

The Effect of Supplemental Probiotics and Spray-Dried Egg Proteins on Piglet Growth Performance Characteristics

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ABSTRACT

This study evaluated the use of a supplemental probiotic, containing spray-dried egg proteins and several different dried bacteria. The objectives of this study were to determine if the probiotic supplement affected growth performance of the piglets, fecal consistency, and the number of *Escherichia coli* and *Lactobacillus* species present in the feces. The supplemental probiotic was administered at three intervals with fecal samples being collected at four intervals. Weights were collected at five intervals. There were no differences ($P > 0.05$) in 21-day, weaning, nursery, or finishing floor weights between treatment and control groups. There were no differences ($P > 0.05$) in fecal scores in the farrowing house, nursery, or finishing floor between treatment and control groups. There were no differences ($P > 0.05$) in the number of *Escherichia coli* or *Lactobacillus* species present in the fecal material, between treatment and control groups, at all intervals measured.

KEY WORDS: piglets, probiotic, egg proteins, growth performance

INTRODUCTION

Consumers today are worried about consuming meat from animals who received sub-therapeutic levels of antibiotics for growth promotion while producers are worried about keeping their animals healthy and having the animals reach market weight as early as possible. These worries make it difficult for producers to please consumers and keep their animals healthy, while at the same time limiting production costs. Lowering the mortality rate of pre-weaned pigs and maintaining weight gains when weaning occurs are major economic factors associated with the profitability of swine operations. When piglets are weaned, they have a limited ability to deal with diseases because the level of immunoglobulins supplied by the colostrum may vary depending on the pathogen level they have faced and their immune system is just beginning to function (Coffey and Cromwell 2001). At weaning, piglets experience dietary and environmental changes that lower feed intake, cause poor performance, and increased susceptibility to diseases

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(Mathew et al. 1998). Producers prefer that their pigs reach market as soon as possible and any days off feed increases the time it takes the pigs to reach market weight, thus cutting into the producers' economical return.

Probiotics are prepared doses of live bacteria, usually in a feed type supplement form, that are given to an animal in order to repopulate the animal's digestive system with beneficial bacteria (Fuller 1989). The addition of beneficial bacteria when piglets are under stress works to improve the intestinal microflora balance, increasing nutrient absorption (Fuller 1989). Beneficial bacteria compete with harmful bacteria for substances in the digestive system. Probiotics give a boost to beneficial bacteria so they can outperform the harmful bacteria. Probiotics also stimulate the piglets to remain on feed, which lessens the amount of days the producers must feed the pigs before they reach market weight. In addition to probiotics, there are prebiotics available which work to promote a healthy intestinal microflora. A prebiotic can be defined as a non-digestible food ingredient that through metabolism promotes the growth of beneficial microflora in the intestines (Manning and Gibson 2004). A prebiotic does not stimulate the growth of harmful pathogens such as toxigenic *Escherichia coli* (*E. coli*) (Manning and Gibson 2004). The two common bacteria that are used to improve intestinal microflora are *bifidobacteria* and *lactobacilli* (Manning and Gibson 2004). The thought is that by increasing lactic acid bacteria, there is an increased stimulation of the immune system, specifically non-specific host defense mechanisms and certain cell types (Manning and Gibson 2004).

There is also public concern over the therapeutic and sub-therapeutic uses of antibiotics in animals. A common public perception is that sub-therapeutic use of antibiotics, which are the inclusion of antibiotics in the feed, might have an effect on the humans who consume the meat, making them resistant to the antibiotics used in the feed (Estienne et al. 2005). Probiotics, for example, could be the answer to replace sub-therapeutic uses of antibiotics. The use of probiotics may provide bacteria that can compete with organisms such as the *E. coli* in the intestine, which can limit the cases of diarrhea and the amount of antibiotics used in piglets. There is a need to find alternatives to antibiotics that will maintain the performance of pre-weaned and weaned piglets (Bhandari et al. 2008). Therefore the objective of this study was to determine if an oral probiotic supplementation affected the growth performance of the piglet, fecal consistency, and the number of *E. coli* and *Lactobacillus* species present in the fecal material.

MATERIALS & METHODS

Study Design. This study was conducted at the Tarleton State University Swine Center in Stephenville, TX, in an all-in/all-out management system. Fifty-nine crossbred (cross and Yorkshire based) sows and their piglets (n = 569) were included in the study. The same sow herd was used over five farrowings (replications) with sows being used two to three times, depending on when they farrowed. The litters were divided into two groups (treatment and control), with standardization occurring within 48 hours after birth to minimize differences in birth weights and litter sizes. When litters were divided, the assignment of the sow's previous litter was not considered. All piglets in the treatment litters received the treatment and all piglets in the control litters received no type of treatment. Control litters did not receive a placebo in order to closer mimic a commercial operation in which piglets would either receive a treatment or receive nothing. Piglets were evaluated for weight gains and fecal consistency as well as bacterial counts of fecal material. A complete randomized designed was used with litters being evenly divided into

treatment and control groups in order to try to maintain an even number of piglets and litters in each group throughout the study.

Treatment Group. The treatment group in this study received an oral supplemental probiotic manufactured by Trouw Nutrition International (Putten, The Netherlands). Ingredients of the supplement can be found in Table 1.

Table 1. Probiotic Supplement.

Ingredient
Lactic Acid Producing Bacteria (<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium thermophilum</i> , and <i>Enterococcus faecium</i>)
Vegetable Oil
Dextrose
Sodium Aluminosilicate
Dried Egg
Sorbitan Monostearate
Dried <i>Bacillus subtilis</i>
Dried <i>Saccharomyces cerevisiae</i>
Vitamin A
Vitamin D3
Vitamin E

Treatment was administered orally when piglets were three days of age (3g/piglet), 18-28 days of age (weaning) (6g/piglet), and 50-65 days of age (6g/piglet) when they were moved to the finishing floor. The control piglets did not receive a placebo. All management/handling practices were the same for both groups and piglets remained on the same treatment for the duration of the study.

Farrowing House, Nursery, and Finishing Floor Procedures. Birth weights were obtained and needle teeth were clipped within 24 hours of farrowing. Each piglet received 1.5 ml of iron, penicillin, and Draxxin (IPD) (48% iron-100mg/1ml, Agri Laboratories, Ltd, St. Joseph, MO; 28% penicillin-300,000 units/ml, Norbrook Laboratories Limited, Newry, Northern Ireland; 24% draxxin-100mg tulathromycin/ml, Pfizer Animal Health, New York City, NY). When birth weights were obtained, each individual had a number written on its back with a permanent marker for identification, which was its pig number for that litter. Piglets were ear notched at 24-48 hours of age for permanent identification. When the piglets were 48-72 hours old they had their tails docked. Also, male piglets were castrated at three to seven days of age. At day seven, all piglets received a 1.5 ml booster shot of IPD as well as 1 ml of Rhinogen BPE (Intervet Inc., Millsboro, DE). When the piglets were 14 days old, they received 2 ml of RespiSure One (Pfizer Animal Health, Exton, PA). At day 21, weights were obtained for all piglets and at day 24, all piglets received a booster of 1 ml of Rhinogen BPE (Intervet Inc., Millsboro, DE) and the first of two immunizations of 2 ml of Circumvent PCV (Intervet Inc., Millsboro, DE). For piglets that were weaned before 21 days of age, the weaning weight was adjusted according to "Livestock and Carcasses": An Integrated Approach to Evaluation, Grading, and Selection. Twenty-one-day weights were used as a measurement point since there is a formula that allows weights to be adjusted to 21 days. Piglets were weaned at 18-28 days with an all-

in/all-out management system. Weaning weights were obtained and used as initial weights going into the nursery period.

Fourteen days after the first Circumvent PCV vaccination, when the piglets were in the nursery, they received a booster of Circumvent PCV (Intervet Inc., Millsboro, DE) vaccination of 2 ml per head. Individual weights were obtained for each piglet when they had been in the nursery for 30 days.

Weights obtained from the piglets at day 30, in the nursery period, served as entry weights for the finishing floor. Individual pig weights were obtained after they had been on the finishing floor for 30 days.

Creep Feed Procedure. All litters had access to creep feed at 14 days of age which was ACCO Showmaster Prestarter, 10 Medicated (ACCO Feeds, Minneapolis, MN) (Table 2). Creep feed intake was measured by weighing the feed before offering it to the piglets. If the creep feed became spoiled in the feeder, it was collected, weighed, discarded, and recorded as Orts. Creep feed was monitored daily and was added as needed so the piglets were allowed to eat *ad libitum*.

Table 2. ACCO Showmaster Prestarter 10 Medicated.

Item	Guaranteed Analysis
Crude Protein	25.00%
Lysine	1.70%
Crude Fat	6.00%
Crude Fiber	5.00%
Calcium	1.25%
Phosphorus	0.50%
Salt	1.25%
Sodium	0.60%
Selenium	0.3 ppm
Zinc	3,000 ppm
Chlortetracycline	400 grams/ton
Tiamulin Hydrogen Fumarate	35 grams/ton

Nursery & Finishing Floor Diets. Each litter received one bag (22.68 kg) of the ACCO Showmaster Prestarter 10 Medicated (ACCO Feeds, Minneapolis, MN) (Table 2), in the nursery and then all litters received ACCO Showmaster Starter (Bannec) Medicated (ACCO Feeds, Minneapolis, MN) (Table 3) for the remainder of the nursery period.

Table 3. ACCO Showmaster Starter (Bannec) Medicated.

Item	Guaranteed Analysis
Crude Protein	23.00%
Lysine	1.61%
Crude Fat	5.00%
Crude Fiber	3.50%
Calcium	1.29%
Phosphorus	0.70%
Salt	1.00%
Sodium	0.60%
Selenium	0.3 ppm
Zinc	3,000 ppm
Chromium	200 ppb
Carbadox	50 grams/ton
Pyrantel Tartrate	96 grams/ton

The ration for all litters on the finishing floor was ADM Alliance 12191APY (ADM Alliance Nutrition, Inc., Quincy, IL) (Table 4).

Table 4. ADM Alliance 12191APY.

Item	Guaranteed Analysis
Crude Protein	18.50%
Lysine	1.10%
Crude Fat	2.50%
Crude Fiber	4.50%
Calcium	0.60%
Phosphorus	0.60%
Salt	0.40%
Selenium	0.3 ppm
Zinc	100 ppm
Carbadox	0.01%

Fecal Scoring. Each litter received a fecal score three times weekly when they were in the farrowing house and in the nursery by the primary researcher. Litters were used instead of individual piglets due to the fact that piglets may die or be sold before the end of the study. To determine the fecal score, all of the feces were observed in the pen and it was assigned a score based on its consistency. The fecal scores were averaged for the litter (score divided by number of times observed) when the piglets left the farrowing house and the nursery. Fecal scores were scored on a scale of 0-3: 0 = normal, 1 = soft feces, 2 = thick fluid feces, and 3 = watery feces (Bhandari et al. 2008; Marquardt et al. 1999). On the finishing floor, litters were monitored for 10 days for changes in fecal consistency.

Fecal Sample Collection. Due to budget restrictions, litters were used for bacterial counts instead of individual piglets. Fecal samples were collected throughout the study from litters to determine if there was a change in the number of *Lactobacilli* species and *E. coli* present. The first fecal sample was obtained when the piglets were 48 hours of age to establish a baseline since the first treatment would be administered at three days of age. The other

fecal samples were collected five days after the first treatment, five days after the second treatment, and five days after the third treatment to determine if there was a change in the number of microorganisms present as the piglets' aged.

At least four fecal samples were collected each time from the litter and combined into one sample. Due to the fact that a piglet could be sold or die any time during the study, the fecal sampling was random to ensure that enough fecal material could be collected for analysis and that consistency remained in having at least four samples. Although both treatment and control piglets were housed in the same pens, contamination of feces did not occur because fecal samples were collected from the rectum or from the top of the feces from a pig that had just defecated. The researcher had to visually see the pig defecate and that the feces did not mix with other feces on the ground.

Using a mini vortex, the sample was mixed with a phosphate buffer solution (PBS) (Table 5).

Table 5. Phosphate Buffer Solution.

Item	grams/2 liters of deionized water
Sodium Dihydrogen Phosphate (NaH_2PO_4)	1.16
Sodium Monohydrogen Phosphate (Na_2HPO_4)	5.00
Sodium Chloride (NaCl)	17.0

Bacterial Culture Analysis & Measurement. For both *E. coli* and *Lactobacilli* species plates, the Petri dishes were labeled with litter number and dilution number for identification. One blank plate with no fecal material was included, each time samples were cultured, to ensure there was no contamination. The dilution samples selected for plating were selected because in this range the number of colonies on the plates were low enough to distinguish individual colonies, which could be counted.

To test for the presence of *E. coli*, dilution samples of 10^{-5} to 10^{-8} were poured on to S-PAK membrane filters (Millipore Corporation, Billerico, MA) and filtered. After filtration, the filters were transferred to the Petri dish containing Difco Modified mTEC agar (Becton, Dickinson, & Company, Sparks, MD). After all samples had been plated, they were incubated for 18-24 hours at 35 degrees Celsius (EPA 2009).

To test for the presence of *Lactobacilli* species, a sample from each litter was mixed with BBL LBS Agar (Becton, Dickinson, & Company, Sparks, MD). The dilutions used were 10^{-3} to 10^{-6} and one milliliter of the dilution sample was placed in a Petri dish. Then 15 to 20 ml of the agar was added to the Petri dish. The Petri dishes were rotated so that the dilution sample and agar mixed. After all samples had solidified at room temperature, the plates were incubated for 72 hours at 35 °C.

Following the appropriate amount of time for incubation, colonies were counted for each sample. Plates containing less than 75 colonies were used to determine if there was a difference in the number of bacteria present. The dilution with the least amount of colonies was entered into a formula to determine the number of coliforming units per gram. If no colonies were present on any of the samples, then zero was used.

Statistical Analysis. Growth performance data, fecal consistency scores, and log transformed bacterial count data were analyzed using Proc GLM of SAS. Mean fecal consistency scores were found for each litter in the farrowing house, nursery, and finishing floor. The model contained the main effect of treatment, main effect of replication, and treatment x replication interaction. A *P*-value of 0.05 was considered significant when

differences were detected; the LSMEANS statement with the PDIF was used for mean separation. The differences in the n values are due to using individual piglets for growth performance and litters for fecal consistency and bacterial counts.

RESULTS & DISCUSSION

Decreasing pre-weaning mortalities as well as decreasing poor performance in the nursery is important to producers in order to limit the number of days to market weight and maintain economical returns. Producers have relied on the use of antibiotics sub-therapeutically to prevent illnesses in their swine herd but, as this practice comes under fire, producers must look for other alternatives to prevent sickness in their herds as well as reducing poor nursery performance.

Growth Performance. There was no difference ($P > 0.05$) in 21-day weight, weaning weight, nursery weight, or finishing floor weight between the treatment and control groups (Table 6).

Table 6. Effect of Supplemental Probiotics and Spray-Dried Egg Proteins on Growth Performance.

Variable	Treatment			Control			P value
	Wt.	n value	SEM ^c	Wt.	n value	SE M ^c	
21-day wt. (kg) ^{a,b}	5.830	240	0.19	5.770	252	0.22	0.63
Weaning wt. (kg) ^a	6.350	238	0.21	6.380	249	0.24	0.85
Nursery wt. (kg) ^a	17.68	228	0.45	17.73	242	0.53	0.89
Finishing Floor wt. (kg) ^a	32.45	140	1.35	32.34	124	1.21	0.90

^a Replication effect ($P < 0.05$).

^b Treatment x replication interaction ($P < 0.05$).

^c Standard Error of the Mean.

For weaning ($P < 0.0001$), nursery ($P < 0.0001$), and finishing floor ($P < 0.0001$) weights there was a difference due to the effect of replication and for 21-day weight there was a treatment x replication ($P < 0.01$) interaction as well as a replication effect ($P < 0.0001$). The effect of replication and replication x treatment interaction could be due to environmental conditions since the farrowings (replications) occurred in five different months.

These results are similar to Bhandari et al. (2008), who found that the addition of a direct-fed microbial to the diet, when compared to a spray-dried porcine plasma diet or a diet containing antibiotics, yielded no difference in growth performance of the piglets who were challenged with a strain of *Escherichia coli*. Estienne et al. (2005) also found that the administration of a probiotic supplement 24 hours after birth had no effect on growth performance at 7 and 14 days of age. However, Mathew et al. (1998) found that the inclusion of yeast in the piglets' diet led to a higher daily gain for the piglets who received the yeast in a pelleted form, over those who didn't receive the yeast or the yeast in a ground form. Marquardt et al. (1999) found that the use of egg-yolk antibodies in piglets exposed to *Escherichia coli*, increased the weight gains of the treatment piglets over the control piglets. However, De Cupere et al. (1992) also found no difference in weekly weight gains between control piglets and piglets that received three different probiotics who were also

infected with *Escherichia coli*. Although Taras et al. (2006) used a supplement that contained *Enterococcus faecium*, researchers reported no difference in weight gains in the nursery period between treatment and control piglets. Increase in weight gains reported in other studies may have been due to the type of probiotic used, dosage, or frequency of the treatment which is different from the current study.

Egg products contain avidin which is thought to bind with biotin, rendering the biotin unavailable to the animal. It is thought that although most egg products go through a heating process, the heating process might not inactivate all of the avidin. It is possible that the avidin in the egg products in this study may have interfered with the levels of biotin that the piglets received. Since this parameter was not measured in the study, it cannot be said if this had an effect on the piglets' growth.

Fecal Consistency. There was no difference ($P > 0.05$) in fecal consistency between the treatment and control litters in the farrowing house or nursery (Table 7). On the finishing floor, there was also no difference ($P > 0.05$) in fecal consistency between treatment and control litters (Table 7).

Table 7. Effect of Supplemental Probiotics and Spray-Dried Egg Proteins on Fecal Consistency.^a

Variable	Treatment			Control			<i>P</i> value
	Score	<i>n</i> value	SEM ^b	Score	<i>n</i> value	SEM _b	
Farrowing House	0.11	29	0.04	0.20	30	0.04	0.12
Nursery	0.19	29	0.05	0.22	30	0.06	0.08
Finishing Floor	1.58	49	0.17	1.67	31	0.19	0.10

^a Fecal consistency scale: 0 = normal, 1 = soft feces, 2 = thick fluid feces, 3 = watery feces.

^b Standard Error of the Mean.

A higher fecal consistency score indicates a loose stool and loose stool tends to indicate that there is some type of intestinal disturbance occurring. In the nursery, the treatment group tended ($P = 0.08$) to have lower fecal scores than the control group. Bhandari et al. (2008) found that the incidences of scouring were lower 24 hours after infection in the group of piglets who received the direct-fed microbial when compared to the other treatment groups. The group who received the direct-fed microbial had lower fecal scores than the control group, although the difference was not significant except for 24 hours after infection. Findings by Marquardt et al. (1999) are similar to findings in this study as well. The researchers found that at 21 days of age piglets treated with egg-yolk antibodies had lower fecal score consistencies except for 24 hours post-infection. De Cupere et al. (1992) also found no difference in fecal consistency between the control group and groups who received *Bacillus subtilis*, *Lactobacilli*, or *Streptococcus faecium*.

Escherichia coli. The number of *E. coli* present in the fecal samples between the groups was not different ($P > 0.05$) (Table 8). However, five days after the first treatment, the treatment piglets tended ($P = 0.09$) to excrete a higher number of *E. coli* in their feces, suggesting that something was occurring to cause more *E. coli* to be excreted in their fecal material. In order to maintain production levels, the sub-therapeutic levels antibiotics were not removed from the feed. By not removing the sub-therapeutic levels antibiotics, they

may have influenced the outcome of this study with regard to the number of *E. coli* present in the fecal material since antibiotics work to destroy harmful bacteria in the body.

Bhandari et al. (2008) found that there was no difference in the number of *E. coli* present in the intestinal mucosa when comparing diets that contained antibiotics, no antibiotics, direct-fed microbial with no antibiotics, spray-dried porcine plasma with no antibiotics, and a diet that contained spray-dried porcine plasma and direct-fed microbial with no antibiotics. Mathew et al. (1998) also found that the addition of yeast to the piglets' diet did not affect the number of *E. coli* present in the gastrointestinal tract. Shen et al. (2009) reported that the inclusion of either a yeast culture or antibiotic growth promoter did not affect the number of *E. coli* present in the rectum.

Table 8. Effect of Supplemental Probiotics and Spray-Dried Egg Proteins on the presence of *Escherichia coli* in fecal samples.

Variable	Treatment			Control			P value
	log CFUs ^b	n value	SEM ^a	log CFUs ^b	n value	SEM ^a	
48 hrs old	20.10	13	0.43	20.07	14	0.42	0.35
5 days post 1st trt	19.69	13	0.39	18.41	14	0.38	0.09
5 days post 2nd trt	8.150	13	2.14	7.850	14	2.10	0.25
5 days post 3rd trt	15.82	9	1.82	17.20	12	1.53	0.12

^a Standard Error of the Mean.

^b CFUs = colony forming units.

Lactobacillus species. The number of *Lactobacillus* species found in the feces of the piglets was not different ($P > 0.05$) between treatment and control groups (Table 9). There was a replication effect present for five days after the first ($P < 0.001$) and third ($P < 0.005$) treatment. The difference could be due to environmental conditions since the replications occurred in five different months. In addition, the number of *Lactobacillus* species present in fecal material may have been affected by the sub-therapeutic antibiotics left in the feed to maintain a certain production level.

Table 9. Effect of Supplemental Probiotics and Spray-Dried Egg Proteins on the presence of *Lactobacillus* species in fecal samples.

Variable	Treatment			Control			P value
	log CFUs ^c	n value	SEM ^b	log CFUs ^c	n value	SEM ^b	
48 hrs old	6.520	13	1.57	8.510	14	1.54	0.43
5 days post 1st trt ^a	12.23	13	0.46	11.79	14	0.45	0.49
5 days post 2nd trt	14.32	13	1.03	14.02	14	1.01	0.68
5 days post 3rd trt ^a	14.37	9	1.19	14.35	12	1.00	0.99

^a Replication effect ($P < 0.05$).

^b Standard Error of the Mean.

^c CFUs = colony forming units.

Bhandari et al. (2008) found no difference in the number of lactic acid bacteria present in the ileum after the inclusion of a direct-fed microbial. Shen et al. (2009) also found no difference in the number of *Lactobacilli* species present in the rectum between piglets who received a yeast culture or antibiotic growth promoter. Mathew et al. (1998)

found that the addition of yeast to the diet of piglets did not affect the number of *Lactobacillus* species present in the gastrointestinal tract. These results also concur with Pollmann et al. (1980), who found that the inclusion of a Probios or *Lactobacillus acidophilus* in the diet did not have a significant effect on the number of *Lactobacillus acidophilus* present in fecal counts between the treatment and control groups.

CONCLUSIONS

The probiotic supplement used in this study did not result in any significant differences in growth performance, fecal consistency, or number of *E. coli* and *Lactobacillus* species present in the fecal material between treatment and control groups. There was not enough of a difference in growth performance to warrant the continued use of this probiotic at the dosage and application method used in this project. Due to the fact that sub-therapeutic level antibiotics were not removed from the feed, this could have had confounding effects on the results of this study.

In the nursery, the treatment group tended ($P = 0.08$) to have lower fecal consistency scores than the control group; however, this is not seen in the farrowing house or finishing floor, and why it is not, cannot currently be explained. After the first treatment of probiotic, the treatment group tended ($P = 0.09$) to have more *E. coli* in their fecal material, although by what mechanism cannot be explained. Also, the number *E. coli* present in the fecal material could have been affected by the fact that both treatment and control piglets were housed together allowing for the ingestion of fecal material from both treatment and control piglets.

At the present time, there is no explanation for why treatment and control groups had nearly the same number of *Lactobacillus* species present in their fecal material, since the treatment piglets received several different *Lactobacillus* species on several different occasions when they received the treatment. Perhaps the *Lactobacillus* species used in this probiotic needed to be at a higher concentration in order for there to be a difference or different *Lactobacillus* species need to be used. Control piglets could have ingested feces from treatment piglets which may have affected the number of *Lactobacillus* species present in the fecal material since treatment and control piglets were housed together.

Recommendations for future research concerning the use of a probiotic include a higher dosage at treatment or replacement of antibiotics in the feed with a probiotic supplement.

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