RESEARCHREPORT
PORK SAFETY

Title: Dietary Manipulation as a Method of Modifying Intestinal Bacteria and Reducing the Need for Sub therapeutic Administration of Conventional Antibiotics - **NPB #01-060**

Investigator: Michael D. Howard,

Institution: National Swine Research & Information Center, USDA/ARS

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Abstract

An experiment was conducted with weaned pigs entering a conventional nursery facility. This experiment was designed to evaluate the effects of feeding graded levels of short chain fructooligosaccharides (SCFOS; 0, 0.1%, and 0.2%) alone or in combination with 100 g Tylan/ton of feed. Response was measured as pig performance and changes in intestinal bacterial populations as exemplified by two lactic acid bacterial genera: Lactobacilli and Bifidobacterium. Pigs fed diets with 0.1% SCFOS tended to have greater feed intake and weight gain than pigs fed 0.2% SCFOS; however, pigs fed 0% SCFOS diets had similar performance to pigs fed 0.1% SCFOS diets. Addition of Tylan significantly increased feed intake and weight gain during weeks 3 and 4 of the 6 week study. However, for the rest of the experiment, performance of Tylan-fed pigs was only modestly better than control pigs. Concentration of Lactobacilli in the feces was not altered by SCFOS or Tylan. The presence of Bifidobacterium could not be verified. This experiment indicates that low level feeding of antibiotics did not dramatically improve pig performance. Likewise addition of SCFOS did not enhance performance.

A second experiment with market weight hogs was conducted to evaluate the efficacy of adding lactic acid to drinking water as a method of reducing rapidly acquired Salmonella infection. This experiment was designed to model the preslaughter conditions of hogs in slaughter plant holding pens. Supplying 0.44% lactic acid in the drinking water was unable to reduce the prevalence of Salmonella in the contents of stomach, cecum, distal colon, ileum and ileocecal lymph nodes. The presence of sub therapeutic Tylan (20 g Tylan/ton of feed) had no effect on Salmonella prevalence.

Introduction

Feeding of antibacterial compounds to animals for the promotion of growth has become a controversially practice and has led some individuals to conclude that increased antibiotic resistant bacteria in humans is due in part to antibiotics fed to animals [1]. This line of reasoning has lead countries of the European Union to ban the feeding of antibiotics to livestock. It is unclear whether feeding sub therapeutic antibiotics will remain legal in the United States, but it is prudent that alternatives be evaluated.

The means by which sub therapeutic antibiotics increase animal performance is not entirely understood. However, one suggestion is that antibiotics sufficiently reduce populations of potentially pathogenic bacteria so that host animal can contribute more nutrients to growth and less to defending against pathogens.

An alternative to using antibiotics to reduce pathogenic bacteria is to selectively increase populations of host beneficial-bacteria and allowing beneficial bacteria to competitively exclude pathogens. Populations of beneficial bacteria can be increased through continually administering bacteria in feed or water: bacteria administered in this manner are referred to as probiotics. Beneficial bacteria can also be selectively fed through dietary manipulation: a dietary ingredient capable of selectively enhancing a bacterial population is referred to as a prebiotic.

Lactic acid bacteria of which, *Lactobacilli* and *Bifidobacterium,* are two important genera, are theorized to exert a protective role against potentially pathogenic bacteria present in the small and large intestine [2]. Benefits of lactic acid bacteria include stimulation of the immune system, preventing pathogenic bacteria from adhering and invading cells lining the gut, prevention of disease symptoms, and improved animal performance.

Objectives

Objective 1: determine if consumption of short chain fructooligosaccharides (SCFOS) containing diets with or without sub therapeutic levels of Tylan would effect animal performance and alter intestinal populations of lactic acid bacteria and Salmonella species.

Objective 2: determine if consumption of SCFOS, with or without sub therapeutic levels of Tylan would reduce the incidence of post weaning diarrhea and alter intestinal populations of lactic acid bacteria.

Objective 3: determine if administering lactic acid, with or without sub therapeutic levels of Tylan could prevent *Salmonella typhimurium* infection of market weight hogs, subjected to conditions approximating abattoir holding-pens. This objective was amended from the original objective of determining if SCFOS would be effective against rapid Salmonella infection. Reasoning for the change was that a study conducted shortly before the start of Experiment 3, found that SCFOS was not effective in preventing this rapid Salmonella infection. Lactic acid is a fermentation by-product of lactic acid bacteria and has been successfully used in other livestock situations to reduce Salmonella prevalence [3, 4].

Procedures

Objective 1.

Pigs (total of 480) were weaned at approximately 18 days of age and weighed. Pigs were stratified by weight and assigned to groups of twenty. One of six dietary treatments was randomly assigned to each group. Dietary treatments were arranged in a 2 by 3 factorial and consisted of 2 levels of Tylan (0 or 100 grams (g) Tylan/ton of feed) and 3 levels of SCFOS (0, 0.1%, and 0.2%; Nutraflora P95, Golden Technologies Co.). Diets were based on complex ingredients and fed in three phases: phases one and two were 2 weeks in length and phase 3 was 15 days long. Measurements of body weight were taken at the beginning of the experiment and at 2-week intervals until the end of study: intermittent weights corresponded with changes in diets. This experiment was conducted in two replications.

At the end of each replication, fecal samples were collected from 60 pigs. During replication one, either a cotton swab of rectal contents or small quantity of freshly voided feces was collected and placed in either 5 milliters (ml) of Trypticase Soy broth or 5 ml of Selenite Cysteine Broth. Broths were incubated at 37°Celsius for approximately 18 hours. A 1 ml sub sample was removed and bacterial DNA extracted. An attempt to further enrich samples for the detection of Salmonella was made by transferring 0.1 ml of sample initially incubated in Trypticase Soy broth into Selenite cysteine broth and incubating for 24 hours in a shaking incubator at 37°C. Polymerase chain reaction was performed on the extracted bacterial DNA to detect the presence of Salmonella species. Forward and reverse primers (25 base pairs) were selected from previously published data. Samples were analyzed on a Strategene RoboCycler. Conditions for polymerase chain reaction were an initial denaturation of 3 minutes at 94°C, this was followed by 40 cycles composed of 35 seconds of denaturation at 94°C, 45 seconds of annealing at 60°C, and 45 seconds of chain length extension at 72°C. After the 40^{th} cycle, the reaction mixture was held at 72°C for 10 minutes then cooled to 4°C. The amplified product was 496 base pairs.

At the end of the 2^{nd} replication, feces were collected and analyzed for the presence of *Salmonella*, *Lactobacillus* and *Bifidobacteria* species. Analysis for *Salmonella* species was the same as previously described. Analysis for *Lactobacillus* species and *Bifidobacteria* species was conducted by serially diluting (1:10) samples in anaerobic peptone yeast broth. Dilutions were placed on agar media presumptively selective for Lactobacilli [5] or Bifidobacterium [6]. Lactobacilli and Bifidobacterium media were incubated anaerobically at 37°C for 72 hours or 96 hours respectively. Bacterial identification was confirmed by gas chromatographic analyses of cell wall fatty acid profile and comparison to a library of standards.

Data were statistically analyzed as a 2 by 3 factorial (0 or 100 grams (g) Tylan /ton of feed and 0, 0.1% or 0.2% SCFOS).

Objective 2

Pigs used in this objective were sterilely derived by Caesarian section and either remained germ-free or were inoculated with bacteria derived from the gastrointestinal tract of naturally born and reared pigs: pigs receiving the bacterial inoculum are referred

to as normalized. This objective was conducted in two replications. In Replication 1, ten pigs were derived by Caesarian section from two sows. Two pigs were returned to a sow with the intention they would nurse and becoming naturally inoculated with bacteria. However, the sow did not initiate lactation and the pigs were placed in nonsterile isolators and fed Esbilac milk replacer. Two days after birth, these pigs were inoculated with approximately 1 g of feces from young healthy pigs. The rationale for normalizing pigs was that these pigs would receive the same source of inoculum and this would minimize variation among pigs. Pigs were randomly assigned to 1 of 6 treatments: germ-free or normalized with no dietary supplements; germ-free or normalized receiving Tylan soluble; germ-free or normalized receiving SCFOS.

Six days after inoculating the two nonsterilely raised pigs, they were euthanized and contents from intestinal tract collected. The contents were equally divided and stored frozen at –80°C.

On day 10, 4 pigs received 2.8 g of intestinal contents diluted with 5 ml of sterile phosphate buffered saline: intestinal contents were added to milk replacer. Four pigs remained germ-free. On day 25, pigs were euthanized and samples of intestinal contents and tissue were collected.

In Replication 2, 12 pigs were derived by Caesarian section. Pigs were randomly assigned to one of three treatments: control, Tylan soluble, or SCFOS. At 6 days of age, all pigs received 2.8 g of intestinal contents diluted in 5 ml of sterile phosphate buffered saline. At 18 days of age, pigs were inoculated with inoculum derived from intestinal contents of two weaned pigs (approximately 2 weeks old and weighing 6 to 9 lbs) that had showed clinical symptoms consistent with post weaning diarrhea. Twenty-four hours after inoculations, pigs were euthanized and samples of intestinal contents and tissue were collected.

Feeding. Replication 1 pigs were fed a nutritionally complete milk replacer powder that was sterilized by irradiation (31 kilo Gray) then mixed with sterile distilled water. Replication 2 pigs were fed a commercially prepared, sterile milk replacer (Esbilac). Short chain fructooligosaccharides were dissolved in distilled water at the rate of 50 g/100 ml. The solution was sterilized by passing through a 0.2-micron filter into a sterile bottle. Three milliliters of solution/day were added to a pigs' milk replacer, providing 1.5 g of SCFOS/day. Administration of SCFOS began the day after birth. Tylan soluble was given in the milk replacer beginning 7 days after birth. The dose was 25 mg/day for 8 days then 50 mg/day for 6 days. Concentration of Tylan was 10 mg/ml and was formulated on the recommended dosage of 100 grams of Tylan/ton of feed used for weaned pigs. The increased dosage of Tylan after 8 days was designed to mimic increased intake of medication that would occur as weaned pigs grow and increase their feed intake.

Bacterial analysis. Intestinal contents from the healthy pigs used to inoculate germ-free pigs were analyzed for enumeration of total anaerobic bacteria. One gram of intestinal contents was serially diluted 1:10 in Peptone Yeast solution. Dilutions were placed (in triplicate) on to rumen fluid-based agar medium. Plates were incubated for 48 hours at 37°C in an anaerobic chamber and total number of colonies counted.

Samples of cecal fluid and colon contents from normalized pigs were analyzed to enumerate *Lactobacilli* species, *Bifidobacterium* species, and *Escherichia coli* strains:

Lactobacilli and *Bifidobacterium* analyses were the same as described for Objective 1. Analysis for E. coli was conducted on Petrifilm® for E. coli/coliforms . Petrifilm medium was aerobically incubated for 48 hours. Confirmation of bacterial identity was performed by analyzing colonies for cell membrane fatty acid profile and comparing this profile against a library of known bacteria.

Objective 3

The swine used in this study were purchased from a high health status herd. Thirty-six pigs weighing approximately 90 kg were brought on site 8 days prior to euthansia. The experiment was conducted in three replications (12 pigs per replication). Fecal samples (approximately 20 g) from all animals were tested for existing Salmonella shedding 3 times prior to treatment administration: 8, 6 and 1 day prior to euthansia.

Pigs were randomly allocated to one of two treatment rooms (6 animals per room). Pigs in Room A received normal municipal water. Pigs in Room B received 0.44% L-lactic acid (Purac FCC 88, Purac America) through an in-line Dosatron 310-RE water medicator. Two typical finishing diets were mixed and pelleted. One diet was formulated to contain 20 g Tylan®/907 kg of feed and the other diet had no antibiotic. Half the pigs in each room received Tylan-diet and the half received nonmediacated feed. The animals were fasted for approximately 12 hours prior to challenge in order to simulate normal preslaughter conditions. Animals were challenged with a Nalidixic-acid resistant strain of *S. typhimurium* χ4232. All animals were anesthetized and exsanguinated prior to necropsy.

Preliminary fecal samples (~20 g) were collected and cultured in Tetrathionate broth and GN Hajna broth (10 g in 90 ml of each medium) at 37°C for 24 hours. After 24 hours, 0.1 ml of each media were transferred into 10 ml Rappaport-Vassiliadis R10 broth, and incubated at 37°C for 24 hours. XLT-4 agar and ChromAgar Salmonella® plates were subsequently streaked and incubated for 24 hours at 37°C. Two suspect Salmonella colonies were streaked onto Rambach agar and incubated for 24 hours a 37° C. Colonies presumptively identified as Salmonella were streaked onto XLT-4 agar containing 50 μg/ml Nalidixic acid to determine if drug resistant mutants existed prior to the start of the study.

Animals were infected with a fecal-slurry seeded with *S. typhimurium* χ4232: slurry contained 1.4 kg wet feces, 800 ml sterile water and 22 ml of *S. typhimurium* χ4232 (1 x 10^8 CFU/ml). Serial dilutions of *S. typhimurium* χ4232 culture were made and spread-plated on Trypticase Soy agar for enumeration. An aliquot of slurry was also serially diluted and spread-plated on to XLT4 with Nalidixic acid for enumeration: concentration in slurry was 1.2 x 10^6 CFU/g of feces. The slurry mixture was spread on the floor of the animals' pen and lightly sprinkled with feed to encourage oral exposure.

At necropsy, the following samples were collected: ileocecal lymph node(s), distal ileum, cecal contents, stomach contents and distal colon contents. Ileocecal lymph nodes were macerated with a rubber mallet, mixed with 25 ml of Buffered Peptone Water, and stomached for 2 min: 10 ml of the mixture were then added to GN Hajna and Tetrathionate broths. Distal ileum (5 cm sections) was transversely cut twice to expose the mucosal surface. Sections were added to both GN Hajna and Tetrathionate broths. Stomach, cecal and distal colon contents (10 g) were placed into 90 ml of GN

Hajna and Tetrathionate broths and incubated at 37° C for 24 h. After 24 h, 0.1 ml of each broth was transferred to 10 ml of Rappaport-Vassiliadis R10 broth, and incubated at 37° C for 24 hours. After 24 h, Rappaport-Vassiliadis R10 broth was streaked onto XLT-4 agar plates for isolation and incubated for 24 hours at 37° C. One suspect colony (if present) was streaked for confirmation onto Rambach agar and incubated for 24 h at 37° C. All bacterial media used for necropsy specimens contained 50 µg/ml Nalidixic acid.

Additional samples from cecal and distal colonic contents were collected and analyzed to enumerate Lactobacilli and Bifidobacterium species using the same methods described in Objective 1.

Lactic acid (L-(+)- lactic) concentration was measured by using a Waters High Pressure Liquid Chromatograph (Millipore Corporation, Milford, MA) equipped with a Waters Model 2414 refractive index detector, column heater, autosampler and computer controller. The separation of lactic acid was done in a Bio-Rad Aminex HPX-87H column (300 X 7.8 mm) (Bio-Rad Chemical Division, Richmond, CA) using 0.012 N sulfuric acid as a mobile phase at a flow rate of 0.8 ml/min with a 20-µl injection volume and a 65ºC column temperature. Concentration of dissociated and undissociated lactic acid was determined by solving the Henderson-Hasselbach equation:

$$pH = pKa + \log \frac{[\text{dissociated}]}{[\text{undissociated}]}$$

where pH = stomach pH and lactic acid pKa = 3.86 [7].

Salmonella prevalence data were analyzed with a nominal logistic model using JMP® software (version 4). Independent variables were lactic acid, Tylan®, replication and lactic acid by Tylan® interaction. Stomach pH and lactic acid concentrations data were analyzed with the mixed model of SAS (SAS System for Windows, Release 8.00). Independent variables were the same as for Salmonella prevalence data. Because of missing observations, least squares means were computed.

Results

Objective 1

Growth performance, feed intake and efficiency of gain data are presented for phases 1, 2, 3 and entire 43 day study (Tables 1, 2, 3 and 4 respectively). Phase 1 feed intake was effected by level of SCFOS consumption P= 0.10: feed intake was highest with 0.1% SCFOS and lowest with 0.2% SCFOS. Effect of SCFOS and Tylan on body weight gain did not reach the statistically significant threshold of $P \leq 0.10$, but did show trends: weight gain was higher with 0.1% SCFOS and lower with 0.2% SCFOS and addition of Tylan gave increased weight gain. Tylan also tended (P = 0.18) to improve feed efficiency.

Phase 2 feed intake and weight gain were strongly effected by addition of Tylan (P = 0.0006 and P = 0.02, respectively): pigs eating Tylan treated diets had 7.8% greater feed intake (1.65 vs. 1.53 lbs per day per pig) and 5.2% greater gain (1.21 vs. 1.15 lbs per day per pig) than pigs not fed Tylan. However, Tylan did not alter feed efficiency. Addition of SCFOS did not affect pig performance during Phase 2.

Phase 3 feed intake tended (P = 0.15) to be effected by SCFOS: 0.1% SCFOS tended to increase intake, while 0.2% SCFOS tended to depress feed intake. Phase 3 weight gain was effected (P = 0.09) by SCFOS and reflected the pattern seen with feed intake: 0.1% SCFOS diets increased weight gain 4.8% compared to the 0.2% SCFOS diet (1.32 vs. 1.26 lbs per day). However, weight gain of pigs fed the 0% SCFOS diets was also 1.32 lbs per day.

For the entire 43 day study, feed intake was effected (P = 0.07) by level of SCFOS: pigs fed the 0.1% SCFOS diet ate 5.3% more feed compared to pigs fed the 0.2% SCFOS diet (1.57 vs. 1.49 lbs per day). Intake of pigs fed 0% SCFOS diet was in between the other diets. Weight gain tended (P = 0.11) to be effected by level of SCFOS. Pigs fed 0.1% SCFOS diets gained 4.1% more (1.02 vs. 0.98 lbs per day) than pigs fed 0.2% SCFOS diets: weight gain of pigs eating 0% SCFOS diets was in between. Expressing weight gain for the 43 day study as a total rather than average daily gain tended (P = 0.11) to favor 0.1% SCFOS over 0.2% SCFOS: pigs fed 0.1% SCFOS diets gained 4.23% more than pigs fed 0.2% SCFOS (43.89 vs. 42.11 lbs per pig). Addition of Tylan tended (P = 0.18) to increase average daily gain compared to Tylan-free diets. Total weight gain tended (P = 0.15) to be higher for pigs fed Tylan compared to pigs eating Tylan-free diets.

The number of Lactobacilli contained in the feces of nursery pigs tended (P = 0.17) to be less when SCFOS was included in the diet (Table 5). The medium presumptively selective for Bifidobacterium species was not successful as no confirmed Bifidobacterium were found. No Salmonella species where detected in any of the fecal samples.

The results of Objective 1 indicate that acceptable pig performance in the nursery phase of production can be obtained without the feeding of antibiotics. Although there was some benefit to Tylan in Phase 2 for feed intake and weight gain, it was apparently short-lived and not cumulatively. Likewise, performance of pigs fed diets supplemented with SCFOS was not significantly improved over performance of pigs consuming SCFOS-free diets.

Objective 2

This objective had many technical problems: of the twenty pigs that were started on one of the two replications, only ten survived to the end of a replication. From Replication 1, one germ-free control pig died from a congenital heart defect and two normalized control pigs (no SCFOS or Tylan) died from apparent septicemia. From Replication 2 (all pigs normalized), one SCFOS pig died at 3 days of age (prior to normalization) and an additional pig on this treatment died at 10 days of age. All four pigs receiving Tylan soluble treatment died by 11 days of age. One control pig died at 9 days of age. During Replication 1, all pigs had very loose stools which was attributed to the strong irradiation of their diet. Because of this condition, Replication 1 pigs were not challenged with the scour-associated intestinal contents. Replication 2 pigs were challenged with the scour-associated intestinal contents. Control pigs (no SCFOS) did show slight symptoms of diarrhea. Because of inadequate numbers of pigs and samples, statistical analyses could not be performed. Table 6, shows the concentrations of E. coli and Lactobacilli in cecal and colonic contents. Consuming SCFOS appeared to increase Lactobacilli concentration in colonic contents, but it is

unclear if this would benefit the pig in context of an E. coli challenge. Tylan appeared to decrease Lactobacilli concentrations. Concentration of E. coli appeared to be lowest in control pigs.

Interpretation and application of these results are difficult due to lack of statistical analyses and wide variation between replications. This model of causing post-weaning diarrhea is not considered successful, so application relative to the original objective of preventing or reducing the severity of post weaning diarrhea through feeding SCFOS is not possible. It does appear that SCFOS did not noticeably increase a pig's ability to survive. Interestingly, Tylan appeared to counterproductive. Tylan is generally recognized as effective against anaerobic spirochetes causing swine dysentery and gram positive bacteria. Lactobacilli and other lactic acid bacteria belong to the gram positive group. This leads to speculation that Tylan might be hindering beneficial gram positive bacteria.

Objective 3

Fecal samples collected prior to inoculation with S. typhimurium χ4232 showed evidence of prior Salmonella exposure: 14 of 36 pigs had at least one Salmonella-positive samples, although no Nalidixic acid resistant strains were found. Data on Salmonella prevalence in the various samples is presented in Table 7. No differences due to lactic acid or Tylan treatments were significant at $P < 0.05$. However, some indications of treatment effects were noted. Cecal contents of pigs drinking water without added lactic acid tended ($P = 0.27$) to have lower Salmonella prevalence than pigs consuming lactic acid (5 of 18 vs. 8 of 18). Distal colon contents of pigs eating Tylan-free diets tended ($P = 0.21$) to have lower Salmonella prevalence than pigs consuming Tylan (5 of 18 vs. 8 of 18). Ileal tissue of pigs drinking lactic acid treated water tended ($P = 0.28$) to have lower Salmonella prevalence than pigs drinking untreated water (7 of 18 vs. 10 of 18).

Results of stomach pH and lactic acid concentration are presented in Table 8. Stomach pH tended ($P = 0.12$) to be higher when pigs were fed Tylan. Neither lactic acid nor Tylan consumption had an effect on stomach concentration of total lactic acid, dissociated or undissociated lactic acid ions.

Concentrations of Lactobacilli species in cecal and colonic contents are presented in Table 9. Neither lactic acid nor Tylan had an effect of the quantity of Lactobacillus.

These results were not encouraging and appear to be contradictory to other research [3, 4]. However, van der Wolf's research found that one production site, where nearly all pigs were seropositive, had no reduction in Salmonella prevalence by adding lactic acid to the drinking water. The research of Byrd was conducted over a longer time period: 8 to 10 hours of Salmonella exposure to lactic acid. This might indicate that lactic acid needs to be in contact with Salmonella longer than 2 to 4 hours in order to be effective in reducing Salmonella. Unfortunately, within the context of a slaughter plant holding pen, 8 to 10 hours of waiting would not be practical. The observation that lactic acid concentration in the stomach was not different between treated and untreated pigs points to one of two possibilities: either the quantity of lactic acid consumed was insufficient to raise it above the normal level present in the stomach or lactic acid was quickly absorbed from the stomach.

These results indicate that controlling the rapid Salmonella infection occurring at slaughter plant holding pens is a very difficult task. Although there is ample evidence that market hogs can markedly increase their Salmonella prevalence in the holding pen [8, 9], breaking the Salmonella cycle through on-farm intervention might be more feasible.

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Table 1. Average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) of nursery pigs fed varying combinations of short chain fructooligosaccharides (SCFOS) and Tylan: Phase 1 (2 weeks).

		ADFI, lbs[a]	ADG, lbs[bc]	G:F[d]
SCFOS				
	0%	0.77	0.52	0.66
	0.1%	0.79	0.53	0.68
	0.2%	0.7	0.48	0.67
	SE[e]	0.028	0.033	0.055
Tylan				
	0 g	0.73	0.49	0.66
	100 g	0.77	0.53	0.68
	SE	0.023	0.031	0.055

[a]Effect of level of SCFOS significant at P = 0.10.
[b]Effect of level of SCFOS significant at P = 0.16.
[c]Effect of Tylan significant at P = 0.12.
[d]Effect of Tylan significant at P = 0.18.
[e]SE= standard error of the mean.

Table 2. Average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (GF) of nursery pigs fed varying combinations of short chain fructooligosaccharides (SCFOS) and Tylan: Phase 2 (2 weeks).

		ADFI, lbs[a]	ADG, lbs[b]	G:F
SCFOS				
	0%	1.57	1.16	0.71
	0.1%	1.62	1.2	0.72
	0.2%	1.59	1.19	0.72
	SE[c]	0.062	0.041	0.072
Tylan				
	0	1.53	1.15	0.72
	100 g/ton	1.65	1.21	0.71
	SE	0.061	0.039	0.072

[a]Effect of Tylan significant at P = 0.0006.
[b]Effect of Tylan significant at P = 0.02.
[c]SE= standard error of the mean

Table 3. Average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (GF) of nursery pigs fed varying combinations of short chain fructooligosaccharides (SCFOS) and Tylan: Phase 3 (15 days).

		ADFI, lbs[a]	ADG, lbs[b]	G:F
SCFOS				
	0%	2.23	1.32	0.59
	0.1%	2.25	1.32	0.58
	0.2%	2.14	1.26	0.59
	SE[c]	0.063	0.059	0.012
Tylan				
	0	2.23	1.31	0.59
	100 g/ton	2.19	1.29	0.59
	SE	0.058	0.058	0.012

[a]Effect of level of SCFOS significant at P = 0.15.
[b]Effect of level of SCFOS significant at P = 0.09.
[c]Standard error of the mean.

Table 4. Average daily feed intake (ADFI), average daily gain (ADG), gain to feed ratio (GF), and total weight gain (TWG) of nursery pigs fed varying combinations of short chain fructooligosaccharides (SCFOS) and Tylan: Phases 1,2 and 3 (43 days).

		ADFI, lbs[a]	ADG, lbs[bc]	G:F	TWG, lbs[bd]
SCFOS					
	0%	1.54	1.00	0.64	43.21
	0.1%	1.57	1.02	0.65	43.89
	0.2%	1.49	0.98	0.64	42.11
	SE[e]	0.035	0.041	0.036	1.714
Tylan					
	0	1.51	0.99	0.64	42.58
	100 g/ton	1.55	1.01	0.64	43.56
	SE	0.033	0.040	0.036	1.683

[a]Effect of level of SCFOS significant at P = 0.07.
[b]Effect of level of SCFOS significant at P = 0.11.
[c]Effect of Tylan significant at P = 0.18.
[d]Effect of Tylan significant at P = 0.15.
[e]Standard error of the mean.

Table 5. Effect of short chain fructooligosaccharides (SCFOS) and Tylan on concentrations of Lactobacillus species in nursery pig feces.

		Lactobacilli, CFU/g[ab]	SE
SCFOS			
	0%	3.24×10^8	5.891×10^7
	0.1%	1.03×10^8	
	0.2%	1.27×10^8	
Tylan			
	0	1.29×10^8	4.865×10^7
	100 g/ton	2.53×10^8	

[a]CFU/g = colony forming units per gram of wet feces.
[b]Effect of SCFOS significant at P = 0.17.

Table 6. Effect of short chain fructooligosaccharides (SCFOS) and Tylan on concentrations of E. coli and Lactobacilli in cecal and colonic contents[a].

	Cecal contents		Colonic contents	
	E. coli	Lactobacilli	E. coli	Lactobacilli
SCFOS	1.14×10^7	3.00×10^8	4.04×10^7	1.51×10^9
Tylan	7.94×10^6	1.43×10^7	2.72×10^7	1.38×10^7
Control	5.92×10^6	1.09×10^8	5.56×10^6	3.27×10^8

[a]Expressed as colony forming units per gram.

Table 7. Comparison of lactic acid and Tylan on proportion of samples positive for Salmonella typhimurium χ4232.

	0% Lactic acid		0.44% Lactic acid	
Sample type	0 Tylan (positive/tested)	20g Tylan (positive/tested)	0 Tylan (positive/tested)	20g Tylan (positive/tested)
Cecal contents	2/9	3/9	4/9	4/9
Colon contents	2/9	4/9	3/9	4/9
Ileal tissue	6/9	4/9	3/9	4/9
Ileal-cecal lymph node	2/9	0/9	3/9	2/9
Stomach contents	8/9	9/9	9/9	7/9

Table 8. Effect of lactic acid and Tylan on stomach pH and lactic acid concentration of stomach fluid.

	0% Lactic acid		0.44% Lactic acid	
Item	0 Tylan	20g Tylan	0 Tylan	20g Tylan
Stomach pH[a]	3.58	5.06	4.36	4.58
Lactic acid concentration, mM	5.25	6.31	6.65	6.3
Dissociated lactic acid concentration, mM	2.47	4.91	4.51	4.58
Undissociated lactic acid concentration, mM	2.77	1.39	2.14	1.72

[a]Effect of Tylan, significant P= 0.12.

Table 9. Effect of Lactic acid and Tylan on concentrations of Lactobacillus species in cecal and colonic contents of market weight hogs[a].

		Cecal contents	SE	Colonic contents	SE
Lactic acid					
	0	3.30×10^8	1.266×10^8	7.47×10^8	8.785×10^8
	0.44%	4.66×10^8	1.229×10^8	1.91×10^9	8.996×10^8
Tylan					
	0	3.76×10^8	1.27×10^8	1.70×10^9	8.996×10^8
	100 g/ton	4.20×10^8	1.23×10^8	9.57×10^8	8.785×10^8

[a]Expressed as colony forming units per gram.