RESEARCH REPORT
ANIMAL SCIENCE

Title: A comparison of administration routes of direct fed microbials to nursery pigs, and the effects on growth performance and gut health - **NPB#: 04-097**

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II. Abstract:

One hundred eighty, crossbred, weanling pigs were used in a 5-wk experiment to investigate the effects of administering a bolus dose of direct fed microbials (DFM) at weaning and/or supplementing the feed with DFM on growth performance and gastrointestinal morphology and physiology. Three dietary phases were fed during the experiment. All diets were formulated to contain adequate levels of all nutrients (NRC, 1998). Treatments included: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics. Six pigs were housed in each pen with 6 pens per treatment. Individual BW and pen feed disappearance were recorded weekly. Eight pigs (4 barrows and 4 gilts) were euthanized, by asphyxiation with CO_2 followed by exsanguination on d 0, and one pig per pen (3 barrows and 3 gilt/trt) was euthanized on d 7, 20, and 34 for collection of duodenal (15 cm post pylorus) and ileal (15 cm prior to the ileo-cecal junction) tissue. In addition, thymus weight and total empty stomach, small intestine, and large intestine weights were recorded. Pigs were injected i.p. with 5-bromo-2´-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) (10 mg:kg BW) to allow for estimation of enterocyte migration rate. Overall, there was no effect of treatment on ADG. Including antibiotics in the diet improved ADG ($P < .05$) during phase 3 compared to pigs fed the negative control diet. Administering a bolus dose of DFM to pigs at weaning with or without subsequent provision of DFM in the feed resulted in similar growth performance to pigs fed either the negative control diet or the diet containing antibiotics. However, providing DFM in the feed alone, resulted in a reduced ADG during phases 1 ($P < .05$) and 2 ($P < .10$) compared to pigs fed the antibiotic containing diet. Overall feed intake was higher for pigs fed the antibiotic diet compared to pigs fed the negative control diet, the diet with DFM to pigs provided a bolus DFM dose at weaning. Pigs provided a bolus dose of DFM at weaning and provided subsequent DFM in the feed had similar feed intakes to pigs fed the antibiotic containing diet. On d 21, pigs fed the diet with antibiotics had deeper duodenal crypts ($P < 0.05$) compared to pigs fed the negative control diet with or without administration of a bolus dose of DFM at weaning. On d 35, duodenal crypts remained deeper ($P < 0.10$) for pigs fed the diet with antibiotics compared to pigs receiving the bolus dose of DFM at weaning with no subsequent treatment. Ileal crypt depths were greater ($P < 0.10$) for pigs fed the antibiotic containing diet compared to all other treatments on d 7. A similar pattern was observed on d 35 ($P <$

0.05) with ileal crypts depths being greater for pigs fed diets containing antibiotics compared to all other treatments, with the exception of pigs receiving a bolus dose of DFM at weaning, which had similar crypt depths. Duodenal enterocyte migration rate on d 21 was greater ($P < 0.05$) for pigs receiving the combination bolus and in-feed DFM treatment compared to pigs receiving the negative control treatment. All other treatments were similar. A similar response was observed in the ileum on d 21, where enterocyte migration rate was faster for pigs receiving the combination bolus and in-feed DFM treatment compared to pigs fed the negative control diet with or without administration of a bolus dose of DFM at weaning. Increased cellular proliferation rates, in duodenal crypts, were observed on d 21 for pigs receiving the combination bolus and in-feed DFM treatment compared to pigs provided DFM through the feed only. Similar results were observed on d 7 in the ileum ($P < 0.05$). However, on d 35, the reverse occurred, with higher cellular proliferation rates occurring in ileal crypts from pigs receiving DFM through the feed only compared to pigs receiving the combination bolus and in-feed DFM treatment and the negative control treatment. Preliminary data using intestinal tissue mounted in modified Ussing chambers indicates that DFM administered in a bolus dose and/or through the feed may increase the rate of nutrient absorption.

III. Introduction:

Weaning and the subsequent nursery phase represents a time of gastrointestinal and immune instability in the pig. Diets are rapidly changed from a pre-weaning milk diet to a corn-soybean meal based diet containing complex carbohydrates by the end of the nursery period. Drastic dietary shifts require the pig's digestive tract to rapidly adjust to newly available substrates. This is further complicated by low feed intakes and instability in HCl secretion by the stomach post-weaning. The combination of unstable gastrointestinal pH and changes in substrate availability in the gastrointestinal tract (GIT), provide the perfect environment for opportunistic, pathogenic bacterial colonization of the GIT causing a decline in health of the pig. To prevent colonization of the GIT by detrimental pathogenic microorganisms, subtherapeutic concentrations of antibiotics are routinely fed to nursery pigs. Previous research has reported improvements in growth performance as a result of this practice (reviewed by Rosen, 1995). However, in-feed antibiotics are coming under increasing public scrutiny as a result of concerns over antibiotic resistance. Therefore, the animal agriculture industry is under increasing pressure to find alternatives to antibiotics. Research investigating antibiotic alternatives has focused on performance data, with very little research investigating the modes of action of antibiotic alternatives or their effects on gut health and integrity. Ultimately, an understanding of the mode of action of antibiotic alternatives, and their effects on gut health, overall health, and gut and systemic immune function will allow scientists to better design, and producers to better utilize antibiotic alternatives.

One strategy for preventing colonization of pathogenic bacteria is to feed probiotics or direct fed microbials, but attempts to do so have produced varying results. In a review of 44 published experiments by Simon et al. (2003), a numerical improvement in ADG was observed in over 70% of the experiments reported. However, only 6.8% of the experiments reported improvements in ADG that were statistically significant ($P < 0.05$). Similar results were reported for ADFI. While evidence exists that probiotics are beneficial under certain conditions, experiments attempting to elucidate the mode of action of probiotics, or the conditions under which they are most effective are minimal. Postulated modes of action, as reviewed by Stavric and Kornegay (1995) include:

1) Competitive inhibition of gut epithelial receptors
2) Competition for nutrients
3) Production and secretion of antimicrobial compounds
4) Stimulation of the immune system

Early and recurrent inoculation of the GIT with probiotics may serve as a method of competitively excluding colonization by detrimental pathogenic microorganisms. However, early inoculation of the GIT by probiotics is difficult as a result of low feed intakes post-weaning. Therefore, during the early post-weaning period, it may be beneficial to deliver a bolus dose of probiotics to the pig. In addition, due to shifts in

microbial populations from the time of weaning to the end of the nursery phase, it may be beneficial to gradually shift the microbial content of the probiotic inoculant. Therefore, this experiment was designed to investigate the effects of inoculating pigs with a bolus dose of direct fed microbials at birth with or without subsequent reinnoculation with in-feed probiotics.

IV. Objectives:

1) To compare bolus delivered direct fed microbials (DFM) at weaning, feed delivered DFM, and the combination on growth performance and gastrointestinal health of weanling pigs.

2) To compare growth performance and measures of gastrointestinal health between pigs receiving supplementary probiotics with pigs receiving sub-therapeutic levels of antibiotics in the feed.

V. Materials and Methods:

One hundred eighty, crossbred, weanling pigs were used in a 5-wk experiment to investigate the effects of administering a bolus dose of direct fed microbials (DFM) at weaning and/or supplementing the feed with DFM on growth performance and gut health. Pigs (19 d of age, 14 lbs IW) were blocked by BW and ancestry, and randomly assigned to treatments. Six pigs were housed in each pen, with 6 pens per diet. An equal number of barrows and gilts were placed on each treatment.

Three dietary phases were fed during the experiment. Phase 1, 2 and 3 diets were fed on days 1-7, 7-20, and 21-34 of the experiment, respectively (Tables 1, 2, and 3). All diets were formulated to contain adequate levels of all nutrients (NRC, 1998). Treatments included: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics. DFM administered in a bolus dose at weaning were formulated to contain 10^9 CFU/L of *Lactobacillus acidophilus* and 10^9 CFU/L of *Entercoccus faecium*. Each pig received 5 ml through an endogastric tube. Pigs not receiving DFM, received an equal volume of sterile water. Diets supplemented with DFMs contained 0.05% Bioplus 2B (*Bacillus licheniformis* and *Bacillus subtilis*).

Individual BW and pen feed disappearance were recorded weekly. Eight pigs (4 barrows and 4 gilts) were euthanized, by asphyxiation with CO_2 followed by exsanguination on d 0, and one pig per pen (3 barrows and 3 gilt/trt) was euthanized on d 7, 20, and 34 for collection of duodenal (15 cm post pylorus) and ileal (15 cm prior to the ileo-cecal junction) tissue. In addition, thymus weight and total empty stomach, small intestine, and large intestine weights were recorded. Pigs were injected i.p. with 5-bromo-2´-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) (10 mg:kg BW) to allow for estimation of enterocyte migration rate.

Gross morphology. Duodenal and ileal tissue samples were rinsed in phosphate buffer and No-Tox fixative (Scientific Device Lab, Des Plaines, IL – alcohol/aldehyde fixative), embedded with paraffin, and stained with Hematoxylin and Eosin for light microscope examination. Determination of gross morphological parameters of intestinal structure (villus height and crypt depth) was conducted according to Gao *et al*. (2000) and Applegate *et al*. (1999a).

Enterocyte proliferation. Additional sections of the same tissues were used to determine cellular proliferation rates by quantifying proliferating cell nuclear antigen (PCNA; Applegate *et al*., 1999b) using an immuno-histochemical PCNA labeling and detection kit (Zymed Laboratories, Inc, San Francisco, CA). Briefly, endogenous peroxidase activity was removed by treatment with 3% hydrogen peroxide in methanol for 10 min followed by a PBS rinse. Sections were then treated with 2N HCL for 30 mins at 37 C to denature DNA, followed by digestion in a 0.1% trypsin solution (Type II-S from porcine pancreas, Sigma Chemical Co.) for 20 mins, and then washed in PBS. Slides were exposed to blocking solution for 10 min, after which a biotinylated antibody (mouse anti-PCNA, Zymed Laboratories, Inc.) was applied for 60 min, followed by streptavidin-peroxidase for 10 min. Sections were stained with diaminobenzidine for 2 min and counter-stained with

Hematoxylin and Eosin. Numbers of PCNA stained cells per crypt (lamina propria to invagination between villi) were determined.

Enterocyte migration rate. Additional sections of the same tissues were stained for BrdU as previously described (Kitchell and Dibner, 1989). Briefly, slides were treated with hydrogen peroxide (3%) to remove endogenous peroxidase, digested using 0.1% trypsin (Type II-S from porcine pancreas, Sigma Chemical Co.) for 30 min, and washed in PBS. A mono-clonal antibody to BrdU (Becton Dickinson, San Jose, CA), diluted at 1:200 in PBS with 0.01% bovine serum albumin was then incubated with slides for 2 h at room temperature. Following incubation, slides were washed with Cadenza buffer (Shandon, Inc., Pittsburgh, PA) and a biotinylated secondary antibody, avidin-biotin complex (Vector Elite Kit, Vector Laboratories, Burlingame, CA), and chromagen indicator (Vector VIP Kit, Vector Laboratories) was used for color development. Slides were then counter-stained with methyl-green.

All data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC), with pen serving as the experimental unit. The Duncan procedure of SAS was use to compare treatment means.

VI. Results:

Growth performance. Pigs were weaned at approximately 19 d of age, with an average initial weight of 13.99 lb. Pigs fed the diet containing probiotics but no oral probiotic dose at weaning had lower ADG ($P < 0.05$) during phase 1 (d 0-7 post-weaning) compared with pigs fed either the positive control diet containing carbadox or the negative control diet with no added antibiotics or probiotics (Table 4). Growth rates were not different ($P > 0.10$) between pigs fed the negative control diet, the positive control diet, and pigs receiving a bolus inoculation of probiotics at weaning. Feed intake and feed efficiency were similar for all diets during phase 1 (d 0-7). During phase 2 (d 7-21), ADG was higher ($P > 0.05$) for pigs fed the positive control diet with carbabdox compared to pigs fed the probiotic supplemented diet but not receiving an oral gavage of probiotics at weaning. Pigs fed the antibiotic supplemented diet had numerically the highest ADG during phase 2, but this difference was not significantly better than pigs fed the negative control diet or pigs fed the probiotic diet and receiving an oral dose of probiotics at weaning. Similar to ADG, ADFI was numerically the highest for pigs fed the carbadox containing diet, but this difference was only significantly ($P < 0.05$) better than pigs fed the probiotic containing diet without an oral gavage of probiotics at weaning. Dietary treatment had no effect on feed efficiency. During phase 3 (d 21-35 post-weaning), pigs fed the diet containing probiotics, but not receiving an oral dose of probiotics at weaning seemed to rebound having similar performance to pigs fed the positive control diet containing antibiotics. Pigs on both of these diets had higher ADG ($P<.10$) compared to pigs fed the negative control diet. Similar to phase 2, pigs fed the positive control diet had numerically the highest feed intake, which also proved to be significantly ($P > 0.05$) better than pigs fed the negative control diet with or without a bolus dose of probiotics at weaning. Overall (d 0-34), ADG was similar for all diets, and ranged from 0.69 lb/d for pigs fed the negative control diet to 0.77 lb/d for pigs fed the positive control diet. Feed intake was higher for positive control fed pigs compare to pigs fed the negative control diet (with or without the bolus dose of probiotics at weaning), and those fed the diet containing probiotics, but not orally dosed with probiotics at weaning combined with in-feed DFM. Overall feed efficiency was not different between dietary treatments.

Tissue data. There were a few differences in wet stomach, intestinal, and thymus weights on d 7, 21, and 34 (Table 5). However, there were very few differences in dry stomach, intestinal and thymus weight. On d 7, dried thymus weights for pigs given an oral bolus of probiotics at weaning were heavier ($P < 0.10$) than pigs not receiving an oral dose of probiotics. This response was not observed at the end of phase 2 or 3. On d 34, pigs fed the positive control diet or the diet containing probiotics but not receiving an oral dose of probiotics at weaning had the lightest intestinal weights on a dry basis. This difference was significant ($P < 0.05$) when compared to pigs fed the negative control diet and receiving an oral dose of probiotics at weaning.

Ussing chamber data. Preliminary data using modified Ussing chambers (Figure 1) indicates that in general, active glucose transport is enhanced in intestinal tissue from pigs receiving probiotics either at weaning, in the feed, or a combination of the two compared to pigs receiving no probiotic. This data is very preliminary, and the response did change over time. On d 7, pigs consuming the diet containing probiotics but

not receiving a bolus dose of probiotics at weaning had numerically the fastest rate of glucose uptake. However, on d 21 there was no effect of the probiotic diet on glucose uptake. While pigs receiving a bolus dose of probiotics at weaning did have numerically higher rates of glucose transport in the small intestine. Finally, on d 34 pigs receiving probiotics demonstrated a stepwise increase in active glucose transport compared to pigs fed the negative control diet with pigs given a bolus dose of probiotics at weaning followed by a diet containing probiotics having numerically the highest rate of active glucose transport, followed by pigs receiving probiotics in their feed only, and then by pigs receiving a bolus dose of probiotics at weaning only.

*Gross morphology***.** Results from the morphological analysis of the duodenum (Table 6) and ileum (Table 7) indicate that there was no effect of treatment on duodenal crypt depth at any time point during the experiment. However, duodenal villus height tended to be increase on d 35 in pigs receiving antibiotics compared to pigs who received a bolus dose of probiotics at weaning. On d 7 ileal crypt depth was significantly greater ($P<.05$) when antibiotics were fed compared to all other dietary treatments.

Enterocyte migration rate. BrdU labeled enterocytes in duodenal sections from pigs killed on d 21 had migrated 14 µm further in pigs that were fed DFM in a bolus dose at weaning and also in feed compared with pigs that were fed neither probiotics nor antibiotics (Table 8: $P<.10$). Also, on d 21 duodenal enterocyte migration rate (µm/h) tended to be faster ($P<.10$) for pigs given a bolus dose of DFM at weaning and provided DFM in the feed compared to pigs that were fed the negative control diet with no probiotics or antibiotics. Duodenal enterocyte migration rate and leading edge height of BrdU labeled enterocyte was not affected by dietary treatment on d 7 or d 35. Similarly, BrdU labeled enterocytes in ileal sections from pigs slaughtered on d 21 had migrated 15 µm further in pigs that were given a bolus dose of DFM at weaning and provided DFM in the feed compared with pigs that were fed neither probiotics or antibiotics (Table 9: $P<.10$). Also, on d 21 ileal enterocyte migration rate (µm/h) tended to be faster ($P<.10$) for pigs provided the combination bolus and in-feed DFM compared to pigs that were fed the negative control diet with no probiotics or antibiotics.

Enterocyte proliferation rate. The number of PCNA positive enterocytes per crypt in duodenual and ileal sections are presented in Tables 11 and 12, respectively. In sections taken from the duodenum on d 21, there tended to be a greater number of proliferating enterocytes in the crypts of pigs who were fed DFM in feed for the duration of the study compared to pigs that received a bolus dose of DFM at weaning along with the DFM in feed (Table 10: $P<.10$). The number of proliferating enterocytes in the duodenal crypts of pigs was not different between dietary treatments on d 7 or d 35. On d 7 sections taken from the ileum demonstrated a tendency for an increase in number of proliferating enterocytes in crypts of pigs provided the combination bolus and in-feed DFM compared with pigs that just received DFM in feed (Table 11: $P<.10$). There was a greater number of proliferating enterocytes in crypts of pigs that received antibiotics compared to pigs that were not supplemented with probiotics by any means or antibiotics on d 21 (Table 11: $P<.05$). However on d 35, pigs that were fed in feed DFM had greater numbers of proliferating enterocytes in their crypt compared to those pigs fed the negative control diet along with those pigs that were fed DFM in fed as well as in a bolus dose at weaning (Table 11: $P<.05$).

VII. Discussion:

Pigs receiving a combination bolus and in-feed DFM treatment grew 7.2% faster than pigs fed a negative control diet with no supplemental DFM, resulting a 1.8 lb. heavier pig at the end of the nursery. However, similar to the majority of the experiments reviewed by Simon et al. (2003), this difference was not significant. A similar response was observed for pigs fed the antibiotic containing diet, where there was an overall numerical improvement in ADG, but this was not significant, although a significant response was observed in phase 3 of the experiment. The lack of a significant growth response to probiotic treatment should therefore be kept in context with the overall lack of a growth response to antibiotics, which would indicate a relatively good health status of the pigs used in this trial. While growth responses were minimal, in-feed antibiotics did increase duodenal crypt depth on d 21 and ileal crypt depth on d 35 relative to pigs receiving no antibiotics or probiotics. Duodenal crypt depth responses to DFM were less predictable, with animal treated with a bolus dose of DFM having smaller crypt depths than pigs receiving antibiotics in the feed on d 21, while pigs receiving DFM in the feed or in a combination bolus and in-feed DFM treatment having crypt depths numerically closer to the antibiotic treatment, but statistically similar to both the negative control treatment and

the antibiotic treatment. On d 35, pigs receiving the diet with antibiotics had deeper ileal crypt depths than pigs receiving all other treatments except the bolus treatment with DFM. This indicates the in-feed antibiotics enhance gut morphology, thus increasing surface area, while the response to DFM in less consistent. Duodenal enterocyte migration rate on d 21 was greater ($P < 0.05$) for pigs receiving the combination bolus and in-feed DFM treatment compared to pigs receiving the negative control treatment. All other treatments were similar. A similar response was observed in the ileum on d 21, where enterocyte migration rate was faster for pigs receiving the combination bolus and in-feed DFM treatment compared to pigs fed the negative control diet with or without administration of a bolus dose of DFM at weaning. An increased migration rate would indicate a faster turnover rate of enterocytes in the gut. Turnover is good in that it is a necessary part of maintaining gut integrity and health. However, it is also an energy expending process and therefore may take energy away from the animal that could be used for growth. Increases in cellular migration rate on d 21 for pigs fed the combination bolus and in-feed DFM treatment was supported by an increased cellular proliferation rate in the duodenal crypts.

Preliminary data using modified Ussing chambers indicates that in general, active glucose transport is enhanced in intestinal tissue from pigs receiving probiotics either at weaning, in the feed, or a combination of the two compared to pigs receiving no probiotic. This data is very preliminary, and the response did change over time. On d 7, pigs consuming the diet containing probiotics but not receiving a bolus dose of probiotics at weaning had numerically the fastest rate of glucose uptake. However, on d 21 there was no effect of the probiotic diet on glucose uptake. While pigs receiving a bolus dose of probiotics at weaning did have numerically higher rates of glucose transport in the small intestine. Finally, on d 34 pigs receiving probiotics demonstrated a stepwise increase in active glucose transport compared to pigs fed the negative control diet with pigs given a bolus dose of probiotics at weaning followed by a diet containing probiotics having numerically the highest rate of active glucose transport, followed by pigs receiving probiotics in their feed only, and then by pigs receiving a bolus dose of probiotics at weaning only. As a result of chamber and treatment numbers, tissue from pigs fed the diet containing anbiotics could not be run in the modified Ussing chambers. Therefore, only the negative control treatment and the DFM treatments were evaluated. Preliminary data from this work is exciting in that it suggests that feeding DFM may enhance nutrient uptake. However, this data is very preliminary and more work needs to be done to determine if this is a repeatable response and to determine the mechanism behind this response.

In summary, responses to DFM treatments in this experiment were relatively small. However, so was the response to in-feed antibiotics. Therefore, it is not surprising that a response to DFM was not observed. However, even with minimal performance responses there were indications that DFM and antibiotics may alter gut morphology and possibly the active transport rates of nutrients by the gastrointestinal tract. Therefore, more research is needed to further elucidate the mechanisms through which DFM function.

VII. Lay Interpretation:

Direct fed microbials (DFM) have been investigated as a potential replacement for sub-therapeutic antibiotics in swine diets with mixed results being reported. Our results, found no overall significant effect of DFM or their mode of administration on growth rate. However, we also did not observe an effect of carbadox on overall growth rate, although ADG was improved during phase 3 when carbadox was added to the diet. A few small improvements in intestinal morphology and indicators of cellular migration and proliferation rate with in the gastrointestinal tract were observed when antibiotics were included in the diet. Adding DFM to the diet or administering DFM as a bolus at weaning followed by a diet containing DFM did return gut morphological measurements to a higher level than the negative control treated pigs which was not different from the antibiotic treated pigs. In summary, responses to DFM treatments in this experiment were relatively small. However, so was the response to in-feed antibiotics. Therefore, it is not surprising that a response to DFM was not observed. However, even with minimal performance responses there were indications that DFM and antibiotics may alter gut morphology and possibly the active transport rates of nutrients by the gastrointestinal tract. Therefore, more research is needed to further elucidate the mechanisms through which DFM function.

Table 1. Dietary composition for phase 1 diets (d 1-7).

	Diet		
Ingredients, %	Neg. control	Pos. control	Probiotics
Corn	42.025	41.305	42.005
SBM, 48% CP	21.550	21.82	21.47
Soybean oil	5.000	5.2	5.05
Limestone	0.610	0.610	0.610
Dicalcium phosphate	0.990	0.990	0.990
Vitamin premix[a]	0.250	0.250	0.250
TM premix[b]	0.125	0.125	0.125
Salt	0.200	0.200	0.200
Fishmeal	4.000	4.000	4.000
Dried whey	20.000	20.000	20.000
Plasma protein	5.000	5.000	5.000
Lysine-HCL	0.1	0.1	0.1
DL-methionine	0.1	0.1	0.1
Carbadox-10	0	0.25	0
Bioplus 2B	0	0	0.05
Se premix[c]	0.05	0.05	0.05

[a]Vitamin premix provided per kg of diet: vitamin A, 6064 IU; vitamin $D_{3,}$ 606 IU; vitamin E, 44.1 IU; menadione, 2 mg; vitamin $B_{12,}$ 35 μg ; riboflavin, 7.1 mg ; d-pantothenic acid, 22 mg; niacin, 33 mg.
[b]Trace mineral premix provided per kg of diet: Iron, 121.2 mg; Zinc, 121.2 mg; Manganese, 15.0 mg; Copper, 11.2 mg; Iodine, .46 mg; Se, .30 mg.
[c]Se premix provided 0.3 mg Se per kilogram of diet.

Table 2. Dietary composition for phase 2 diets (d 7-21).

	Diet		
Ingredients, %	Neg. control	Pos. control	Probiotics
Corn	51.185	50.682	51.115
SBM, 48% CP	27.950	27.97	27.95
Soybean oil	4.005	4.188	4.025
Limestone	0.520	0.520	0.520
Dicalcium phosphate	0.840	0.840	0.840
Vitamin premix[a]	0.250	0.250	0.250
TM premix[b]	0.125	0.125	0.125
Salt	0.300	0.300	0.300
Fishmeal	4.500	4.550	4.500
Dried whey	10.000	10.000	10.000
Lysine-HCL	0.150	0.150	0.150
DL-methionine	0.075	0.075	0.075
Threonine	0.05	0.05	0.05
Carbadox-10	0	0.25	0
Bioplus 2B	0	0	0.05
Se premix[c]	0.05	0.05	0.05

[a]Vitamin premix provided per kg of diet: vitamin A, 6064 IU; vitamin $D_{3,}$ 606 IU; vitamin E, 44.1 IU; menadione, 2 mg; vitamin $B_{12,}$ 35 μg ; riboflavin, 7.1 mg ; d-pantothenic acid, 22 mg; niacin, 33 mg.
[b]Trace mineral premix provided per kg of diet: Iron, 121.2 mg; Zinc, 121.2 mg; Manganese, 15.0 mg; Copper, 11.2 mg; Iodine, .46 mg; Se, .30 mg.
[c]Se premix provided 0.3 mg Se per kilogram of diet.

Table 3. Dietary composition for phase 3 diets (d 21-35).

	Diet		
Ingredients, %	Neg. control	Pos. control	Probiotics
Corn	62.570	62.09	62.48
SBM, 48% CP	30.850	30.88	30.85
Soybean oil	3.000	3.2	3.04
Limestone	0.810	0.810	0.810
Dicalcium phosphate	1.690	1.690	1.690
Vitamin premix[a]	0.250	0.250	0.250
TM premix[b]	0.125	0.125	0.125
Salt	0.350	0.350	0.350
Lysine-HCL	0.200	0.200	0.200
DL-methionine	0.05	0.05	0.05
Threonine	0.055	0.055	0.055
Carbadox-10	0	0.25	0
Bioplus 2B	0	0	0.05
Se premix[c]	0.05	0.05	0.05

[a]Vitamin premix provided per kg of diet: vitamin A, 6064 IU; vitamin $D_{3,}$ 606 IU; vitamin E, 44.1 IU; menadione, 2 mg; vitamin $B_{12,}$ 35 μg ; riboflavin, 7.1 mg ; d-pantothenic acid, 22 mg; niacin, 33 mg.
[b]Trace mineral premix provided per kg of diet: Iron, 121.2 mg; Zinc, 121.2 mg; Manganese, 15.0 mg; Copper, 11.2 mg; Iodine, .46 mg; Se, .30 mg.
[c]Se premix provided 0.3 mg Se per kilogram of diet.

Table 4. The effects of direct fed microbials on growth performance.

	Neg. ctrl.	Bolus	In feed prob.	Bolus + in feed prob.	Pos. ctrl.	SE
Initial BW, lbs	13.96	13.99	14.00	14.00	13.96	0.031
Phase 1 (d 0 to 7)						
ADG, lbs	0.175^{a}	0.152^{ab}	0.102^{b}	0.154^{ab}	0.188^{a}	0.0317
ADFI, lbs	0.333	0.325	0.284	0.287	0.318	0.0275
G:F	0.48	0.58	0.32	0.51	0.58	0.110
d 7 BW, lbs	15.04	15.05	14.71	14.79	15.28	0.245
Phase 2 (d 7 to 20)						
ADG, lbs	0.55^{xy}	0.57^{xy}	0.49^{y}	0.57^{xy}	0.60^{x}	0.033
ADFI, lbs	0.96^{xy}	0.93^{xy}	0.88^{y}	0.94^{xy}	1.03^{x}	0.047
G:F	0.58	0.62	0.55	0.62	0.58	0.027
d 20 BW, lbs	22.23^{xy}	22.51^{xy}	21.08^{y}	22.26^{xy}	23.08^{x}	0.557
Phase 3 (d 20 to 34)						
ADG, lbs	1.10^{b}	1.15^{ab}	1.26^{a}	1.24^{ab}	1.27^{a}	0.056
ADFI, lbs	1.90^{y}	1.93^{y}	2.00^{xy}	2.03^{xy}	2.26^{x}	0.084
G:F	0.58	0.60	0.63	0.62	0.56	0.027
d 34 BW, lbs	36.55	37.46	37.47	38.35	39.50	1.120
Overall (d 0 to 34)						
ADG, lbs	0.69	0.71	0.71	0.74	0.77	0.034
ADFI, lbs	1.21^{y}	1.21^{y}	1.21^{y}	1.25^{xy}	1.38^{x}	0.052
G:F	0.55	0.59	0.59	0.61	0.56	0.024

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.
[xyz]Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.

Table 5. The effects of direct fed microbials on intestinal and thymus weights.

	Neg. ctrl.	Bolus	In feed prob.	Bolus + in feed prob.	Pos. ctrl.	SE
d 7 tissue weigh, g						
Wet stomach	47.64^{ab}	41.75^{b}	49.08^{a}	47.32^{ab}	48.03^{ab}	2.633
Dry stomach	7.01	6.54	7.25	7.20	7.28	0.392
Wet sm. Intestine	211.21	200.59	213.53	225.75	238.52	15.359
Dry sm. Intestine	23.81	22.58	24.52	24.60	27.28	1.848
Wet lg. intestine	85.33^{ab}	71.77^{b}	93.80^{a}	79.11^{ab}	83.85^{ab}	7.871
Dry lg. intestine	10.29	8.98	11.32	9.98	10.56	0.989
Wet thymus	10.28^{yzb}	12.73^{xya}	9.29^{zb}	13.35^{xa}	10.34^{yzb}	0.878
Dry thymus	2.24^{ybc}	2.63^{xyab}	2.14^{ybc}	3.08^{xa}	1.98^{yc}	0.228
d 21 tissue weigh, g						
Wet stomach	89.89	97.20	97.74	103.61	104.85	7.40
Dry stomach	15.45	15.95	15.91	17.29	17.17	1.531
Wet sm. Intestine	494.11	558.09	585.39	559.71	585.03	34.988
Dry sm. Intestine	68.34	70.24	77.03	73.77	76.36	5.774
Wet lg. intestine	172.84^{ab}	169.91^{ab}	160.19^{b}	179.41^{ab}	194.73^{a}	12.165
Dry lg. intestine	22.96	20.09	20.12	22.87	24.28	1.812
Wet thymus	6.50^{b}	9.65^{a}	7.57^{ab}	7.11^{ab}	7.75^{ab}	1.055
Dry thymus	1.19	1.85	1.56	1.45	1.82	0.263
d 34 tissue weigh, g						
Wet stomach	157.53	151.74	149.53	164.45	163.49	8.056
Dry stomach	28.67	27.50	27.50	30.00	29.67	1.586
Wet sm. Intestine	842.45^{xyab}	887.25^{xa}	767.92^{yb}	800.81^{xyab}	770.33^{yb}	35.549
Dry sm. Intestine	122.67^{xyab}	131.00^{xa}	112.83^{yb}	117.00^{xyab}	112.33^{yb}	5.644
Wet lg. intestine	304.57^{ab}	312.78^{ab}	245.95^{b}	326.25^{a}	278.63^{ab}	28.244
Dry lg. intestine	44.33	43.83	38.00	46.67	39.33	4.169
Wet thymus	21.14^{ybc}	18.85^{yc}	18.12^{yc}	27.96^{xa}	24.33^{xyab}	2.077
Dry thymus	3.50	3.17	2.83	4.00	3.33	0.516

^{abc}Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.
^{xyz}Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.

Table 6. The effects of probiotics or antibiotics on duodenal morphology.

	Treatment[1]					
	1	2	3	4	5	SEM
d 7						
Villus height[2], μm	67.9	64.7	61.5	64.6	59.3	5.85
Crypt depth[3], μm	55.1	49.5	58.3	55.1	57.2	4.52
d 21						
Villus height, μm	63.5	69.7	62.5	68.7	68.7	2.67
Crypt depth, μm	62.7^{b}	62.7^{b}	72.0^{ab}	75.0^{ab}	78.1^{a}	4.79
d 35						
Villus height, μm	72.2^{xy}	65.2^{y}	71.4^{xy}	73.1^{xy}	80.9^{x}	4.22
Crypt depth, μm	70.7	63.7	70.9	69.2	60.3	5.45

[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[2]Villus height is measured as the distance from the lamina propria to villus tip.

[3]Crypt depth is measured as the distance from the lamina propria to invagination between adjacent villi.

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

[xyz]Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.

Table 7. The effects of probiotics or antibiotics on ileal morphology.

	Treatment[1]					
	1	2	3	4	5	SEM
d 7						
Villus height[2], μm	55.7	52.8	53.8	48.0	53.3	3.56
Crypt depth[3], μm	44.7^{y}	38.2^{y}	44.7^{y}	39.7^{y}	55.5^{x}	2.67
d 21						
Villus height, μm	53.5	52.6	58.0	60.5	60.2	4.40
Crypt depth, μm	56.7	55.0	52.5	50.7	54.0	2.95
d 35						
Villus height, μm	60.3^{b}	66.0^{ab}	61.9^{ab}	67.7^{a}	62.1^{ab}	2.47
Crypt depth, μm	46.0^{b}	54.2^{a}	46.0^{b}	45.0^{b}	55.2^{a}	3.15

[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[2]Villus height is measured as the distance from the lamina propria to villus tip.

[3]Crypt depth is measured as the distance from the lamina propria to invagination between adjacent villi.

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

[xyz]Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.

Table 8. The effects of probiotics or antibiotics on bromodeoxyuridine (BrdU) labeled enterocyte height of duodenal villi, relative bromodeoxyuridine (BrdU) labeled enterocyte height (% of crypt-villus axis migrated) of duodenal villi and bromodeoxyuridine (BrdU) labeled enterocyte migration rate (μm/h) of duodenal villi.

	Treatment[1]					
	1	2	3	4	5	SEM
d 7						
BrdU height, μm	27.52	31.64	31.88	26.31	24.62	6.551
Relative BrdU height, %	36.77	44.86	51.96	57.21	41.11	10.013
Migration rate, μm/h	1.15	1.32	1.33	1.10	1.03	0.273
d 21						
BrdU height, μm	32.62[b]	40.92[ab]	34.49[ab]	46.62[a]	43.10[ab]	4.688
Relative BrdU height, %	50.68	58.25	55.10	67.62	60.92	7.390
Migration rate, μm/h	1.36[b]	1.71[ab]	1.43[ab]	1.94[a]	1.79[ab]	0.196
d 35						
BrdU height, μm	40.96	37.07	47.73	46.22	43.25	5.957
Relative BrdU height, %	58.31	57.11	64.92	63.67	51.61	7.596
Migration rate, μm/h	1.71	1.55	1.99	1.93	1.80	0.248

[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

Table 9. The effects of probiotics or antibiotics on bromodeoxyuridine (BrdU) labeled enterocyte height of ileal villi, relative bromodeoxyuridine (BrdU) labeled enterocyte height (% of crypt-villus axis migrated) of ileal villi and bromodeoxyuridine (BrdU) labeled enterocyte migration rate (μm/h) of ileal villi.

	Treatment[1]					
	1	2	3	4	5	SEM
d 7						
BrdU height, μm	26.72	11.98	20.25	26.86	22.45	4.684
Relative BrdU Height, %	48.63[ab]	21.73[b]	38.26[ab]	59.24[a]	42.88[ab]	9.397
Migration rate, μm/h	1.11	0.50	0.84	1.12	0.93	0.195
d 21						
BrdU height, μm	25.96[b]	25.88[b]	33.43[ab]	41.11[a]	32.45[ab]	4.892
Relative BrdU Height, %	48.66	48.14	57.81	68.36	52.18	7.584
Migration rate, μm/h	1.08[b]	1.08[b]	1.39[ab]	1.71[a]	1.35[ab]	0.204
d 35						
BrdU height, μm	47.34	45.23	42.58	46.32	42.70	4.667
Relative BrdU Height, %	78.31	69.32	69.24	68.25	67.04	6.464
Migration rate, μm/h	1.97	1.88	1.77	1.93	1.78	0.194

[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

Table 10. The effects of probiotics or antibiotics on the number of proliferating cell nuclear antigen (PCNA) positing cell in the crypt region of the duodenum.

Region	Treatment[1]					
Crypt[2]	1	2	3	4	5	SEM
d 7 # PCNA stained enterocytes	8.47	6.42	11.57	11.25	9.72	1.998
d 21 # PCNA stained enterocytes	10.67[ab]	13.50[ab]	15.73[a]	9.48[b]	13.90[ab]	2.100
d 35 # PCNA stained enterocytes	15.45	13.27	11.85	11.17	14.33	1.857

[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[2]Crypt is the lamina propria to invagination between adjacent villi

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

Table 11. The effects of probiotics or antibiotics on the number of proliferating cell nuclear antigen (PCNA) positing cell in the crypt region of the ileum.

Region	Treatment[1]					
Crypt[2]	1	2	3	4	5	SEM
d 7 # PCNA stained enterocytes	20.48^{ab}	16.72^{ab}	15.63^{b}	22.65^{a}	20.37^{ab}	2.128
d 21 # PCNA stained enterocytes	22.28^{y}	26.97^{xy}	26.38^{xy}	24.65^{xy}	31.65^{x}	2.715
d 35 # PCNA stained enterocytes	27.10^{y}	34.35^{xy}	43.72^{x}	28.43^{y}	36.37^{xy}	3.542

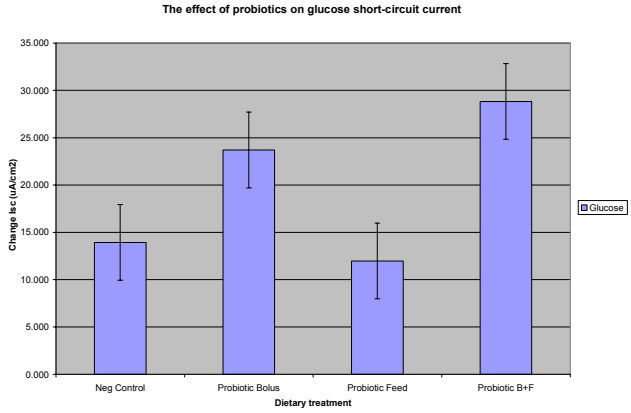
[1]Treatments: 1) negative control with no supplementation of DFM or antibiotics, 2) Trt. 1 + DFM administered in a bolus dose at weaning, 3) Trt. 1 + DFM administered through the feed for d 1-35, 4) Trt. 1 + DFM administered in a bolus dose at weaning, and through the feed for d 1-35, and 5) Trt. 1 + in feed antibiotics.

[2]Crypt is the lamina propria to invagination between adjacent villi

[abc]Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.

[xyz]Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.

7 d post-weaning



21 d post-weaning

35 d post-weaning

Figure 1. The effects of probiotics on glucose uptake as measured by changes in transepithelial current, following a glucose challenge in pigs at 7, 21, and 35 days post-weaning.