lactobacillus and coliform fecal counts for the first 48 hours is presented in Figures 18, 19, and 20. Figure 18 presents the average development of the treated and control pigs studied; and Figures 19 and 20 present the individual development in 20 randomly selected treated and control pigs (10 treated and 10 control). On the average the lactobacilli began their development earlier than the coliforms, however, after eight hours the coliforms surpassed the lactobacilli in number and remained near 10 times greater in number to 48 hours. In the 20 randomly selected pigs studied individually, the Lactobacillus development began earlier than the coliform development in eight of the 20; it began at the same time and at nearly the same rate in the other 12 of the 20; and the Lactobacillus counts reached the same or higher levels than the coliforms in only four of the 20 during the first 48 hours. There were no obvious differences in the development of the Lactobacillus and coliform flora between the 10 pigs from the treated sows and the 10 pigs from the control sows.

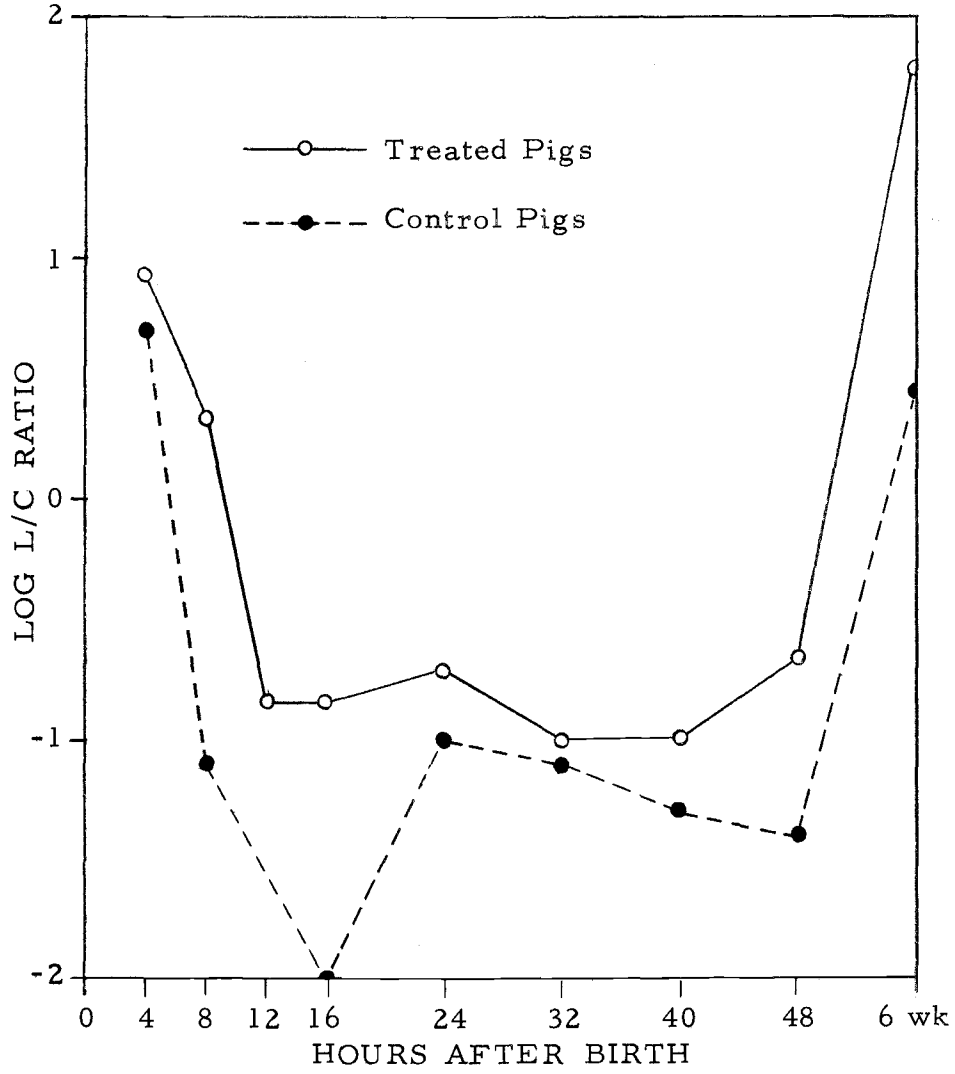


Figure 17. L/C ratios of geometric means in Lactobacillus MLC water-treated and control pigs from the sows in Experiment No. 3 for the first 48 hours after birth.

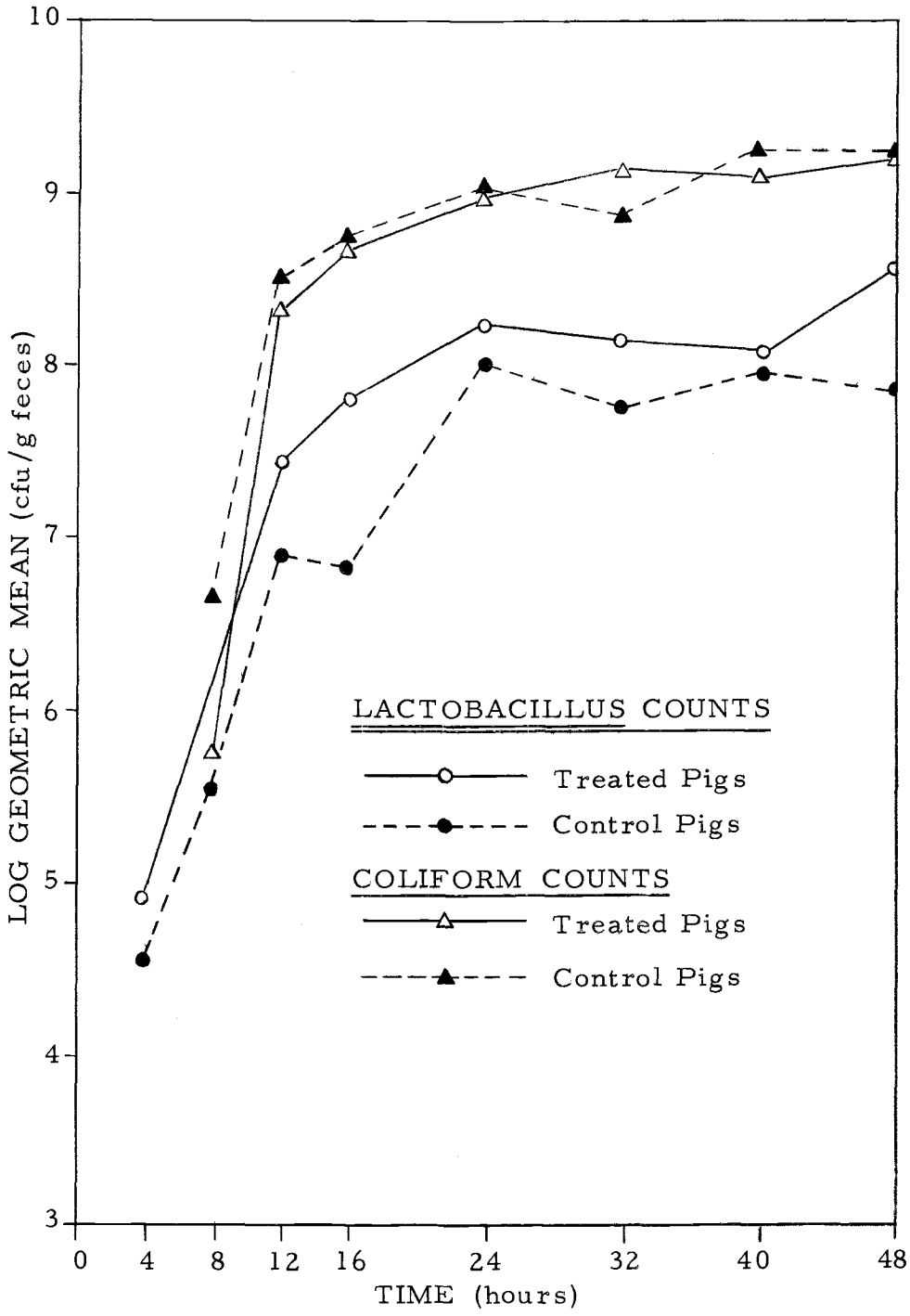


Figure 18. Pattern of development of geometric mean Lactobacillus and coliform counts in 36 pigs from treated and 24 pigs from control sows for the first 48 hours after birth.

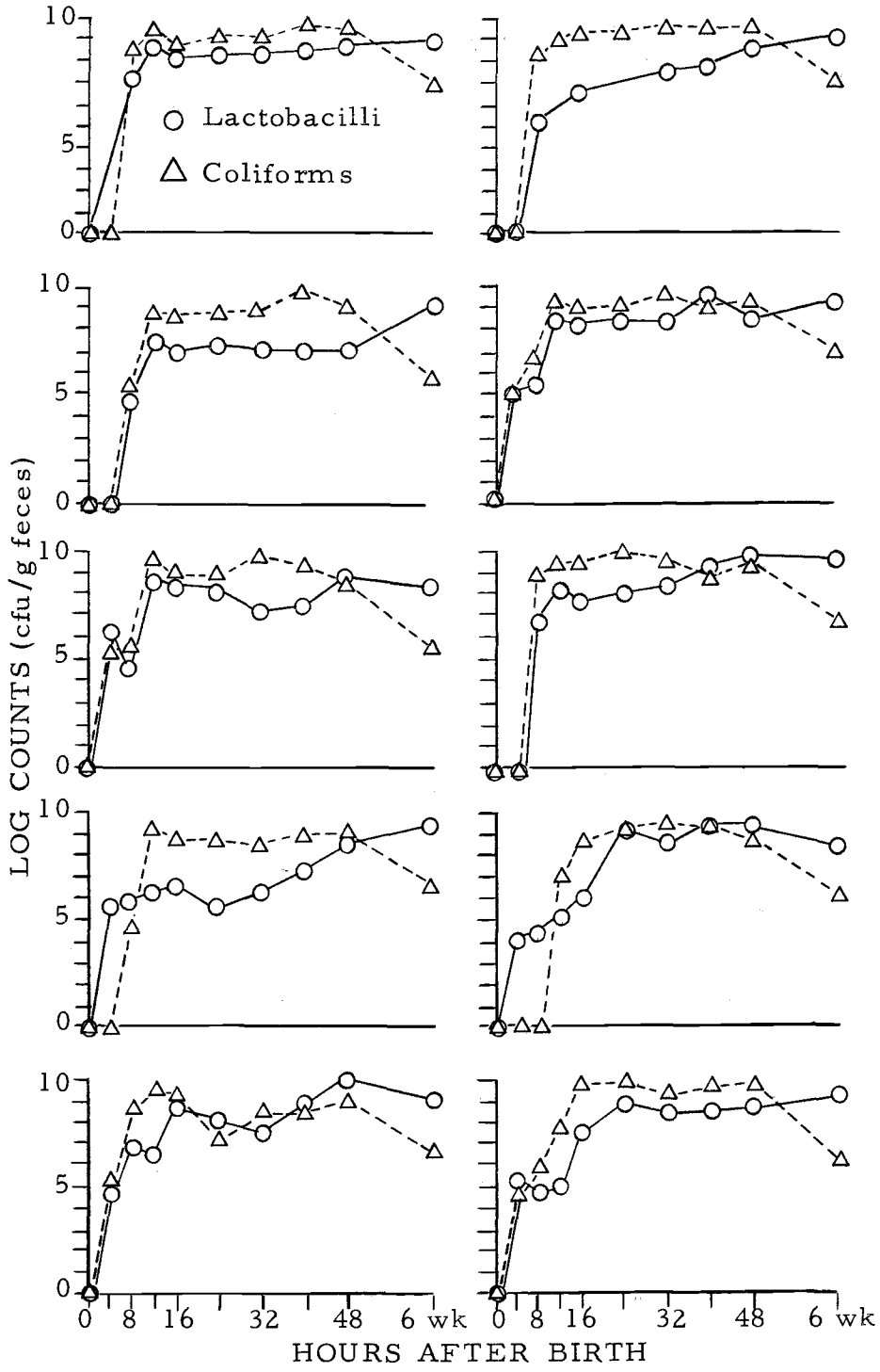


Figure 19. Development of Lactobacillus and coliform flora for the first 48 hours after birth in 10 randomly chosen pigs from litters of Lactobacillus MLC treated sows.

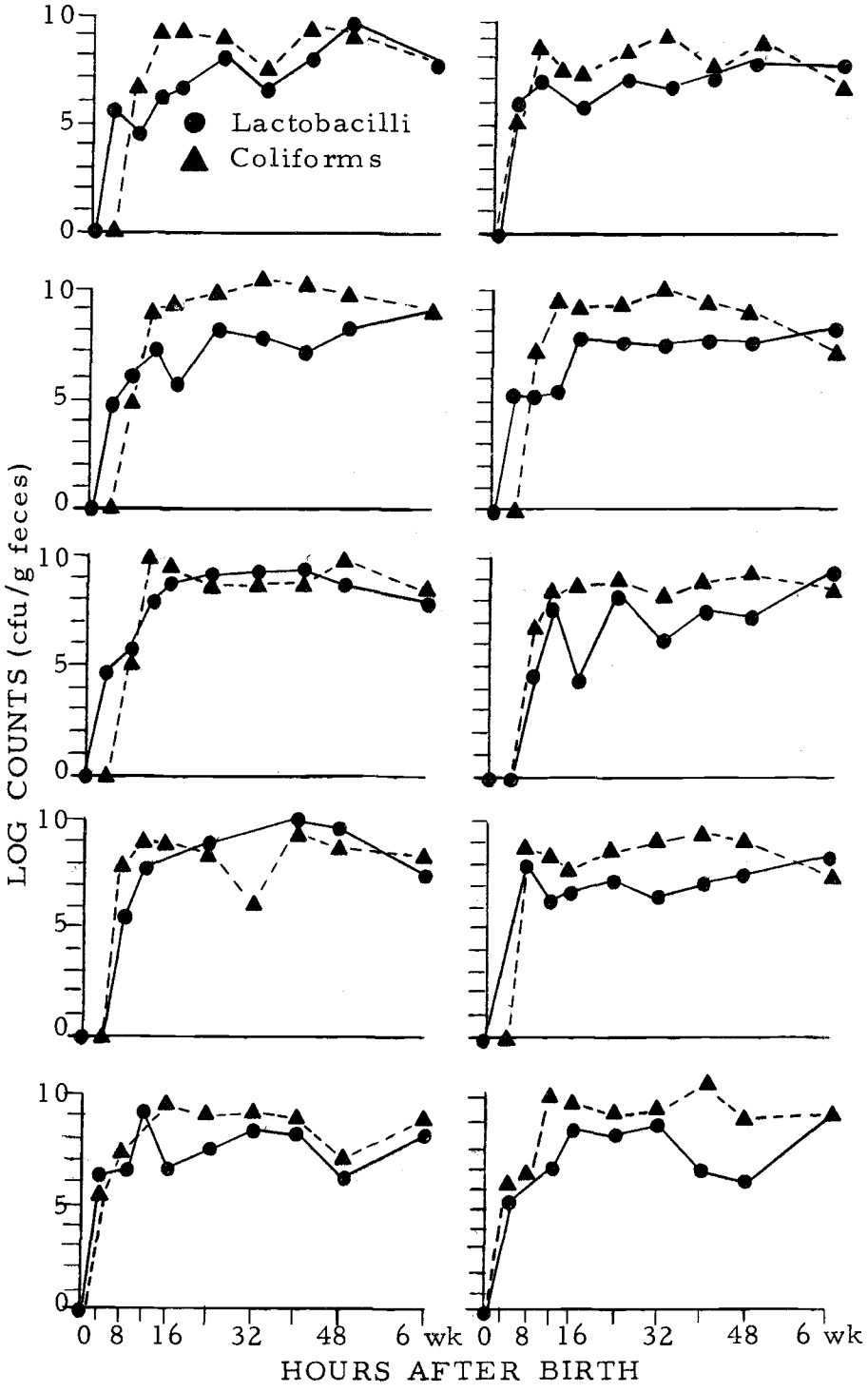


Figure 20. Development of Lactobacillus and coliform flora for the first 48 hours after birth in 10 randomly chosen control pigs.

At weaning (six weeks age) the Lactobacillus MLC treated pigs from the treated sows had significantly lower coliform counts and significantly higher Lactobacillus counts than the control pigs from the control sows (Table 22). Figure 21 also demonstrates a much greater reduction in coliform count in the treated pigs than in the control pigs from 48 hours to weaning. This confirms the observations from Experiment No. 2. (The pigs in both experiments had received rations free of antibiotics.) Correlation and regression analysis revealed no correlation between Lactobacillus or coliform counts at weaning time and Lactobacillus or coliform counts during the first 48 hours.

The Lactobacillus to coliform ratio of geometric mean counts at weaning time was 62:1 for the treated pigs and about 3:1 for the control pigs (Table 22 and Figure 17). The higher Lactobacillus to coliform ratio in the treated pigs compared with the control pigs is also observed in the 20 individual pigs studied (Figure 19 and 20).

Table 23 summarizes the scouring data from birth to weaning for the litters of the sows studied. There was essentially no difference between the treated and control pigs with regard to scouring.

Table 24 summarizes the weight gain performance of the litters of the sows studied. Again, the Lactobacillus MLC treated pigs from the treated sows showed no advantage over the untreated pigs in weaning weights or in average daily gains. Correlation and regression

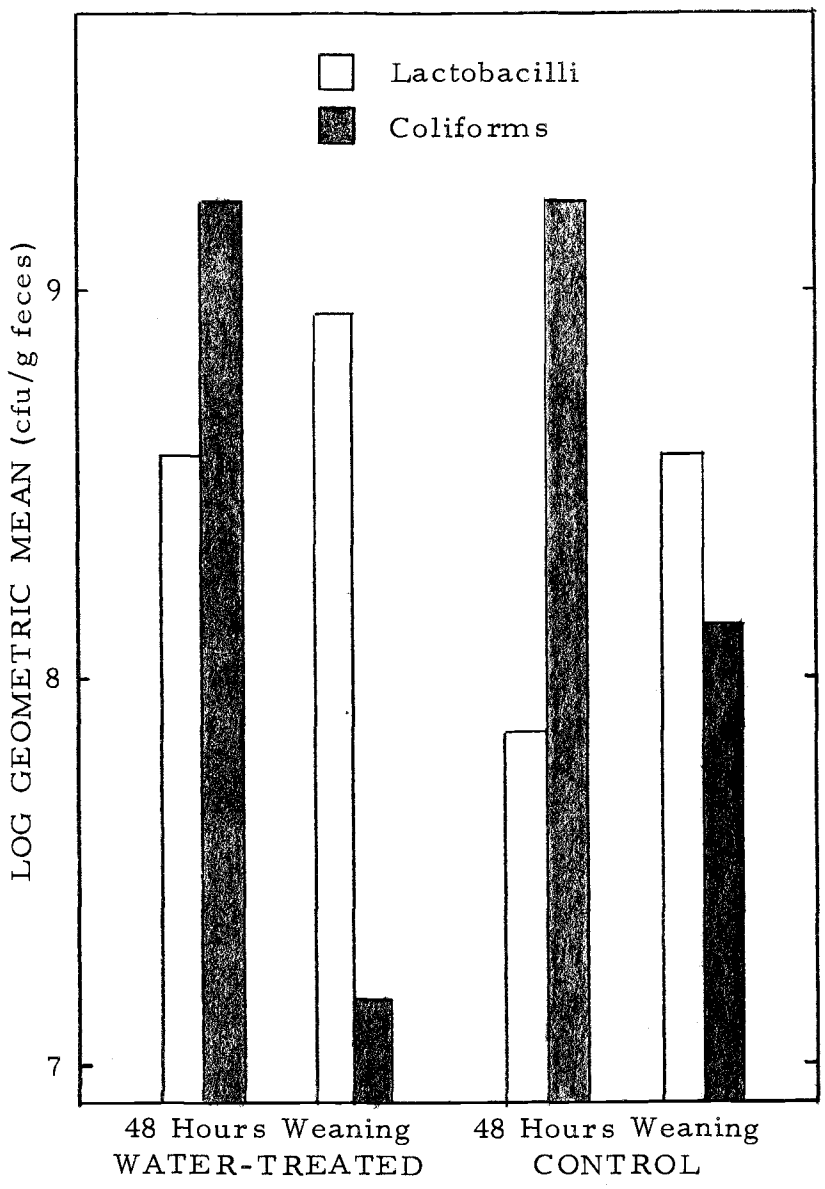


Figure 21. Relative changes in fecal Lactobacillus and coliform geometric means from 48 hours after birth to weaning in Lactobacillus MLC water-treated and control pigs in Experiment No. 3.

Table 23. Summary of scouring incidence for the pigs in Experiment No. 3 from birth to weaning.

	Water-Treated	Control
No. Pigs	141	87
No. Litters	17	10
No. Pigs Scouring	56	36
% Pigs Scouring	39.7	41.4
No. Litters Scouring	8	6
% Litters Scouring	47.0	60.0
No. Deaths Due to Scours	1	2

Table 24. Growth performance of pigs in Experiment No. 3.

	Water-Treated	Control
Suckling Pigs		
No. Pigs	117	73
Age at Weaning (days)	45	45
Weaning Weight (lb)	22	21
Weaned Pigs		
No. Pigs	117	73
Final Age (days)	100	100
Final Weight (lb)	59	62
Average Daily Gain	0.71	0.78

analysis failed to show a correlation between weaning weight and weaning Lactobacillus or coliform counts in the treated or control pigs that supports the premise that high Lactobacillus counts and low coliform counts are associated with favorable production performance. Neither were Lactobacillus counts for the first 48 hours after birth in treated pigs correlated favorably with weaning weights. However, in the control pigs a positive correlation between Lactobacillus counts during the first 48 hours and weaning weights was seen and the regression of weaning weights on Lactobacillus counts was found to be significant at the 95% confidence level. A negative correlation between weaning weights and coliform counts during the first 48 hours (i.e.--low coliform counts during the first 48 hours after birth were associated with high weaning weights) was found in the pigs from treated sows, but no such correlation was found in the pigs from the control sows. Again the regression for the weaning weights on the early coliform counts in the treated pigs was significant at the 95% confidence level. This provides somewhat tenuous support that a favorable balance in lactobacilli and coliforms early in the life of the pig may be associated with higher weight gains later.

DISCUSSION

The potential importance of these studies received emphasis in the cover story of the November, 1973, issue of Animal Nutrition and Health (1973). In a study conducted on over 10,000 first sow litters at eight midwest agricultural experiment stations to determine factors related to baby pig deaths, it was found that 30% of U. S. baby pigs die soon after birth. According to mid-1973 prices it costs about $80 to produce a nine-pig litter based on the feed bill for the sow and the labor involved. This means each pig at birth is worth $9.00 and its value increases each day. Research has shown that if the pork producers could save one extra pig per litter, an $80 million national annual savings to the industry in feed costs alone would result. Baby pig deaths now cost the industry about $250 million per year.

It has been well-documented that a substantial portion of these baby pig losses are a result of intestinal disease in young pigs. Among pigs which survive cases of scouring, a loss to the industry is still reflected in a lower efficiency of feed utilization and in slower rates of weight gain, plus the added number of man-hours spent with these animals.

The idea which led to this research effort that intestinal disorders can be treated or prevented by feeding Lactobacillus organisms is certainly not a new idea. Metchnikoff (1908) believed that

this genus was beneficial to man and animals. On the other hand, as pointed out by Fuller (1972), the view that the healthy intestine contains a collection of harmless bacteria living amicably together in a long tube of food could not be further from the truth. The same constant struggle for survival exists among the intestinal bacteria as is typical among other mixed populations. In addition to antagonism among the different intestinal bacterial species, the host also struggles to keep the bacterial population under control and maintain the balance typical of its species.

Man and animals have evolved into a symbiotic interrelationship with their respective autochthonous Lactobacillus species. The animal provides the substrate and the optimum environment for its symbiote, while the Lactobacillus organism helps its animal host to control the bacterial population and maintain the proper balance.

The evolution of this relationship has continued from the time animals first encountered these organisms in fermented food materials and milk. The degree of specialization of this relationship varies from one animal species to another. Recent studies on the Lactobacillus flora associated with the crop-epithelium in fowl (Fuller, 1973) have demonstrated that the association in bird species is a highly specific and intimate one. Even though some colonizing strains in chickens closely resembled certain named species (e.g. L. salivarius and L. fermenti) in physiological characteristics,

differences in surface structure seemed to account for the fact that the named species would not colonize the crop epithelium of chickens. A similar relationship was shown to exist between the mouse and its Lactobacillus flora (Dubos et al., 1965). On the other hand, an indigenous human strain of L. lactis was found to colonize the intestinal epithelium in pigs by Muralidhara (1974).

Lactobacilli are highly versatile organisms and can survive in the acid stomach of many animals. In many host species they can establish themselves throughout the entire length of the gut. The range of other intestinal bacteria is much more restricted.

It has been noted (Rettger et al., 1935) that a Lactobacillus organism must be bile tolerant in order to implant in the intestine. The organism used in this study (L. lactis MLC), an indigenous human intestinal Lactobacillus, was nevertheless found to be inhibited by 2% taurocholate even though it was shown to colonize the intestinal tracts of the pigs studied; this indicates that the property of bile tolerance is not a strict requirement for intestinal colonization.

Some confusion exists centered around the idea that L. acidophilus is effective in disease therapy, while L. bulgaricus is not. This is based on early reports by Herter and Kendell (1908), and Rahe (1915). Today there exists a tendency to designate a Lactobacillus organism thought to be effective in therapy or otherwise healthful as L. acidophilus. The bacterium used in this study was, nevertheless,

found to be L. lactis (Muralidhara, 1974).

Considerable inaccuracy may occur in attempts to study and enumerate bacteria in material as bacteriologically complex as feces. However, the counts made daily from the feces of young pigs during this study were so consistent and followed such a definite pattern that there was justification for concluding that the behavior of some types of fecal bacteria was being studied with reasonable accuracy. Reassurance was also obtained from the fact that, where comparisons were possible, counts of various types of bacteria in the pigs resembled in general those obtained by other workers using different techniques (Haenel and Müller-Beuthow, 1956).

In determining the normal bacteria flora of healthy non-scouring pigs, it was noted that the Lactobacillus counts were nearly always greater than the coliform (E. coli) counts. On the other hand, in scouring animals, invariably the coliform counts were greater than the Lactobacillus counts. This is in agreement with work done by Chopra et al. (1963a) who observed an increase in coliform and reduction in Lactobacillus counts in scouring pigs. Dubos et al. (1963) also noted that lactobacilli were predominant in the intestinal tract of mice maintained under unusually clean conditions. This balance between lactobacilli and coliforms is sometimes altered due to unknown stress factors. During these times EEC may increase in numbers in the intestines resulting in colibacillosis and reduced animal growth.

Further, there is indication that the widespread use of antibiotics may alter the balance between intestinal lactobacilli and E. coli in some instances and hereby cause colibacillosis.

The bacterial results from all the experiments reported here where pigs have been fed concentrations of the MLC L. lactis strain have been quite pronounced and nearly the same in each case, whether the organisms were bottle fed or fed through the drinking water. Significant reductions in coliform counts (near 100%) and favorable shifts in the balance of the population of lactobacilli and coliforms were demonstrated to be a result of the treatment.

It should be noted that these favorable bacterial changes remained stable in the pigs' intestines for at least 30 days following discontinuation of the treatment with the lactobacilli.

It is also interesting to note that in spite of feeding large numbers of L. lactis MLC, the fecal Lactobacillus counts did not increase significantly higher in the treated pigs than in the control pigs in the experiment in which antibiotics were included in the diet. This is in agreement with results obtained by Speck et al. (1973), and Paul and Hoskins (1972). However, when antibiotics were not included in the diet, increases in Lactobacillus count were significantly higher in the treated pigs than in the control pigs.

Several other differences in the bacteriological results were seen between the experiment in which antibiotics were included in the diet and the experiments in which antibiotics were withheld. When a

ration containing antibiotics was fed, the coliform counts in control pigs were reduced significantly (though they were still significantly higher than in the Lactobacillus MLC treated pigs) by the seventh week of age. No similar reduction in coliform counts was noted in control pigs from which antibiotics were withheld. Similarly, the Lactobacillus to coliform ratio in treated pigs without antibiotics in the ration became markedly greater than in the treated pigs with antibiotics in the ration.

The bacteriological results obtained from the sows treated with the Lactobacillus MLC concentrate were unremarkable. After feeding the concentrate to the sows for over four months, no significant changes in the numbers or ratios of fecal or vaginal lactobacilli and coliforms were seen. However, the numbers of fecal coliforms were much lower in the sows to begin with than in normal young pigs. In fact the numbers of fecal coliforms in the control sows were in the same range as the numbers seen in the Lactobacillus MLC treated pigs after three or four weeks of treatment. Similarly the percentages of lactobacilli were much higher and the percentages of coliforms were much lower in sows than in young pigs. These findings are in agreement with those of Smith and Crabb (1961). The lower counts in the sow are probably due to an increased resistance with age to colonization by E. coli. This is substantiated by findings of Moon and Whipp (1970) and others.

The inability of the organisms (L. lactis MLC) fed to have any effect on the sow's fecal flora might be explained as follows. The sow's immune system, being highly developed, could have a lethal effect on the organisms being fed since they may act as antigens. This may be true especially since the Lactobacillus MLC strain fed is not indigenous to swine. In fact, the human strain fed may have been able to implant in the young pigs intestines only because their immune system was not developed. It was seen that after three or four weeks treatment of the pigs with the MLC L. lactis, an equilibrium point seemed to be reached (seven or eight weeks age) at which no further reduction in coliform (E. coli) counts took place.

In view of the reductions in coliforms which took place in the young pigs studied, it was surprising and somewhat perplexing to find little or no improvement in either scouring or weight gain performance. The Lactobacillus MLC treated pigs with the antibiotic-containing ration did demonstrate an improvement in scouring incidence over the controls, and there were indications of weight gain improvement over the controls in this group also. However, in the antibiotic-free experiments, no improvement in either scouring or weight gain performance was detected in the treated pigs over the controls; and as a whole, the pigs without antibiotics exhibited much poorer production performance than normal for the Oregon State University swine herd. Even though there is not enough data

available, the suggestion is that improvements in production performance might be seen when MLC Lactobacillus and antibiotics are used together.

It is obvious that much more must be known concerning colonization by Lactobacillus and the nature of its protective effect in the intestinal tract before we can fully answer the question of why we cannot show an improved performance by feeding MLC Lactobacillus concentrate, especially when we are clearly able to greatly reduce the numbers of E. coli. This question becomes more perplexing when studies by Muralidhara (1974) on the colonization of the MLC Lactobacillus strain in the pig's intestine and the resulting effect on colonization by EEC are viewed. We have clearly been able to protect baby pigs from EEC challenge by feeding the MLC Lactobacillus. In addition, we have been able to show conclusively, through direct evidence obtained in histological studies and fluorescent antibody tests, that the MLC Lactobacillus strain will colonize the intestinal epithelium of baby pigs and prevent colonization by challenge EEC.

The degree to which the E. coli flora must be depressed in order to prevent symptoms of colibacillosis is not entirely clear at the present. During the recent American Society of Microbiology meetings Gorbach and Pierce (1973) reported that at least 10^5 EEC must be present per gram of intestinal tissue in order to produce typical symptoms of colibacillosis. On the other hand, Meyer (1972)

has stated that as few as 50 toxin producing E. coli cells can cause the death of gnotobiotic pigs within 48 hours. The few numbers of organisms regarded in the latter case is no doubt related to the microbial status of gnotobiotic pigs which do not have a normal indigenous flora, especially lactobacilli. In the present study, we were able to depress the fecal E. coli counts to between 10^6 and 10^7 per gram feces. Other workers (Muralidhara, 1974) in our laboratory showed that the numbers of E. coli colonizing the intestinal epithelium could be reduced to less than 10^2 per gram of intestinal tissue in baby pigs by feeding the MLC Lactobacillus concentrate.

Again, the degree of specificity of the host-symbiote relationship which exists between an animal species and its autochthonous Lactobacillus flora should be mentioned as a possible explanation for the lack of a favorable production response in the pigs to the MLC Lactobacillus strain. It is entirely possible that, even though the MLC strain was able to colonize in the baby pigs and prevent colonization by E. coli, unknown factors (immune system of the pigs, etc.), as the pigs grew older, prevented this strain from acting as vigorously in competing with E. coli in the pig intestine as it normally would in its human intestine niche. This is supported by general ecological principles. Populations of geographically isolated members of the same species whose habitats vary in environmental conditions from one another usually have specializations which allow them

to match their own community and environmental conditions optimally. If one population of the species is transplanted into the niche of the other population it will usually be able to survive in its new niche, but will be more susceptible to adverse environmental conditions and will not have the full competitive ability of the population which is indigenous in the niche. The range of tolerance of the two populations allows them to survive in one another's niche; each, however, has an optimum level of response to the particular set of conditions of its own normal habitat (Salt, 1971). For this reason the MLC Lactobacillus may have to put more energy into maintaining itself in an environment that is sub-optimal for the population.

In order to explore the full potential of feeding Lactobacillus organisms to pigs to prevent scouring and improve production performance characteristics, then, it may be absolutely necessary to use a Lactobacillus strain isolated from the herd in which its use is intended, or at least from swine. This is supported by the studies of the ecology of lactobacilli in the fowl crop by Fuller (1973). Savage (1972) also indicated that for an organism to become established in the intestinal tract it should be an autochthonous type, should be anaerobic, and should colonize in the early life of the individual to become part of the indigenous flora.

The environment into which a pig is born has an important influence on the subsequent early development of its intestinal

bacterial flora. This was greatly emphasized in the results of experiments by Coalson and Leece (1973). When pigs are farrowed in a clean environment the majority of organisms that colonize their alimentary tract are largely derived from their dams. One factor affecting the speed of colonization is the degree of contact between newborn animals and the source of colonizing organisms. Pigs which are active at birth soon come into contact with an environment highly contaminated by feces from the sow. In addition, the pig's stomach produces little gastric acid for the first day and the pH of the contents are in the region of 5.3 to 5.9. These factors provide pressure for rapid colonization of the alimentary tract by E. coli, Cl. welchii, and streptococci. After one day when the pH is lower, the pressure for colonization favors lactobacilli which eventually become established as the principle component of the flora of the stomach and small intestine, and serve to inhibit the overpopulation by E. coli and other organisms.

This early pressure for colonization by E. coli and lactobacilli is substantiated with our studies on the development of the fecal flora in newborn pigs for the first 48 hours. Lactobacilli were detected in the feces of newborn pigs as early as four hours after birth, whereas coliforms (E. coli) were generally detected around four hours later. The coliforms soon surpassed the lactobacilli in number during the first day and remained near ten times greater in number than the

lactobacilli up to 48 hours. By six weeks age the coliforms had gradually decreased in number and the lactobacilli had gradually increased in number to become the dominant flora. These findings are similar to those of Smith and Crabb (1961) and Smith (1965) except that these workers indicated that the development of the lactobacilli did not begin until two days age. Our findings agree, however, that E. coli are dominant for the first two days. Mata et al. (1972) documented this same pressure for a natural rapid colonization by the intestinal bacteria in a study conducted with Guatemalan children who were born in less than sanitary conditions. They found the presence of bacteria in the feces of these children as early as four hours after birth. Similarly, the streptococci and gram-negative bacilli (E. coli) began their development first, followed by the bifidobacteria (closely related to lactobacilli) which became established as the dominant flora by the end of one week.

Since there was no apparent effect of the MLC Lactobacillus on the fecal flora of the sows to which it had been fed, it was not expected that the development of the fecal flora of baby pigs from these sows would develop any differently from the fecal flora of baby pigs from untreated sows. There was no reason to believe that the pigs from treated sows would be exposed to a bacterial environment any different from that which pigs from untreated sows were exposed. This proved to be true. There were no significant differences

between the development of the fecal flora for the first 48 hours in pigs from treated sows and in pigs from untreated sows.

It was found that low coliform and high Lactobacillus counts in pigs during the first 48 hours after birth were positively correlated with high weaning weights, and the regression of weaning weight on early coliform and Lactobacillus counts in the pigs studied was found to be significant ($P < .01$). This provides evidence that production performance is somehow related to colonization of the intestinal tract early in the life of the pig by the bacteria which become its indigenous flora. The pattern of development of the flora, and especially any methods designed to prevent colonization by E. coli immediately after birth may be very important to the swine producer in increasing the efficiency of his production. In this sense then, the bottle-feeding of newborn pigs with Lactobacillus organisms capable of implanting in the intestinal tract as soon as possible after birth may be beneficial. Since the organisms fed act as antigens (especially if they are not autogenous) they should be fed before the immune system of the pigs begins functioning.

Experiments with feeding Lactobacillus MLC organisms (Muralidhara, 1974) have shown that not much benefit of any kind was seen when the pigs were fed at 0, 12, and 24 hours of age. From histological studies, however, it was shown that when pigs were fed Lactobacillus MLC every two hours from the first 24 hours and every four

hours for the next 48 hours, the bacteria colonized the small intestine, prevented colonization by EEC, and prevented colibacillosis.

It is obvious that this research has raised more questions than it has answered and a considerable amount of work has yet to be completed before we can define precisely how lactic acid bacteria can best be used to benefit man and animals at the intestinal level. A large area of consideration yet to be developed lies in the precise nature of the host-symbiote relationship each animal species has with its native colonizing intestinal flora. Studies should fully explore the advantage of feeding autogenous Lactobacillus organisms (derived from the population to which it is fed) over feeding non-native organisms which are capable of colonizing but which may lack unknown factors that prevent the full expression of their protective ability in the intestinal tract.

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