EFFECT OF FEEDING BACILLUS SUBTILUS SPORES ON SOW AND BABY PIG PERFORMANCE AND BACTERIAL POPULATIONS

by

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INTRODUCTION

Of the infectious causes of preweaning mortality, neonatal diarrhea is the most common accounting for approximately 11% of these deaths (Bergeland, 1980). Enteritis costs the swine industry over $30 million a year.

Probiotics have been proposed as a possible method of controlling enteritis. Though lactobacillus cultures are presently the most common probiotic, they tend to easily lose their viability when introduced into feed (Pollmann and Bandyk, 1982). Bacillus subtilus is a spore-forming bacteria with an indefinite shelf-life currently being marketed as a possible alternative. In this study we investigated the effect of Bacillus subtilus on sow and baby pig performance and on the bacterial populations of the gastrointestinal tract of the newborn pig.

LITERATURE REVIEW

Diseases of the gastrointestinal (GI) tract are a major source of economic loss in the swine industry. Of the infectious causes of preweaning mortality, neonatal diarrhea is the most common accounting for approximately 11% of these deaths. It has been estimated that, in 1981, 30 million pigs were affected by enteritis and that this disease costs the swine industry over two million dollars a year. Much research has been done to determine the cause and solution to this costly problem.

The resident fecal flora of the young pig from the first to the 23rd week after birth is composed of Lactobacillus, Bifidobacterium, Bacteroides, Streptococcus, Clostridium, and E. coli. E. coli and streptococcus are the predominant organisms soon after birth but fluctuate greatly after that. Lactobacillus and Bifidobacterium become the most predominant within several weeks after farrowing and then maintain fairly constant. There are no changes of the principal constituents of the fecal flora between morning and evening (Uchida et al., 1965).

Pathogenic E. coli has been isolated as the principle cause of enteritis (Kenworthy and Crabb, 1963; Chopra et al., 1964). Of 1004 isolates of E. coli, 12.4% were enterotoxigenic based on the ability to distend ligated intestine of the young calf (Myers and Guinee, 1976).

Enteropathogenic E. coli stimulate the movement of fluid into the intestinal lumen of the pig producing a disease called neonatal colibacillary enteritis, enteritis, neonatal diarrhea, or gut edema. The first signs of enteritis can be seen from 12 h to 4 d after birth with the pig usually dying by day 7 if it is fatal. Enteritis is associated with an increase in coliforms and a decrèase in lactobacilli (Chopra et al., 1963) and in dogs, this resulted in no change in the numbers of viable organisms (Ishikawa et al., 1982).

It has been shown that the highest levels of E. coli in the small intestine are achieved between 6 and 18 h after birth (Smith and Jones, 1963) and that the initial changes in the ileal absorptive cells of E. coli-challenged pigs were observed 8 h after infection (Drees and Waxler, 1970).

Two requirements are necessary for E. coli to cause enteritis: (1) the production of entertoxin (Smith and Halls, 1967; Smith and Jones, 1970; Sack, 1980), and (2) the ability to adhere to the epithelial cells of the small intestine with pili (Gaastra and De Graaf, 1982). The pili can be K88 (Smith and Linggood, 1971; Jones and Rutter, 1972; Jones and Rutter, 1974), K99, 987P, or $3P^-$ ETEC (Awad-Masalmeh et al., 1982).

The pathogenesis of enteritis is as follows: (1) infection of the pig with enterotoxigenic E. coli, (2) adhesion to the villi of the small intestine, (3) production of enterotoxin, and (4) effective toxic action in causing fluid secretion.

Beachey (1980) suggested that the sum of the surface charges of both eukaryotic and prokaryotic cells are negative. It is thought that fimbriae increase attachment by counteracting repulsive electrostatic forces. The more hydrophobic the bacterial cell, the more likely it will move toward the negatively charged epithelial cell and so allow the ligands (fimbriae) on the bacterial cell and the receptors on the epithelial cell to interact with each other to form specific bonds of high affinity. This reaction is not just molecule-cell but is cell-cell which means that there are a large number fimbriae interacting with a comparable number of receptors. It is for this reason that the attachment of the bacterial cell to the epithelium is nearly irreversible since it is unlikely that all of these bonds would be broken simultaneously. The receptors are thought to be sugar moities (Anderson et al., 1980) and this attachment occurs instantly (Wilson and Hohmann, 1974).

Pigs removed from the sow at two days of birth were given a pathogenic strain of E. coli. Attachment to the small intestine villi was not observed until 6 h after inoculation. Bacterial adhersion was most prominent in the anterior small intestine and started in the basal region of the villi and then progressed anterior (Arbuckle, 1969).

In two other studies (Smith and Halls, 1967; Nielsen et al., 1968), enteropathogenic E. coli was inoculated into ligated loops of the small intestine. The loops in the anterior intestine resulted in the accumulation of much larger volumes of fluid than in comparable loops in the ileum. Smith and Jones (1963) and Kenworthy and Crabb (1963) also concluded that pigs with enteritis are characterized by a great proliferation of E. coli in the small intestine, particularly in the anterior section.

It was reported (Nielsen et al., 1968) that the probable mechanism for fluid movement into the lumen is through increased active solute transport. The cells of the crypts of Lieberkuhn respond to musosal injury by secreting a unidirectional flow of sterile fluid which would help wash out any irritants. In this case the irritant would be the enterotoxin. The more pathogenic E. coli present, the more enterotoxin produced, and so the more severe the condition.

Penetration of the epithelial cells by E. coli is not necessary to cause the disease (Bertschinger et al., 1972). One of the problems in working with this disease is the great difficulty in typing E. coli. Since coliforms are abundant in the GI tract their presence does not necessarily indicate pathogenicity. In culturing E. coli, color or morphologic characteristics are not correlated with enterotoxigenicity (Myers, 1975). Thus, more research is needed to quantitate the E. coli pathogenicity.

E. coli and other pathogenic bacteria aid in a number of bodily functions. Peristalsis moves the chyme and bacteria from the small and into the large intestine. Glandular secretions from the salivary glands, stomach, duodenum, pancreas, and liver supply approximately 150 ml of sterile fluid (for a 10-kg pig) into the duodenal end of the intestine every hour. This serves to wash out a great deal of bacteria (Nielsen et al., 1968). Antibodies can protect the pig from enteritis most likely by the neutralization of the entertoxin (Kohler, 1966; Kohler, 1967). The host bacterial population can also suppress some invasive bacteria through means such as competition for a common energy source (Ozawa and Freter, 1964).

The stomach pH of pigs in the first day after birth is markly higher than when older (Smith and Jones, 1963). This is not associated with the time of feed intake. This high pH may permit bacteria to pass the stomach safely and proliferate in the GI tract.

There was no difference in susceptibility to dilatation of ligated segments of intestine between colostrum-fed and colostrum-deprived pigs (Smith and Halls, 1967).

Antibiotics have been widely used to control enteritis. One problem, though, is the development of resistant strains of E. coli, which causes the fear that an outbreak may occur

which would not respond to antibiotic treatment. Bacteria with purified pili are effective in stimulating the host immune system to be prepared for an invasion of these pathogens (Porter et al., 1974). Unless several different types of pili are used together, the response will only be to the one which was injected (Gaastra and De Graaf, 1982).

Probiotics have been proposed as a possible alternative to antibiotic therapy and bacteria usage. These may be simplier and more economic. Probiotics involve using bacteria to either stimulate or suppress particular bacteria already present in the host GI tract or to prevent pathogens from colonizing. Presently, lactobacillus cultures are the most common probiotics used. When used in weaning pigs at three weeks of age, it resulted in less diarrhea for shorter periods of time than those pigs not given the culture (Hill et al.,1970). Chicks given lactobacillus showed significantly less mortality and a lower pH in the crop, cecum, and rectum after being challenged with pathogenic E. coli (Watkins et al., 1982). Mitchell and Kenworthy (1976) concluded that Lactobacillus bulgaricus produced an anti-enterotoxic substance.

One problem with many commercially available lactobacillus cultures is they tend to easily lose viability when introduced into the feed (Pollmann and Bandyk, 1982). Bacillus subtilus is currently being marketed as a possible alternative. Because it is a spore-former, it has an

indefinite shelf life not affected by heat nor by a lack of moisture. It can be shipped as a pure spore in a calcium carbonate/whey carrier. It can either be mixed in with the complete feed and pelleted. After entering the GI tract the spore germinates and is theorized to increase the host lactobacillus while suppressing E. coli.

Two conditions must be met for sporulation to occur: (1) a particular stage is reached where vegetative cell growth stops and sporulation is stimulated, and (2) all of the components necessary for the spore to form are available (Grelet, 1957).

It has been concluded that calcium dipicolinate is a major constituent of the spore structure and accounts for approximately 50 to 60% of the dry matter excreted by the cell during germination (Powell, 1957).

In the transformation of a spore into a vegetative cell there are three sequential processes involved: activation, germination, and outgrowth.

Activation breaks the dormancy of the spore and poises it for germination. This process is reversible. Russell (1982) lists five common methods of activating bacterial spores:

1. Heat (usually 60 to 75 C) has been shown to significantly increase the rate of germination (Curran and Evans, 1944; 1945; Keynan et al., 1964).
2. Calcium dipicolinate (Russell, 1982).
3. Ethyl alcohol (Hyatt and Levinson, 1968).

4. Optimum pH for B. cereus was found to be between 2 and 3 (Keynan et al., 1964, Keynan and Halvorson, 1965).
5. Exposure to water vapor has also been shown to increase spore activation (Hyatt and Levinson, 1966).

Germination is the change of activated spores from a dormant to a metabolically active state and only requires a few minutes. L-alanine is required for the germination of bacillus spores. D-alanine will completely reverse this L-alanine stimulation (Levinson and Hyatt, 1955).

The average time required for B. cereus to germinate is only 235 s. After 6 min all of the changes are completed (Vary and Halvorson, 1964). However, the time for a population to germinate is dependent on the span of time during which the individuals begin the process and so will take longer than 6 min. In slowing germinating populations this span may be an hour or more (Hansen et al., 1970).

Bicarbonate has been found to retard the germination of B. subtilus in the absence of heat shock. It is thought that CO_2 has either a physical or chemical inhibitory action (Barker and Wolf, 1977).

Outgrowth is the development of a vegetative cell from a germinated spore. If germination is stimulated but the environment is insufficent to produce a vegetative cell then either (1) development stops, or (2) the outgrowing cell may proceed to form a second spore without intervening cell division which is a process called microcycle.

It was shown that it takes 90 min from the time of initiation of germination for B. subtilus to elongate and begin cell division (Santo and Doi, 1974).

Spores are basically metabolically inactive and so would not be expected to have any effect in the GI tract. It is only the germinated cell which would be able to influence either lactobacillus or E. coli and this effect, as previously seen, must occur in the anterior small intestine.

B. subtilus was shown to reduce E. coli in the GI tract of rabbits, reduce diarrhea, and improve performance (Hattori, Y., M. Suzuki, T. Uchida, H. Kitamura, M. Kozasa, N. Watanabe, and N. Watanabe, unpublished data; Hattori, Y., M. Kozasa, J. Brenes, unpublished data). B. subtilus improved number of pigs weaned per litter at 21 d and average weaning weight (Danielson, unpublished data).

METHODS AND MATERIALS

This study was divided into two trials, the first being performed in April and the second in October of 1983. These trials were identical in protocol and differed only in numbers and animals used.

This experiment was conducted at the Kansas State University Swine Research Center using Yorkshire-Duroc sows. The Bacillus subtilus product[1] contained 10% of the bacteria in a pure spore form with a carrier of 55% calcium carbonate and 35% sprayed dried whey. The concentration was 10^9 colony-forming units (CFU) per g of product.

This experiment was basically divided into two parts. The first was the effect of B. subtilus on sow and baby pig performance and sow fecal populations. The second involved the quantitation of bacterial populations of the gastro-intestinal (GI) tract of the newborn pig.

The sows were kept in dirt-lot gestation pens until one week before farrowing when they were moved into the farrowing house. They were fed once daily in individual feeding stalls during gestation. Sows received no medication but did get an

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[1] Floramate ^{R}, PBI/Gordon, Kansas City, Mo

Escherichia coli milk vaccine containing several strains at approximately 21 d prior to farrowing.

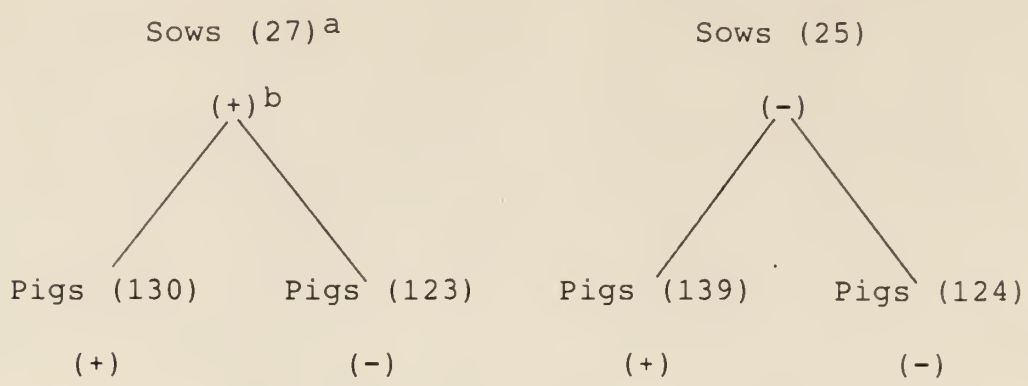
The environmentally regulated farrowing house had 29 stalls and an oxidation ditch for waste disposal. The floor was plastic coated woven wire placed over concrete slats. There was a 250-watt heat lamp hanging in each stall for the baby pigs. The farrowing house was cleaned with a high pressure cleaner and disinfected between groups. Sows were washed with warm water and soap before moving into the farrowing crate. Pigs were weaned at approximately 21 days of age.

The sows were randomly divided into two groups: treated (+) and control (-). The treated sows received 5 g of the bacillus spore product per head per day top-dressed on their feed starting approximately 14 d pre-farrowing and continuing to 14 d post-farrowing. Control sows did not receive any of the product. In the first trial (April) there were 13 primiparious females in each group. In the second trial, sows were allotted by parity with 14 treated sows and 12 controls with a total of 10 first parity and 16 second parity. None of the sows in the second trial were the ones used in the first.

The litter of each individual sow was also randomly divided into two groups: treated (+) and control (-). The

treated baby pigs received one g of the bacillus product mixed with 1 ml of safflower oil given orally using a 20 cc syringe and 8 cm of tygon tubing. The control pigs received only 1 ml of safflower oil. This was done within 24 hours of birth and after the pigs were processed. The odd-numbered ear-notched pigs received the product whereas the even-numbered ear-notched pigs were the controls. Thus, there were four baby pig treatments: (+) sow (+) pig, (+) sow (-) pig, (-) sow (+) pig, and (-) sow (-) pig. The breakdown of the numbers for each treatment for trials one and two and the totals respectively were as follows: (+) (+) 63, 67, 130; (+) (-) 60, 63, 123; (-) (+) 68, 71, 139; and (-) (-) 58, 66, 124. The experimental design is illustrated in figure 1.

FIGURE 1. EXPERIMENTAL DESIGN



[a] Numbers in paranthesis are totals for both trials.

[b] (+) = fed 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing; (-) = none.

Fecal samples were collected from sows at five different time periods. The first collection time (-14) was approximately 14 days before farrowing, immediately before any bacillus product was administered, and was used as a baseline. The second collection time (-7) was seven days later. The third (0) was within 24 hours after farrowing. The fourth (7) was seven days and the fifth (14) fourteen days post-farrowing. A grab sample of 5-10 g was taken using a separate plastic glove for each sow and placed into a sterile,

labeled whirl-pak bag[2]. The first two collection periods were based on expected dates; whereas the last three collections were based on the actual farrowing dates.

The fecal samples were taken to the laboratory within one hour of collection to initiate the counting procedure. One g (± .001) aliquot was weighed from each sample and placed into individual sterile stomacher bags. To each bag was added 99 ml of diluent (see Appendix A) and then homogenized in a Stomacher 400[3] for 1 minute. An aliquot of this (approximately 6 ml) was poured into a sterile test tube. Serial dilutions were prepared with the blanks containing 9 ml of diluent (see Appendix A) to a final dilution of 1:10,000,000.

Fecal samples were cultured for Bacillus subtilus, lactobacillus, and E. coli. The bacillus was cultured on specially prepared medium (see Appendix B) containing chloramphenicol and polymyxin B as selective agents. Lactobacillus was cultured on MRS medium (see Appendix C) and E. coli was cultured on Violet Red Bile Agar[4].

Each of the media was prepared, autoclaved, and allowed to cool for 30 min to 1 h. They were dispensed using a

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[2] Nasco, 901 Janesville Ave., Fort Atkinson, Wisconsin 53538

[3] Tekmar Company, P.O. Box 371856, Cincinnati, Ohio 45222

[4] Difco Laboratories, Inc., Detroit, Michagin 48201

Masterflex fixed-speed drive peristaltic pump[5] with a 460 ml/min pump head. The pump was controlled by an electronic digital timer operated by a footswitch which would automatically reset. Approximately 1.2 seconds would dispense 10 ml of media. For each media 1.1 m tygon tubing was autoclaved before each use. After the media was cooled, the tubing was fit into the pump head and one end carefully lowered into the media. The other was held by hand to dispense the media onto disposible petri dishes[6].

Initially, three dilutions of each sample was done in duplication. These were as follows: B. subtilus, 10^2 to 10^4; lactobacillus, 10^6 to 10^8; and E. coli, 10^4 to 10^6 except on the day of farrowing when E. coli was increased to 10^7 to 10^8. As the experiment progressed the ranges became easier to predict and so, oftentimes, only 2 dilutions, done in duplicate, were necessary.

One ml of inoculum taken from the appropriate dilution with a disposible 1 ml serlogical pipette[7] was put on the prepared media plate. This would then be covered with

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[5] Cole-Parmer, 7425 North Oak Park Ave., Chicago, Ill. 60648

[6] American Scientific Products, Kansas City, Mo. 64116

[7] Dow-Corning, Midland, Michigan 48201

approximately 2-4 ml of a sterile agar overlay (see Appendix D) using the peristatic pump with sterilized tygon tubing. The agar was kept in a 55 C water bath to prevent hardening. The plate was swirled to dispense the overlay evenly over the media and left to harden.

The bacillus plates were aerobically incubated for 48 h at 37 C. The white/yellowish colonies were from two to ten mm with an irregular border and a distinct raised central point. E. coli was aerobically incubated for 24 h at 37 C and produced small, purple colonies. Lactobacillus was anearobically incubated in CO_2 for 48 h at 37 C. This was maintained by continually supplying CO_2 from a pressurized cylinder. The colonies were generally small and off-white.

All counting was done on a Fisher Accu-Lite Bacterial Colony Counter[8]. All the numbers were eventually recorded in log CFU per g of wet feces.

Sows were weighed at parturition and weaning with the difference being lactation loss. Backfat thickness was determined by ultrasonic measurements[9] at last rib. Feed intake during lactation, and number of pigs born alive, dead,

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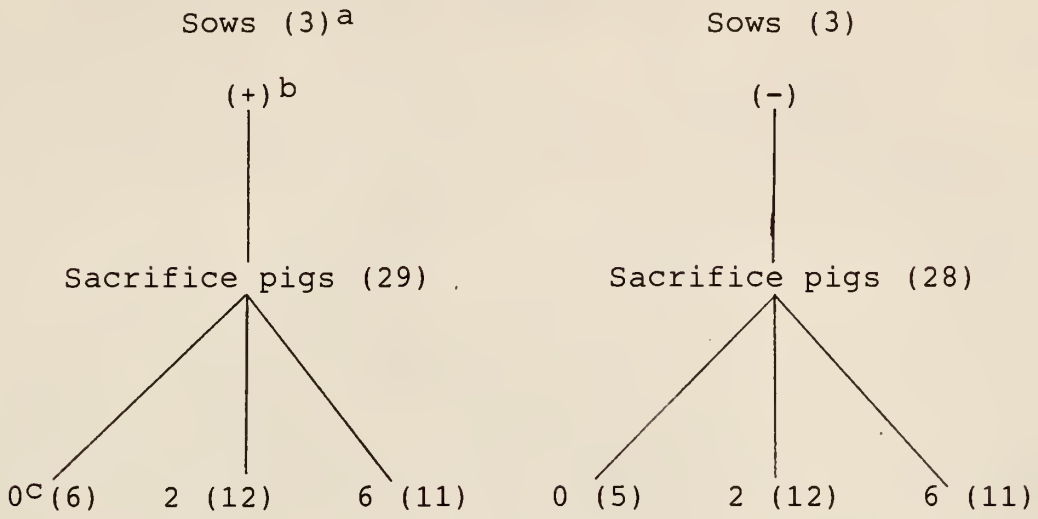
[8] Fisher, 1241 Ambassador Bvld., St. Louis, Mo. 63178

[9] International Livestock Improvement Services Corp., P.O. Box 1870, Ames, Iowa

and mummified were recorded. Percent pig survival was the difference between the number of pigs born alive and those weaned. Pigs were transferred to sows in the same treatment to equalize litter size. Pigs were weighed within one d after birth and were weaned at three weeks. Weaning weights were standardized to 21 d.

The second part of this experiment was to evaluate the effect of B. subtilus on baby pig GI tract bacterial populations. Six litters were sacrificed, three from the treated sows and three from the control. There were three sacrifice times: 0 days, which was after farrowing and before any of the bacillus was administered, two days (2) and six days (6) post-farrowing. One or two pigs were sacrificed on day 0 as a baseline, four on day 2, and four or five on day 6. Half of the pigs on day 2 and 6 were treated. Two litters were sacrificed in April (one from each sow treatment) and four in October (figure 2).

FIGURE 2. EXPERIMENTAL DESIGN



a Numbers in parenthesis are totals for both trials.

b (+) = fed 1 g; (-) = none.

c 0, 2, 6 are d of sacrifice.

The pigs were transported from the swine farm to the necropsy room in a plastic covered container and sacrificed within an hour via electrocution. Five different sites were taken from the GI tract: stomach (cardiac region), duodenum (1 m from pylorus), jejunum (15 cm from the pylorus posterior to the bile and pancreatic ducts), whole cecum, and rectum (posterior colon). These were put into individual, labeled whirl-pak bags and cultured as before to B. subtilus, lactobacillus, and E. coli. The pH of the stomach contents

was also measured within five min after electrocution using a standard glass combination electrode[10].

Daily subjective diarrhea scores were recorded starting when the first sow farrowed and continuing through to weaning. A score of 3 indicated severe enteritis, identified by very loose stool covering the posterior part of the pig and a noticeable weight loss; 2 being moderate enteritis, identified by loose stool; and 1 meaning that there were no evident signs of the disease. These scores were recorded only as one number for an entire litter.

This entire experiment was statistically analyzed as a split-split-split plot design with the sow being the first whole plot (litter (sow treatment) was error a), the baby pigs being the second whole plot (treatment by litter (sow treatment) was error b), and each individual pig being the third (location by treatment by litter (sow treatment) was error c).

Sow performance traits were tested comparing treated vs control. For baby pig performance, sow treatment, pig treatment, and sow treatment by pig treatment interaction were analyzed as a split-plot with sow treatment as the first whole

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[10] Beckman Instruments, Inc., Scientific Division, Ca. 92713 Model 34105-520

plot (sow (sow treatment) as error a) and pig treatment as the second whole plot (pig treatment by sow (sow treatment) as error b). Sow fecal populations were compared across treatment and time. The bacterial intestinal populations were compared across sow treatment, pig treatment, sow treatment by pig treatment interaction, time and site. Scours score was compared across sow treatment, pig treatment, and day; whereas pH was by day and sow treatment by pig treatment interaction.

RESULTS

Sow performance

B. subtilus did not influence any of the sow performance traits (table 1). Since primpiparous sows have smaller litters and consume less feed during lactation, parity had a significant effect in avg birth wt and sow avg lactation daily feed intake and so was included in their model statements. The latter inclined toward significance (P = .07) in the first trial, but when pooled with the second trial was not significant (P = .26).

Pig Performance

Neither treating the sows, the pigs, nor any interaction caused a difference in avg birth wt, avg weaning wt, avg daily feed intake, or percent survival (table 2).

TABLE 1. EFFECT OF FEEDING BACILLUS SUBTILUS SPORES ON SOW PERFORMANCE

Item	Control	Treated[1]	SE	Parity[2]
Number born alive	10.25	10.02	.54	
Number born dead	.56	.55	.19	
Number mummies	.28	.34	.13	
Avg birth wt, kg	1.43	1.42	.05	X
Number weaned	8.38	8.23	.45	
Pig 21 d wt, kg	5.35	5.35	.21	
Avg scour score	1.17	1.12	.04	
D to estrus	5.00	5.08	.27	
Sow avg lactation daily feed intake, kg	5.5	5.8	.20	X
Sow lactation loss, kg	9.85	9.92	1.9	
Backfat, mm	22.31	22.23	.98	

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Those marked "X" included parity in the model statement.

TABLE 2. EFFECT OF FEEDING BACILLUS SUBTILUS SPORES ON BABY PIG PERFORMANCE

	Control sows		Treated sows[1]		
Item	Control pigs	Treated[2] pigs	Control pigs	Treated pigs	SE
No. pigs inital[3]	110	125	107	117	
Birth wt, kg	1.3	1.3	1.3	1.3	.10
Survival rate, %	85.5	88.0	86.9	85.5	
Weaning wt, kg	5.3	5.1	5.2	5.3	.19

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

[3] Does not include those pigs which were sacrificed.

Sow Fecal Populations

Table 3 illustrates the bacterial population of sow feces of the three bacteria cultured. Bacillus was, as expected, virtually undetected at the first collection period which was before any of the product was administered. The .13 mean resulted from one sow which, it is thought, was moved in with the group of sows whose farrowing date was one day sooner than her own and so was given the product a day earlier then she

should have. We would have made this collection, then, approximately one day after she had ingested the bacillus. This number, though, was not different ($P > .05$) from zero.

The control sows, likewise, had essentially no detectable bacillus. The 1.00 and .19 means resulted from two different sows (and different from the one mentioned previous) who were accidently given the bacillus for apparently one time; however, these means were not different ($P > .05$) from zero.

The treated sows consistently averaged around log 5.5 CFU or half a million bacillus organisms per g of wet feces throughout the time of their treatment. This was higher ($P < .05$) than the first collection time and the control.

There was no difference between the treated and control sows at any time period for E. coli. However, on the day of farrowing, there was an increase ($P < .05$) of about 1.5 log CFU from one week pre-farrowing and one week post-farrowing. Even with this increase the bacillus did not have a suppressive effect.

There was no difference in lactobacillus either across time or between treatments. The slightly lower, though not significant numbers for the fifth period were from the CO_2 tank running out on the last day of incubation. If this had not occured the numbers would have, very probably, been closer to the other four periods.

TABLE 3. EFFECT OF BACILLUS SUBTILUS SPORES ON BACTERIAL POPULATION OF SOW FECES (Log CFU per g of wet feces)

Bacterium	Sow Treatment	Period, d -14	-7	0	7	14	SE
E. coli	Treated[1]	6.64[a]	6.81[a]	8.23[b]	6.36[a]	6.19[a]	.13
	Control	6.62[a]	6.74[a]	8.34[b]	6.37[a]	6.39[a]	.14
Lactobacillus	Treated	8.28	8.49	8.70	8.46	8.04	.13
	Control	8.05	8.51	8.58	8.32	7.91	.14
Bacillus	Treated	.13[a]	5.33[b]	5.54[b]	5.67[b]	5.42[b]	.10
	Control	.00[a]	1.00[a]	.00[a]	.19[a]	.00[a]	.10

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[a,b] Means with different superscripts in same row or column of same bacterium are different ($P < .05$).

Baby Pig Intestinal Populations

All of the results for the intestinal populations of the baby pig was analyzed by three ways. The first was by sow treatment (sowtrt) which means that all of the results were pooled considering only whether or not the sow received the product regardless of whether the baby pig did. The second was by pig treatment (pigtrt) in which the results were pooled by

whether or not the baby pig received the oral administration of product regardless of whether the sow was treated or not. The third was by time which did not consider any treatment groups but only looked at the results on days 0, 2, and 6.

For all three bacteria there were no sow treatment by pig treatment, sow treatment by time, pig treatment by time, nor sow treatment by pig treatment by time interactions at any location.

Bacillus subtilus

There was no difference between the treated and control groups' intestinal population due to sow treatment (table 4). The most noticeable, however, was that in the stomach and small intestine the bacillus numbers were exceedingly small being less than 100 organisms per g. It was only in the cecum and rectum that the numbers became larger ($P < .05$).

The treated pigs had more bacillus ($P < .05$) in the stomach, duodenum, and jejunum, but not in the cecum and rectum. However, the numbers for the treated pigs were still very small, being less than 1000 organisms per g of wet feces. As with sow treatment, it was not until the cecum and rectum that the bacillus organism became more numerous ($P < .05$).

In examining the bacillus populations across time on day 0 there was a detectable, though very small, amount of bacillus. Two days after the pigs orally received the product

these numbers increased ($P < .05$) except in the jejunum where the increase was only numerical. Even though this may represent the highest numbers of bacillus attained, they were probably far too low in the stomach and small intestine to have any appreciable effect.

Six days after farrowing the bacillus in the stomach essentially passed through; the level of .2 not being different ($P < .05$) from zero. The small intestine also showed that the bacillus was nearly completely out of this region. The numbers in the large intestine, however, remained basically the same.

TABLE 4. EFFECT OF FEEDING BACILLUS SPORES ON COUNTS OF BACILLUS SUBTILUS IN GASTROINTESTINAL TRACT OF THE BABY PIG (Log CFU per g)

Item	Variable	Stomach	Duodenum	Jejunum	Cecum	Rectum	SE
Sowtrt	Treated[1]	1.5[a]	1.3[a]	1.4[a]	4.1[b]	4.8[b]	
	Control	1.0[a]	1.0[a]	1.7[a]	4.0[b]	4.6[b]	
Pigtrt	Treated[2]	2.1[a]	2.1[a]	2.5[a]	4.7[b]	5.4[b]	.11
	Control	.6[c]	.3[c]	.5[c]	3.4[d]	4.1[b,d]	.11
Time, d	0	1.0[a]	.5[c]	1.3[a]	1.5[a]	1.3[a]	.15
	2	2.5[a]	1.9[a]	2.1[a]	4.3[b]	5.2[b]	.12
	6	.2[c]	.7[c]	1.1[a,c]	4.9[b]	5.4[b]	.13

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

[a,b,c,d] Means with different superscripts in same row or column are different ($P < .05$).

E. coli

There was no difference between the treated and control sows on E. coli counts (table 5). There was, as one would expect, an increase ($P < .05$) in E. coli in the large intestine as compared to the small intestine.

There was, again, no difference between the treated and control pigs on E. coli counts (table 5). The same E. coli increase in the large intestine was observed as above.

There were no differences across time at any location and, again, an increase ($P < .05$) at each time period in E. coli numbers in the large intestine.

TABLE 5. EFFECT OF FEEDING BACILLUS SPORES ON COUNTS OF E. COLI IN THE GASTROINTESTINAL TRACT OF THE BABY PIG

(Log CFU per g)

Item	Variable	Stomach	Duodenum	Jejunum	Cecum	Rectum
Sowtrt	Treated[1]	4.7[a]	4.9[a]	5.2[a]	7.7[b]	8.1[b]
	Control	4.9[a]	4.7[a]	5.1[a]	8.1[b]	8.5[b]
Pigtrt	Treated[2]	4.6[a]	4.9[a]	5.4[a]	7.9[b]	8.1[b]
	Control	4.9[a]	4.6[a]	4.8[a]	7.8[b]	8.4[b]
Time, d	0	4.8[a]	5.2[a]	5.4[a]	8.0[b]	7.7[b]
	2	4.8[a]	4.7[a]	5.0[a]	7.9[b]	8.7[b]
	6	4.7[a]	4.7[a]	5.2[a]	7.8[b]	8.1[b]

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

[a,b] Means with different superscripts in same row or column are different ($P < .05$).

Lactobacillus

There was no difference between the treated and control sows on lactobacillus counts (table 6). The stomach had a greater concentration ($P < .05$) of lactobacillus than the small intestine with the cecum and rectum being even higher ($P < .05$) than the stomach.

There was no difference between the treated and control pigs with the treated pigs having the same pattern as mentioned above. For the control pigs, however, though the stomach had a greater level of lactobacillus than the small intestine this difference was not significant. The large intestine did have higher numbers ($P < .05$) than the stomach.

At day 0 the duodenum and jejunum were not different from each other but the duodenum did have lower numbers ($P < .05$) than at any other location. The concentration of lactobacillus in the cecum and rectum was less ($P < .05$) here than at any other time.

On day 2 the stomach had more ($P < .05$) lactobacillus than the small intestine but less ($P < .05$) than the large intestine.

Six days after birth the small intestine maintained the same levels as at the other time periods but the level in the stomach increased so that there was now no difference between

it and the large intestine. There was also a significant increase ($P < .05$) over the other time periods.

TABLE 6. EFFECT OF FEEDING BACILLUS SPORES ON COUNTS OF LACTOBACILLUS IN THE GASTROINTESTINAL TRACT OF THE BABY PIG (Log CFU per g)

Item	Variable	Stomach	Duodenum	Jejunum	Cecum	Rectum	SE
Sowtrt	Treated[1]	7.4^{a}	6.7^{b}	6.7^{b}	8.3^{c}	8.2^{c}	
	Control	7.4^{a}	6.9^{b}	6.7^{b}	8.1^{c}	8.3^{c}	
Pigtrt	Treated[2]	7.5^{a}	6.7^{b}	6.7^{b}	8.4^{c}	8.4^{c}	.08
	Control	7.2^{a}	$6.9^{a,b}$	$6.7^{a,b}$	7.9^{c}	8.1^{c}	.08
Time, d	0	7.1^{a}	6.5^{b}	$6.7^{a,b}$	7.1^{a}	7.1^{a}	.11
	2	7.5^{a}	6.9^{b}	6.9^{b}	8.9^{c}	8.7^{c}	.09
	6	8.1^{c}	7.0^{b}	6.9^{b}	8.6^{c}	8.7^{c}	.10

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

^{a,b,c} Means with different superscript in same row or column are different ($P < .05$).

pH of the stomach contents

There was no difference in the pH between the treated and control groups in sow treatment, pig treatment, or sow treatment by pig treatment interaction (table 7). However, on day 0 the pH was less acidic ($P < .05$) than on days 2 and 6.

TABLE 7. EFFECT OF FEEDING BACILLUS SPORES ON pH OF THE STOMACH OF THE BABY PIG

	Control sows		Treated sows[1]		
Day	Control pigs	Treated[2] pigs	Control pigs	Treated pigs	Mean
0	4.56	5.16	3.62	3.82	4.29[a]
2	3.33	3.43	2.97	3.04	3.19[b]
6	3.54	3.45	3.33	3.08	3.35[b]

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

[a,b] Means with different superscript in same column are different ($P < .05$).

Scours score

There was no difference in the level of scours evaluated for pig treatment nor for day (table 8). The control sows' litters had a greater scours problem ($P < .05$) than the treated sows but this was only for those who were considered to have severe scours (evaluated as a "3"). For the other two categories there were no differences.

TABLE 8. EFFECT OF FEEDING BACILLUS SPORES ON SCOURS SCORE

	Sow treatment		Pig treatment		Day		
Score	Control	Treated[1]	Control	Treated[2]	0	2	6
1 (none)	11	13	11	13	1	10	13
2 (some)	0	3	2	1	0	2	1
3 (severe)	5[a]	0[b]	4	1	1	4	0

[1] Treated with 5 g bacillus spores per head per d from 14 d pre- to 14 d post-farrowing.

[2] Treated with 1 g bacillus spores.

[a,b] Means with different superscript in same row are different ($P < .05$).

DISCUSSION

Effect of B. subtilus on sow performance

Administering the bacillus product to the sows did not have any effect on performance. Because the proposed mode of action of the product was in surpressing E. coli, this would be somewhat expected since E. coli is not considered as being inhibitory to sow performance.

Since the sows received the bacillus only 14 d pre-farrowing, it would not affect such items as number born alive, number born dead, mummies, or sow body wt change.

There were two principle reasons for giving the sow the product. The first was to see whether the bacillus could be transmitted to the pig either prenatal or through the sow feces. The second was that the baby pigs are infected with E. coli from some source. If this source is from the sow feces it was hoped that the bacillus might reduce this source of E. coli and thus reduce possible exposure to the pig.

It was also necessary to determine if the culture should be given both to the sow and baby pig or if only one of these was sufficient.

Effect of B. subtilus on baby pig performance

Enteritis causes economic loss in two ways: one is by death due to severe dehydration and the other is by reduced weight gain and poor growth (Bergeland, 1980).

The number of pigs which actually die from enteritis may not be as significant as reduced performance. The disease may not be severe enough to cause many deaths. Also, not a great number of pigs usually die before weaning and those that do can be from such a large number of causes (crushing, exposure, other diseases, etc) that those which, in fact, do die from enteritis may not be noticeable. Many more pigs that survive till weaning may have suppressed growth because of enteritis. For this reason weaning weight and avg daily gain would be better indicators of whether enteritis was controlled or not than survival data. The bacillus probiotic could be most effective here.

But, as was seen previously, there was no effect of bacillus on any of these indicators. It could be that there was, at this time, no problem with enteric colibacillus and, thereby, nothing for the bacillus to act upon. There was, in fact, no real noticeable problem with enteritis in either sow group and the scours score was only a little above a "1" for both.

It could also be that the bacillus, in the way administered here, was not effective. Data quantitating the bacteria in five sections of the GI tract provide additional information on the value of B. subtilus.

Intestinal population of B. subtilus

The lack of a difference of bacillus numbers between the treated and control sows seems to indicate very little, if any, bacillus was ingested from the sow's feces or milk.

However, the treated baby pigs had a greater number ($P < .05$) of bacillus indicating that feeding the culture to the pig was more effective. Less of the product would be used, therefore, making this method more economical (1 g total for each pig vs 140 g total for each sow which, for an average litter of 10 live pigs, would be 14 g per pig).

The small number detected for the control pigs may have been either from the sow or may have been picked up from their treated litter mates.

The bacillus on day 0 was most likely obtained from the sow. The numbers on day 2 were expected. The first several days after the oral administration would have the most bacillus present; but by day 6 most of the bacillus had passed out of the stomach and small intestine. B. subtilus, being a transient organism, does not colonize. This being the case, it is apparent that, since E. coli is thought to have its

pathogenic effect in the upper small intestine (Smith and Halls, 1967; Nielsen et al., 1968; Arbuckle, 1969) , the bacillus culture must definitely have its effect within six days (or less) of administration. The higher numbers maintained in the cecum and rectum indicate that this passing of the bacillus was sequential beginning in the stomach and moving down the tract with the posterior colon being the last section with bacillus.

The most important aspect of these data was that the numbers in the stomach and small intestine were consistently very low, being less than 1000 organisms per g, throughout every treatment and time period. This indicates that the B. subtilus may not be germinating until the cecum. The spore state, which was what the pig ingested, was basically metabolically inactive (Russell, 1982). Therefore, except for some physical inhibition which was very unlikely, the bacillus would have no effect on the E. coli or lactobacillus populations. It was only when the conditions seemed more favorable in the cecum that the bacillus germinated and built up significant numbers for the potential inhibition. By this time, however, it may have been too late to have any influence on preventing enteritis.

The lack of any sow or pig performance response may have been due to this late germination of the spore and not to

bacillus being ineffectual in controlling enteritis. The method of administration rather than the bacillus may have been the problem. It may be necessary to germinate the bacillus before it is ingested or determine how to germinate it in the upper GI tract. Should this be economically and conveniently possible then bacillus might prove to be useful in controlling enteritis. The product should be retested under these conditions.

Bicarbonate does have an inhibitory effect on the germination of bacillus (Barker and Wolf, 1977). This product contained approximately 55% calcium carbonate. It may be that the bacillus was prevented from germinating until it got far enough removed from the carbonate carrier which, in this case, would be the cecum. It is possible that the results would have been very different if the bacillus were in a different carrier.

The concentrations between the duodenum and jejunum and between the cecum and rectum were not different ($P > .05$). This means that it is necessary to culture from only one of each of these two sections. This would save considerable time and money.

Effect of B. subtilus on E. coli

The lack of response in the stomach and upper part of the GI tract may be explained by the bacillus not having

germinated. The reason for the lack of a response in the cecum and rectum is more complicated since there was, seemingly, enough numbers of bacillus to elicit a response. One obvious answer may be that the bacillus was not effective. Since no effort was made to distinguish between whether the bacillus was spores or germinated cells when originally cultured, it was difficult to know if all of the bacillus quantitated in the cecum and rectum were germinated. A good percentage of the bacillus may still have been in spore form. Since spores would have no effect the remaining germinated cells might have been too few in number to have any substantial effect. It could also be that the mode of action of the bacillus was ineffectual beyond the small intestine.

It was apparent, from these data from day 0, that within several hours after birth E. coli had firmly established itself completely throughout the GI tract (Ducluzeau, 1983). Pathogenic E. coli would be expected to be present and producing enterotoxin at this time (Myers, 1975). It would be benefical for the bacillus to be introduced to the pig by this time or else it would be attempting to influence an already firmly entrenched pathogen. This level of E. coli appears to be maintained at least through day 6 (Uchida et al., 1965; Barrow et al., 1977).

There were no significant differences between the concentrations in the duodenum and jejunum nor between the cecum and the rectum. Therefore, only one site from each of these sections needs to be cultured and quantitated in further studies.

Effect of B. subtilus on lactobacillus

As with E. coli, the lack of response in the stomach and small intestine may have been due to bacillus' extremely low numbers or lack of germination. The higher concentration of lactobacillus in the stomach than the small intestine was expected, as was the still higher numbers in the large intestine (Uchida et al., 1965; Barrow et al., 1977).

The levels of lactobacillus in the cecum and rectum being the same between the treated and control groups can be explained the same as those given for E. coli.

On day 0, lactobacillus had completely colonized the entire GI tract within several hours after birth (Ducluzeau, 1983). Two days later the levels in the large intestine did increase and by day 6 the lactobacillus reached a higher number than during previous collections. This level was generally maintained throughout the lifetime of the pig (Uchida et al, 1965).

The role of lactobacillus in the suppression of E. coli by bacillus is only a theoretical mode of action in which

bacillus actually increases the host's natural lactobacillus which lowers the GI pH thus inhibiting E. coli (Watkins et al., 1982). If, however, this bacillus probiotic does not involve lactobacillus in this inhibition then lactobacillus levels would not be expected to change. The only way in which lactobacillus would increase in numbers if, in fact, it had no role in any bacillus model would be that as E. coli decreased, lactobacillus increased simply as a function of the extra space and nutrients.

As with the bacillus and E. coli, there were no differences between the numbers of lactobacillus between the duodenum and jejunum and between the cecum and rectum. In further experiments, it would be necessary to choose only one site from each of these two sections as the representative in culturing any of these three bacteria.

Effect of B. subtilus on stomach pH

The reason for measuring the pH was because if the bacillus increased the lactobacillus concentration then more lactic acid would be produced, thereby, lowering the stomach pH (Watkins et al., 1982). Since the level of lactobacillus was, in fact, not changed it was not surprising that neither did the pH.

It was concluded that the stomach pH of the newborn pig becomes more acid after one or two days (Barrow et al., 1977).

Because the optimum pH for bacillus germination is from 2 to 3 (Keynan et al., 1964, Keynan and Halvorson, 1965), this may be the reason the bacillus could have remained a spore.

Effect of B. subtilus on scours score

The difference observed in the scour score for the sow did not seem to have any effect on subsequent baby pig performance. Indeed, this did not even reflect in a significant difference in the overall scours score. This may have been because three litters of the treated sows were considered to have mild diarrhea, but none severe; whereas the control sows had five severe cases, but none which were mild. These may have balanced out each other.

The overall scours score being so low (1.12 for the treated and 1.17 for the control sows) may indicate that, as previously stated, there may not have been any E. coli enteritis problem at the times of this experiment and so there was nothing for the bacillus to react to. To answer this problem either a challenge study or one conducted with continually scouring pigs (with a significantly high scours score during the experiment) would have to be done.

CONCLUSION

Feeding Bacillus subtilus spores to sows or to baby pigs had no effect on their performance.

Though the bacillus counts were significantly higher in the feces of the treated sows, it had no effect on sow fecal E. coli or lactobacillus populations. On the day of farrowing, E. coli counts increased by approximately 1.5 log colony forming units compared to pre- and post- farrowing fecal populations.

The bacillus also had no effect on E. coli or lactobacillus levels in the five sites of the gastrointestinal tract of the baby pig. The bacillus may not have been germinating until the cecum.

This lack of effect may have been due to: 1) Bacillus subtilus being ineffective as a probiotic. 2) The calcium carbonate carrier inhibiting the germination of the bacillus. 3) An inadequate environment in the stomach and small intestine to stimulate germination. 4) There may have not been a significant enteritis problem during the time of the experiments.

The counts between the duodenum and jejunum and between the cecum and rectum were not significantly different and so only one site from each of these two sections needed to cultured.

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APPENDIX A

Phosphate Buffer Concentrate

Add 17 g KH_2PO_4 to 250 ml deionized, distilled water in a 500 ml volumetric flask. Adjust the pH to 7.2 with 1N NaOH. Bring to volume. Add 1.25 ml of this stock solution to 1 liter of distilled water and stir to make the final buffer used.

1. To make dilution bottles, dispense 99 ml into French Square 250 ml bottles. Cap tightly and autoclave at 121 C and 15 psi for 15 min.

2. To make dilution tubes, dispense 9 ml into screw top test tubes. Cap tightly and autoclave at 121 C and 15 psi for 15 min.

APPENDIX B

Selective Medium for Bacillus in Feed

Ingredient	Amount	
Peptone	10	g
Meat Extract	5	g
NaCl	5	g
Agar	15	g
Chloramphenicol	2.5	mg
Polymyxin B	12,500	units

Adjust pH to 7.2 ± .1

Add water to make 1000 ml

APPENDIX C

MRS Medium for Lactobacillus (deMan, Rogosa and Sharpe. 1960. J. Appl. Bact. 23(1):130)

Ingredient	Amount	
Tryptone	10	g
Beef extract	10	g
Yeast extract	5	g
Glucose	20	g
K_2HPO_4	2	g
Sodium citrate	2.5	g
Tri-ammonium citrate	2	g
$MgSO_4$, $7H_2O$	.1	g
$MnSO_4$, $4H_2O$	.1	g
Tween 80	1	ml
Agar	20	g

Adjust pH to 5.5 ± .1

Add water to make 1 liter

APPENDIX D

Agar Overlays

Add 7.5 to 10 g of agar to 1 liter of distilled water. The amount of agar may be adjusted depending on how quickly the agar hardens (or does not) once dispensed on the plate. This is autoclaved at 121 C and 15 psi for 15 min and then immediately put into a 55 C water bath to prevent solidification. It should be allowed to cool down to this point before dispensing. Anywhere from two to four ml should be dispensed per plate; although this can be changed depending on how much is needed to mix with the inoculum and then quickly (approximately 1 min) harden.

APPENDIX E

TABLE 1 - EFFECT OF BACILLUS CULTURE ON SOW PERFORMANCE

Treatment:	Treated		Control	
Trial:	1	2	1	2
No. born alive	10.46±.766[a]	9.57±.738	11.00±.766	9.50±.797
No. born dead	.46±.273	.64±.263	.54±.273	.58±.284
No. mummies	.62±.184	.07±.177	.31±.184	.25±.191
Avg birth wt, kg	1.24±.06	1.58±.10	1.25±.06	1.63±.10
No. weaned	8.46±.612	8.00±.637	8.46±.612	8.30±.698
Avg weaning wt, kg	5.09±.290	5.61±.315	5.15±.290	5.55±.331
Avg scour score	1.16±.060	1.08±.058	1.18±.060	1.15±.062
Lactation loss, kg	18.06±2.77	1.78±2.67	21.06±2.77	-1.36±2.88
Avg daily feed intake, kg	4.99±.288	6.71±.429	4.95±.290	5.95±.446

[a] Least square mean with standard error

TABLE 2 - BACILLUS SUBTILUS IN GASTROINTESTINAL TRACT OF THE BABY PIG (Log CFU per g)

		Control sow		Treated sow	
Location	Day	Control pig	Treated pig	Control pig	Treated pig
Stomach	0	1.30	.14	.00	1.66
	2	.00	4.14	1.17	4.39
	6	.06	.00	.66	.03
Duodenum	0	1.28	.08	1.10	1.37
	2	.00	3.86	.00	4.00
	6	.78	2.19	.55	.95
Jejunum	0	.00	.14	.00	1.41
	2	.00	3.66	.00	3.64
	6	.06	.67	1.14	1.22
Cecum	0	1.45	.42	1.16	2.01
	2	.74	6.01	4.27	5.15
	6	4.67	5.21	4.78	5.04
Rectum	0	1.90	.37	2.24	.21
	2	1.36	7.06	4.65	6.46
	6	5.40	5.38	5.16	5.59

TABLE 3 - E. COLI IN GASTROINTESTINAL TRACT OF THE BABY PIG

(Log CFU per g)

		Control sow		Treated sow	
Location	Day	Control pig	Treated pig	Control pig	Treated pig
Stomach	0	6.45	5.86	6.05	4.96
	2	4.85	4.62	4.44	4.88
	6	4.52	4.40	5.33	3.63
Duodenum	0	6.13	5.45	4.74	5.81
	2	4.82	5.03	4.72	5.25
	6	4.53	5.29	4.99	5.84
Jejunum	0	6.05	5.12	5.15	5.13
	2	4.62	4.40	4.55	5.29
	6	4.13	4.80	4.90	4.57
Cecum	0	7.69	7.51	7.14	8.78
	2	8.14	8.10	7.86	7.66
	6	7.95	8.17	7.84	7.34
Rectum	0	7.82	7.29	8.62	5.94
	2	8.63	8.61	8.79	8.22
	6	8.56	8.51	7.74	7.98

TABLE 4 - LACTOBACILLUS IN GASTROINTESTINAL TRACT OF THE BABY PIG (log CFU per g)

		Control sow		Treated sow	
Location	Day	Control pig	Treated pig	Control pig	Treated pig
Stomach	0	7.64	5.83	7.08	7.62
	2	7.44	7.47	7.48	7.57
	6	7.85	7.75	7.36	6.85
Duodenum	0	6.48	5.58	7.01	5.79
	2	7.01	7.12	6.45	7.15
	6	6.55	7.24	6.58	7.07
Jejunum	0	6.68	5.41	5.98	7.04
	2	7.02	6.93	6.49	7.28
	6	6.37	7.44	7.30	6.65
Cecum	0	7.47	6.40	7.78	7.20
	2	8.82	9.10	8.84	8.74
	6	9.11	8.76	8.81	7.71
Rectum	0	7.74	5.47	7.73	
	2	8.68	8.93	8.45	8.57
	6	9.19	9.05	8.62	8.07

APPENDIX F

TABLE 1. SOW PERFORMANCE - NUMBER BORN ALIVE

Source	Df	SS	PR > F
Treatment	1	.707	.76
Trial	1	18.511	.13
Treatment by trial	1	1.205	.69

TABLE 2. SOW PERFORMANCE - NUMBER BORN DEAD

Source	Df	SS	PR > F
Treatment	1	.001	.97
Trial	1	.166	.68
Treatment by trial	1	.060	.80

TABLE 3. SOW PERFORMANCE - NUMBER OF MUMMIES

Source	Df	SS	PR > F
Treatment	1	.054	.73
Trial	1	1.173	.11
Treatment by trial	1	.766	.19

TABLE 4. SOW PERFORMANCE - AVERAGE BIRTH WEIGHT

Source	Df	SS	PR > F
Treatment	1	.030	.72
Trial	1	.052	.64
Treatment by trial	1	.070	.59
Parity	1	3.864	.0002
Treatment by parity	1	.012	.83

TABLE 5. SOW PERFORMANCE - NUMBER WEANED

Source	Df	SS	PR > F
Treatment	1	.267	.82
Trial	1	1.151	.63
Treatment by trial	1	.267	.82

TABLE 6. SOW PERFORMANCE - AVERAGE WEANING WEIGHT

Source	Df	SS	PR > F
Treatment	1	.002	.99
Trial	1	11.854	.14
Treatment by trial	1	.187	.85

TABLE 7. SOW PERFORMANCE - AVERAGE SCOUR SCORE

Source	Df	SS	PR > F
Treatment	1	.029	.44
Trial	1	.035	.39
Treatment by trial	1	.012	.61

TABLE 8. SOW PERFORMANCE - DAYS TO ESTRUS

Source	Df	SS	PR > F
Treatment	1	.036	.83
Trial	0		
Treatment by trial	0		

TABLE 9. SOW PERFORMANCE - AVERAGE DAILY FEED INTAKE

Source	Df	SS	PR > F
Treatment	1	7.267	.18
Trial	1	18.873	.03
Treatment by trial	1	17.418	.04
Parity	1	38.162	.003
Treatment by parity	1	2.672	.41

TABLE 10. SOW PERFORMANCE - LACTATION LOSS

Source	Df		PR > F
Treatment	1	.318	.98
Trial	1	23535.832	.0001
Treatment by trial	1	594.407	.27

TABLE 11. SOW PERFORMANCE - BACKFAT LAST RIB

Source	Df	SS	PR > F
Treatment	1	.038	.96
Trial	0		
Treatment by trial	0		

TABLE 12. PIG PERFORMANCE - AVERAGE BIRTH WEIGHT

Source	Df	SS	PR > F
Parity	1	15.290	.01
Sow treatment	1	.143	.80
Parity by sow treatment	1	1.847	.37
Sow(sow treatment) error a	38		
Pig treatment	1	.244	.31
Parity by pig treatment	1	.582	.12
Sow treatment by pig treatment	1	.131	.45
Parity by sow treatment by pig treatment	1	.001	.95
Sow by pig treatment(sow treatment) error b	38		

TABLE 13. PIG PERFORMANCE - AVERAGE WEANING WEIGHT

Source		Df	SS	PR > F
Sow treatment		1	17.842	.54
Sow(sow treatment)	error a	37		
Pig treatment		1	8.801	.21
Sow treatment by pig treatment		1	11.303	.15
Sow by pig treatment(sow treatment)	error b	37		

TABLE 14. SOW FECAL BACTERIAL POPULATION - BACILLUS SUBTILUS

Source	Df	SS	PR > F
Treatment	1	316.585	.0001
Parity	1	.711	.20
Treatment by parity	1	.856	.16
Sow(treatment by parity) error a	4		
Period	4	304.215	.0001
Treatment by period	4	246.945	.0001
Parity by period	4	3.979	.11
Treatment by parity by period error b	4	11.764	.0003

TABLE 15. SOW FECAL BACTERIAL POPULATION - ESCHERICHIA COLI

Source		Df	SS	PR > F
Treatment		1	.007	.93
Parity		1	.326	.55
Treatment by parity		1	1.791	.20
Sow(treatment by parity)	error a	4		
Period		4	99.446	.0001
Treatment by period		4	.245	.96
Parity by period		4	4.700	.03
Treatment by parity by period	error b	4	1.358	.52

TABLE 16. SOW FECAL BACTERIAL POPULATION - LACTOBACILLUS

Source		Df	SS	PR > F
Treatment		1	.196	.48
Parity		1	2.576	.05
Treatment by parity		1	.012	.85
Sow(treatment by parity)	error a	4		
Period		4	8.183	.0001
Treatment by period		4	.416	.77
Parity by period		4	4.929	.0004
Treatment by parity by period	error b	4	.103	.98

TABLE 17. PIG INTESTINAL POPULATION - BACILLUS SUBTILUS - STOMACH

Source	Df	SS	PR > F
Sow treatment	1	1.639	.09
Litter(sow treatment) error a	4	1.278	.93
Pig treatment	1	17.133	.0017
Time	2	58.932	.0001
Pig treatment by time	2	50.474	.0001
Sow treatment by pig treatment	1	.544	.55
Sow treatment by time	2	.843	.76
Sow treatment by pig treatment by time error b	2	6.959	.11

TABLE 18. PIG INTESTINAL POPULATION - BACILLUS SUBTILUS - JEJUNUM

Source		Df	SS	PR > F
Sow treatment		1	3.600	.32
Litter(sow treatment)	error a	4	11.297	.06
Pig treatment		1	29.696	.0001
Time		2	21.456	.0005
Pig treatment by time		2	33.911	.0001
Sow treatment by pig treatment		1	.266	.64
Sow treatment by time		2	2.451	.36
Sow treatment by pig treatment by time	error b	2	2.094	.42

TABLE 19. PIG INTESTINAL POPULATION - BACILLUS SUBTILUS - DUODENUM

Source	Df	SS	PR > F
Sow treatment	1	.015	.95
Litter(sow treatment) error a	4	11.448	.27
Pig treatment	1	26.288	.0011
Time	2	11.335	.08
Pig treatment by time	2	44.937	.0002
Sow treatment by pig treatment	1	.122	.81
Sow treatment by time	2	3.434	.45
Sow treatment by pig treatment by time error b	2	2.802	.52

TABLE 20. PIG INTESTINAL POPULATION - BACILLUS SUBTILUS - CECUM

Source	Df	SS	PR > F
Sow treatment	1	5.204	.47
Litter(sow treatment) error a	4	33.049	.0009
Pig treatment	1	15.751	.0019
Time	2	96.979	.0001
Pig treatment by time	2	28.294	.0003
Sow treatment by pig treatment	1	2.668	.18
Sow treatment by time	2	5.314	.17
Sow treatment by pig treatment by time error b	2	22.043	.0014

TABLE 21. PIG INTESTINAL POPULATION - BACILLUS SUBTILUS - FECES

Source	Df	SS	PR > F
Sow treatment	1	2.261	.37
Litter(sow treatment) error a	4	8.823	.21
Pig treatment	1	5.192	.06
Time	2	103.926	.0001
Pig treatment by time	2	56.304	.0001
Sow treatment by pig treatment	1	4.208	.09
Sow treatment by time	2	5.495	.16
Sow treatment by pig treatment by time error b	2	12.737	.02

TABLE 22. PIG INTESTINAL POPULATION - ESCHERICHIA COLI - STOMACH

Source	Df	SS	PR > F
Sow treatment	1	.376	.58
Litter(sow treatment) error a	4	4.063	.39
Pig treatment	1	2.207	.14
Time	2	6.084	.06
Pig treatment by time	2	2.178	.33
Sow treatment by pig treatment	1	.399	.52
Sow treatment by time	2	.379	.82
Sow treatment by pig treatment by time error b	2	2.361	.30

TABLE 23. PIG INTESTINAL POPULATION - ESCHERICHIA COLI - JEJUNUM

Source	Df	SS	PR > F
Sow treatment	1	.065	.88
Litter(sow treatment) error a	4	9.972	.01
Pig treatment	1	.003	.94
Time	2	3.671	.06
Pig treatment by time	2	.802	.52
Sow treatment by pig treatment	1	.224	.55
Sow treatment by time	2	1.125	.40
Sow treatment by pig treatment by time error b	2	2.834	.11

TABLE 24. PIG INTESTINAL POPULATION - ESCHERICHIA COLI - DUODENUM

Source	Df	SS	PR > F
Sow treatment	1	.004	.97
Litter(sow treatment) error a	4	10.014	.01
Pig treatment	1	2.234	.07
Time	2	2.060	.22
Pig treatment by time	2	.776	.55
Sow treatment by pig treatment	1	1.363	.16
Sow treatment by time	2	1.653	.29
Sow treatment by pig treatment by time error b	2	1.069	.45

TABLE 25. PIG INTESTINAL POPULATION - ESCHERICHIA COLI - CECUM

Source	Df	SS	PR > F
Sow treatment	1	.265	.67
Litter(sow treatment) error a	4	5.074	.11
Pig treatment	1	.264	.52
Time	2	.221	.84
Pig treatment by time	2	1.320	.36
Sow treatment by pig treatment	1	.275	.51
Sow treatment by time	2	1.084	.43
Sow treatment by pig treatment by time error b	2	2.489	.15

TABLE 26. PIG INTESTINAL POPULATION - ESCHERICHIA COLI - FECES

Source	Df	SS	PR > F
Sow treatment	1	.897	.57
Litter(sow treatment) error a	4	9.615	.01
Pig treatment	1	2.308	.06
Time	2	5.188	.02
Pig treatment by time	2	2.449	.15
Sow treatment by pig treatment	1	1.027	.20
Sow treatment by time	2	.821	.51
Sow treatment by pig treatment by time error b	2	1.384	.33

TABLE 27. PIG INTESTINAL POPULATION - LACTOBACILLUS - STOMACH

Source	Df	SS	PR > F
Sow treatment	1	.001	.98
Litter(sow treatment) error a	4	1.951	.24
Pig treatment	1	.724	.15
Time	2	.841	.30
Pig treatment by time	2	.724	.35
Sow treatment by pig treatment	1	.942	.10
Sow treatment by time	2	2.550	.03
Sow treatment by pig treatment by time error b	2	2.109	.05

TABLE 28. PIG INTESTINAL POPULATION - LACTOBACILLUS - JEJUNUM

Source	Df	SS	PR > F
Sow treatment	1	.182	.76
Litter(sow treatment) error a	4	6.968	.002
Pig treatment	1	.188	.45
Time	2	1.989	.06
Pig treatment by time	2	.235	.70
Sow treatment by pig treatment	1	.517	.21
Sow treatment by time	2	.343	.59
Sow treatment by pig treatment by time error b	2	6.891	.0002

TABLE 29. PIG INTESTINAL POPULATION - LACTOBACILLUS - DUODENUM

Source	Df	SS	PR > F
Sow treatment	1	.001	.98
Litter(sow treatment) error a	4	4.081	.34
Pig treatment	1	.004	.95
Time	2	2.236	.29
Pig treatment by time	2	3.027	.19
Sow treatment by pig treatment	1	.001	.97
Sow treatment by time	2	.458	.77
Sow treatment by pig treatment by time error b	2	.523	.74

TABLE 30. PIG INTESTINAL POPULATION - LACTOBACILLUS - CECUM

Source	Df	SS	PR > F
Sow treatment	1	.096	.88
Litter(sow treatment) error a	4	13.926	.0001
Pig treatment	1	2.623	.012
Time	2	17.206	.0001
Pig treatment by time	2	2.363	.06
Sow treatment by pig treatment	1	.127	.57
Sow treatment by time	2	2.364	.06
Sow treatment by pig treatment by time error b	2	.586	.47

TABLE 31. PIG INTESTINAL POPULATION - LACTOBACILLUS - FECES

Source	Df	SS	PR > F
Sow treatment	1	1.471	.44
Litter(sow treatment) error a	4	8.029	.013
Pig treatment	1	2.594	.035
Time	2	13.523	.0001
Pig treatment by time	2	5.026	.02
Sow treatment by pig treatment	1	.186	.56
Sow treatment by time	2	.644	.56
Sow treatment by pig treatment by time error b	2	.053	.76

TABLE 32. pH OF PIG STOMACH

Source	Df	SS	PR > F
Sow treatment	1	4.959	.14
Sow(sow treatment) error a	4		
Pig treatment	1	.092	.6101
Day	2	10.340	.0001
Pig treatment by day	2	.769	.35
Sow treatment by pig treatment	1	.186	.47
Sow treatment by day	2	1.444	.15
Sow treatment by pig treatment by day	2	.087	.88
Pig treatment by Sow treatment by day(sow treatment) error b	20		

TABLE 33. SCOURS SCORE

Source	Df	Chi^2	Prob
Sow treatment	2	8.167	.02
Pig treatment	2	2.184	.34
Day	4	6.124	.19

EFFECT OF FEEDING BACILLUS SUBTILUS SPORES ON SOW AND BABY PIG PERFORMANCE AND BACTERIAL POPULATIONS

by

ROBERT RUSSELL LA FORGE

B. S., Kansas State University, 1979

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Enteric colibacillosis is a major cause of mortality and depressed performance in nursing pigs. Probiotics are theorized as an alternative to antibiotics and vaccines as a method of controlling this disease.

A Bacillus subtilus probiotic was fed 5 g per head per d to sows from 14 d pre-farrowing to 14 d post-farrowing. Control sows did not receive the product. Also, 1 g of culture mixed with 1 ml of safflower oil was given orally to half of the baby pigs in each litter within 12 h of birth. The other half only received the oil. Feeding B. subtilus spore had no effect on sow or baby pig performance.

B. subtilus, lactobacillus, and Escherichia coli from sow feces and from five sites of the gastronitestinal (GI) tract of baby pigs were cultured. Fecal E. coli populations in sows increased significantly on the d of farrowing. Bacillus inoculum did not affect lactobacillus and E. coli populations in the GI tract. The bacillus may not be germinating until the cecum which would explain why there may not have been any performance nor bacterial response.