RESEARCHREPORT

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PORK SAFETY

Title: Effect of oligosaccharides and organic salts on the health and performance of growing-finishing pigs – **NPB #99-219**

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Abstract

Two experiments were conducted to determine the influence of two prebiotics (fructooligosaccharide and arabinogalactan) and two probiotics (*Bifidobacterium breve* and a bacillus) on pig performance and on intestinal microbial populations. In Exp. 1 pigs were given the experimental treatments orally for 4 d prior to weaning (23d) and throughout in the feed from weaning to market weight. Treatments were CON = control with no antibiotic, AB = antibiotic, FOS = fructooligosaccharide, AG = arabinogalactan, AG+B = arabinogalactan + *B. breve*. Pigs in Exp. 2 were given treatments in the feed for 4 d prior to weaning (17d) till two weeks after entering the grow/finish building. Treatments were CON, AB, FOS, AG and PRO where PRO = bacillus. In Exp. 1, liveweight gains were increased by 9.5% and feed efficiency improved by 4.5% with AB compared to CON with liveweight gains and feed efficiency not changed by the other treatments. There was a tendency of reduced intakes with the AG and FOS containing diets. In Exp. 2, liveweight gains were increased by 14.6%, 6.6% and 10.4% for AB, FOS and PRO, respectively, compared to the CON. Feed efficiency was increased (4.6%) the most with the PRO diet compared to CON. Feed consumption was increased 11.6% with AB, 4.3% with FOS and 5.4% with PRO compared to CON. There were no effects of treatment on total anaerobes and bifidobacteria in either experiment. Salmonella were not detected in either experiment. Coliform numbers were lower ($P < .05$) for AG and higher for AG+B at weaning in Exp. 1, but no differences were detected in the nursery and grow phase of Exp. 1. FOS fed to nursery pigs reduced ($P < .01$) clostridia in the intestinal system. Coliforms were not changed by treatment in Exp. 2. When pigs are growing close to their maximum potential, it is difficult to demonstrate effects of growth promotant antibiotics, or their alternatives, on animal performance. The general lack of a significant influence of the growth promoting antibiotics on performance suggests that animals were in good health and not stressed. Under these conditions the treatments containing the probiotic tended to give performance values between CON and AB. A standardized stress model would be useful for determining efficacy of these and other compounds that may potentially be used to replace subtherapeutic use of antibiotics.

Introduction

Health management of the pig presents one of the most significant challenges to pork producers. The stress of weaning and movement to another environment increases the potential for disease, poor feed intake and nutritional disorders. In the past, subtherapeutic antibiotics have been an effective means of reducing disease and increasing animal performance. However, concerns of increasing antibiotic resistance has lead the European Union to ban use of subtherapeutic antibiotics and there are efforts to reduce or eliminate their use in the United States. In light of this trend, effective alternatives to subtherapeutic antibiotics need to be developed. Because the intestinal microbiota plays an important role in suppressing colonization by pathogens, there is much interest in manipulating the intestinal microbiota to reduce disease, enhance performance and improve human food safety. Two approaches are (1) to selectively enrich beneficial bacteria by feeding compounds, primarily oligosaccharides, that reach the lower intestinal tract and act as substrates for bacterial populations with increased numbers/activities to suppress pathogen colonization, or (2) by feeding probiotic organisms that consequently populate the intestinal system and suppress pathogens.

However, feeding complex carbohydrates, primarily oligosaccharides, from natural or processed sources, has given inconsistent results on pig performance and microbial populations in the intestinal digesta. Nutritional factors affecting intestinal *E. coli*, *Salmonella* and other pathogen concentrations include antibiotics, nitrogen sources and levels, carbohydrate sources, fiber content, volatile fatty acids (VFA), and vitamins (Sutton and Patterson, 1996). Fructooligosaccharides have been shown to reduce *Salmonella sp*. in the ceca of poultry (Bailey, et. al. (1991). In addition, use of competitive exclusion approaches to control *Salmonella enteriditis* has been accomplished in poultry ceca by administering specific cultures in probiotics. Recent work by Orban, et. al. (1997) indicates that another oligosaccharide, sucrose thermal oligosaccharide caramel, added to broiler diets enhances *Bifidobacteria sp*. and broiler performance, especially if dietary mineral levels are limited. Similar responses were noted with a study using European diets fed to pigs (Houdijk, et al. 1997). FOS was observed to reduce *Clostridium perfringens*, pathogenic *E. coli* and *Salmonella typhimurium* and increased *Lactobacillus bifidus*, *Lactobacillus acidophilus* and *Bifidobacteria* in *in vitro* cultures (Mul and Perry, 1994). Prohaszka and Lucas (1984) determined that VFA concentrations in the large intestine of pigs had an antibacterial affect on *Serpulinia hyodysenteria* and *E. coli* due to lower pH levels. They suggested that proper diet control possibly through certain fiber additions or specific oligosaccharides could reduce the risk of clinical microbial abnormalities and potential infections in the GI tract. Low pH (5.0 vs. 7.0) reduced protein synthesis and internal pH in *E. coli* as compared to *Salmonella typhimurium* (Hickey and Hirshfield, 1990). Expression of adhesive filaments is also dependent on the physiological environment surrounding the E. coli cell (Jacobs and De Graaf, 1985) and thus pathogenecity may be influenced by nutritional factors, other microbial species and their fermentation products. Probiotics also have an inconsistent history, partially because of poor processing methods that assure viability. As a consequence, research is needed with commercial type operations to evaluate the potential of oligosaccharides and probiotics on pig performance and intestinal microflora at different stages of growth.

Objective

Determine the effects of oligosaccharides, probiotics and combinations of oligosaccharides and probiotics added to non-antibiotic diets on pig performance and intestinal microbial populations.

Procedures

Two experiments were conducted to compare efficacy of pre- and pro-biotics on pig performance and intestinal microbial populations. Each experiment used five replicate pens with seven pigs per pen for each treatment. Pens were allocated according to sex with the remaining pens containing mixed sexes in the same combinations. Pigs were housed in slotted floor buildings. All pigs had ad libitum access to food and water. The experiments were approved by the Purdue University Animal Care and Use Committee.

Experiment 1 – Litters were allocated to treatments based upon litter weight at d 19 and pigs were orally dosed with 5 ml of a treatment solution daily until weaned at 23d. At weaning, pigs within preassigned treatments were allocated to pens based upon sex and weight. Treatments were CON=control, AB=antibiotic, FOS=fructooligosaccharide, AG=arabinogalactan and AG+B=Arabinogalactan + *Bifodobacterium breve*. FOS was donated by Encore Technologies (Minneapolis, MN), AG by Larex, Inc. (White Bear Lake, MN), and a human probiotic strain of *Bifodobacterium breve* donated by Chr. Hansen, Inc. (Milwaukee, WI). Pigs were transferred to the grow/finish building after 4 weeks in the nursery and were fed the treatments until reaching finishing weight. Liveweights and feed consumption data were collected 4 d prior to weaning, at weaning, weekly during the nursery phase, bi-weekly during the first month of the grow/finish phase and monthly until reaching finishing weight. The diets are shown in Table 1.

Rectal samples were obtained from one pig per pen (at average pen weight) at weaning, after two weeks in the nursery and 3 d after being transferred to the grow/finish building. Samples were weighed and a 10 fold (w/v) amount of anaerobic peptone broth was added to the tubes. Samples were vortexed until homogenized, serially diluted in anaerobic peptone broth and transferred into an anaerobic chamber (Coy). Samples (0.025 ml) were inoculated onto 60 mm petri dishes of the respective culture media and incubated for 3 d at 37°C before counting. The initial anaerobic dilution was also used to then make an aerobic dilution series and 0.5 ml were inoculated onto 100 ml petri dishes of McConkey agar medium to enumerate coliforms. The initial anaerobic dilution (0.2 ml) was also inoculated in Rappaport-Vassiliadis R 10 broth (Difco), incubated for 42 h at 42°C and then streaked on XLD4 (Difco) agar plates and incubated at 37°C overnight. Black colonies were presumptively identified using TSI and LIA (Difco) slants. Anaerobic culture media included Medium 98-5 (Bryant and Allison, 1961) for enumeration of total anaerobes, Beerens medium (1991) for bifidobacteria and reinforced clostridial agar (Difco, Detroit, MI) was used to enumerate spores from dilutions that had been heated at 95 °C for 10 min.

Experiment 2 – Litters were allocated to treatments based upon litter weight at 14d of age and pig treatments were mixed into nursery feed and offered from d 15 until weaning at 17d. At weaning, pigs within preassigned treatments were allocated to pens based upon sex and weight. Treatments were CON=control, AB=antibiotic, FOS=fructooligosaccharide, AG=arabinogalactan and PRO=bacillus. FOS was donated by Encore Technologies (Minneapolis, MN), AG by Larex, Inc. (White Bear Lake, MN), and the bacillus by Chr. Hansen, Inc. (Milwaukee, WI). Pigs were transferred to the grow/finish building after 4 weeks in the nursery and were fed the treatments through the nursery phase and through the first two weeks of the grow phase. Liveweights and feed consumption data were collected 4 d prior to weaning, at weaning, weekly during the nursery phase and after the second week of the grow phase. The diets are shown in Table 2. Microbial enumeration was as described above.

Statistical Analysis

The GLM analyses of ANOVA was conducted on all performance data with the pen serving as the experimental unit. Microbial data was transformed to $\log_{10}$ basis and statistically analyzed by GLM procedures of ANOVA.

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Results and Discussion

Tables 3 through 6 summarize the effects of the oligosaccharides, probiotics and antibiotics on pig performance and intestinal microbial colonies of pigs fed the experimental diets during the weaning, nursery and growing phases. There were no significant effects of treatments on pig liveweight gain, feed/gain or feed intake. However, there were trends of treatment effects where, liveweight gains were increased by 9.5% and feed efficiency improved by 4.5% with AB compared to CON in Exp. 1. Liveweight gains and feed efficiency were not changed by the other dietary treatments. Part of this effect may have been due to a tendency of reduced feed intakes with the AG and FOS containing diets. Houdijk, et al., (1997) and Klein Gebbink, et al., (2000) noticed a reduced feed intake in the pig when fed 5% FOS in nursery diets. There were no effects of treatment on total anaerobes and bifidobacteria in either experiment. Salmonella were not detected in either experiment. However, coliform numbers were lower ($P < .05$) for AG and higher for AG+B at weaning in Exp. 1, but no differences were detected in the nursery and grow phase of Exp. 1. Evidently, the addition of arabinogalactan (AB) was sufficient to change the microbial populations and/or the metabolic environment to suppress coliforms. However, the addition of a Bifidobacteria culture did not restrict coliforms growth. Potentially AG increased VFA production in the intestine causing a lower pH which could have reduced coliform survival, growth or metabolism

In Exp. 2, liveweight gains were increased by 14.6%, 6.6% and 10.4% for AB, FOS and PRO, respectively, compared to the CON. Feed efficiency was improved (4.6%) the most with the PRO diet compared to CON. Feed to gain ratio was only improved from 2.1% to 3.5% for the remaining dietary treatments. However, feed consumption was increased 11.6% with AB, 4.3% with FOS and 5.4% with PRO compared to CON. FOS fed to nursery pigs reduced ($P < .05$) colonies from spoors in the rectal samples. The increase in colonies from spoor in the PRO treatment in the nursery and grow phases, but not the preweaning phase suggests that the bacillus probiotic was alive throughout the digestive tract and was either colonizing the intestinal tract, or enough were fed to increase fecal numbers over ten fold. Coliforms were not changed by dietary treatments in Exp. 2. The status of the microbial populations tested in the intestine of the pigs did not correlate with animal performance in this experiment. When pigs are growing close to their maximum potential, it is difficult to demonstrate effects of growth promotant antibiotics, or their alternatives, on animal performance. The general lack of a significant influence of the growth promoting antibiotics on performance suggests that animals were in good health and not stressed. Under these conditions the treatments containing the probiotic tended to give performance values between CON and AB and in come cases exceeded the AB in feed efficiency (PRO in Exp 2). Even though the facilities used in this study have been known to exhibit disease pressure, the conditions during this study did not show evidence of stress. These compounds may show greater efficacy under more stressful conditions.

Table 1 Diets for Experiment 1

Ingredient	Nursery 1	Nursery 2	Grower 1	Grower 2	Finishing 1	Finishing 2
Corn	53.37	68.88	73.11	78.71	84.46	88.34
SBM, 48%	27.15	26.98	23.20	17.73	12.34	8.67
Dicalcium Phosphate	.74	1.29	1.08	.92	.74	.54
Limestone	.39	.72	.75	.78	.81	.83
Salt	.25	.35	.35	.35	.25	.25
Animal Fat	0	1.0	1.0	1	1.0	1.0
Soy Bean Oil	3.0	0	0	0	0	0
Lysine HCl	.15	.15	.15	.15	.15	.15
DL Methionine	.05	0	0	0	0	0
Vitamins	.25	.25	.15	.15	.1	.1
Trace minerals	.12	.12	.09	.09	.05	.05
Selenium 600	.05	.05	.05	.05	.025	.025
Dried whey	10	0	0	0	0	0
Menhaden Fish Meal	4	0	0	0	0	0
Zinc oxide	.38	0	0	0	0	0
Copper sulfate		.08	0	0	0	0
Carbadox (10 g/lb)[1]	.25	.25	0	0	0	0
CTC (50g/lb)[1]		0	.1	.1	.1	0
Tylan [1]		0	0	0	0	.05
Banminth (48g/lb)		.1	0	0	0	0
Phytase (600Pu/g)	.1	.1	.08	.08	.08	.05
FOS[2]	.1	.1	.1	.1	.1	.1
AG[3]	.1	.2	.1	.1	.1	.1
B. breve [4] (10^9cfu/0.1lb)	.1	.1	.1	.1	.1	.1

[1] For antibiotic treatment only

[2] For FOS treatment only

[3] For AG treatment and AG + B treatment

[4] For AG + B treatment only

Table 2 Diets for Experiment 2

Ingredient	Nursery 1	Nursery 2	Grower 1
Corn	63.73	64.45	72.51
SBM, 48%	13.19	25.30	23.20
Soy isolate	4.0	0	0
Spray dried plasma	5.0	0	0
Fishmeal	2.5	4	0
Starch	.6	.6	.6
Dicalcium Phosphate	1.96	.97	1.08
Limestone	.63	.65	.75
Salt	.35	.35	.35
Animal Fat	0	0	1.0
Soy Bean Oil	5.0	3.0	0.0
Lysine HCl	0	.1	.15
DL Methionine	.1	.05	0
Vitamins	.25	.25	.15
Trace minerals	.15	.125	.09
Selenium 600	.05	.05	.05
Copper sulfate[1]	0	.078	0
Carbadox (10 g/lb)[1]	.25	.25	0
CTC (50g/lb)[1]		0	.1
Banminth (48g/lb)		.1	0
Phytase (600Pu/g)	.1	.1	.08
FOS[2]	.1	.1	.1
AG[3]	.1	.2	.1
bacillus [4] (10^9cfu/0.1lb)	.1	.1	.1

[1] For antibiotic treatment only
[2] For FOS treatment only
[3] For AG treatment
[4] For PRO treatment only

Table 3. Exp. 1 Animal Performance (41 days after weaning)

	Feed Consumption	Gain	F/G
Con	2.34	1.27	1.85
AB	2.45	1.39	1.76
AG	2.18	1.18	1.84
FOS	2.16	1.19	1.81
AG+B	2.26	1.24	1.82

Con = Control, AB=antibiotic, FOS=fructooligosaccharide, AG= Arabinogalactan, AG+B=Arabinogalactan + *Bifidobacterium breve*. Pigs were weaned at 23d and were given treatments orally (5 ml) for 4 d prior to weaning.

Table 4. Exp. 1 Microbial populations (colonies Log$_{10}$)

	Preweaning				Nursery				Grow			
Trt	Total	Bifido	Clost	Colif	Total	Bifido	Clost	Colif	Total	Bifido	Clost	Colif
Con	9.37	8.24	4.90	6.44^{ab}	9.82	8.58	4.23	6.06	9.81	9.17	5.23	6.51
AB	9.41	8.42	5.95	6.44^{ab}	9.52	8.58	3.52	5.64	9.84	9.27	5.01	7.17
AG	9.23	8.50	5.03	5.36^{b}	9.54	8.13	3.72	6.32	9.85	9.30	5.39	7.49
AG+B	9.40	8.59	5.22	7.49^{a}	9.55	8.33	3.86	5.38	9.78	9.10	5.62	6.81
FOS	9.33	8.38	5.17	6.86^{ab}	9.65	8.70	4.03	6.39	9.56	9.14	4.98	6.89
SEM	0.19	0.34	0.61	0.52	0.16	0.27	0.39	0.55	0.10	0.16	0.41	0.36

Con = Control, AB=antibiotic, FOS=fructooligosaccharide, AG= Arabinogalactan, AG+B=Arabinogalactan + *Bifidobacterium breve*. Pigs were weaned at 23d and were given treatments orally (5 ml) for 4 d prior to weaning.

Table 5. Exp. 2 Animal Performance (49 days after weaning)

	Feed Consumption	Gain	F/G
Con	1.53	.82	1.86
AB	1.70	.94	1.81
AG	1.52	.85	1.79
FOS	1.59	.88	1.82
PRO	1.61	.91	1.77

Con = Control, AB=antibiotic, FOS=fructooligosaccharide, AG= Arabinogalactan, PRO=a probiotic culture from Chr. Hansen. Pigs were weaned at 17d and were given treatments orally (5 ml) for 4 d prior to weaning.

Table 6. Exp. 2 Microbial populations (colonies Log$_{10}$)

	Preweaning				Nursery				Grow			
Trt	Total	Bifido	Clost	Colif	Total	Bifido	Clost	Colif	Total	Bifido	Clost	Colif
Con	9.91	9.16	5.70	8.52	9.56	8.61	3.70^{a}	7.14	9.73	8.79	4.56^{b}	6.44
AB	9.72	8.93	5.41	8.52	9.68	8.58	4.79^{a}	6.65	9.62	8.75	4.38^{b}	6.93
AG	9.64	8.67	5.43	8.51	9.48	8.83	4.62^{a}	6.75	9.59	8.64	3.93^{b}	7.04
FOS	9.82	9.30	5.38	7.98	9.70	9.04	1.44^{b}	6.11	9.63	8.70	4.41^{b}	7.19
PRO	9.78	8.93	5.80	8.70	9.74	8.98	5.73^{a}	6.56	9.76	9.07	5.85^{a}	6.74
SEM	0.16	0.26	0.23	0.25	0.12	0.17	0.62	0.41	0.11	0.21	0.32	0.48

Con = Control, AB=antibiotic, FOS=fructooligosaccharide, AG= Arabinogalactan, PRO=a probiotic culture from Chr. Hansen. Pigs were weaned at 17d and were given treatments orally (5 ml) for 4 d prior to weaning.