

Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 10 *Swine Day (1968-2014)*

Article 220

1982

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Recommended Citation

Pollmann, D S.; Kennedy, G A.; Koch, B A.; and Allee, G L. (1982) "Influence of nonviable lactobacillus fermentation product in artificially reared pigs challenged with E. coli," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 10. <https://doi.org/10.4148/2378-5977.6060>

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Influence of nonviable lactobacillus fermentation product in artificially reared pigs challenged with E. coli

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K**S****U** Influence of Nonviable Lactobacillus Fermentation Product
in Artificially Reared Pigs Challenged with E. coli**U**D.S. Pollmann, G.A. Kennedy, B.A. Koch,
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Summary

Two trials were conducted to determine the influence of nonviable lactobacillus fermentation product (LFP) in artificially reared pigs removed from sows at 24 to 36 hours postpartum. The pigs were fed a non-medicated milk replacer for 21 days in individual cages in an environmentally controlled room. In Trial I, 5 levels (0, .25, .5, 1.0, and 2.0 ml per pig per day) of LFP were used to determine the dosage rate on growth, feed efficiency, mortality rate, white blood cell count, and hematocrit (8 pigs per treatment). No detectable dosage rate was observed in Trial I. In Trial II, a study was conducted to determine the effect of LFP on lactobacillus and coliform (E. coli) counts, histopathology of the small intestine, growth and blood parameters. When pigs were 14 days old they received an inoculum of either a broth containing E. coli (strain K88,91; approximately billion organisms for two days) or broth without E. coli. Pigs were fed three levels of the LFP at 0, .5, and 1.0 ml per day. Pigs were sacrificed five days and seven sections of gastrointestinal tract and feces were excised to enumerate lactobacillus and coliform populations. A dose rate of .5 ml per day increased gain ($P<.08$) and suppressed E. coli count in the stomach area without affecting lactobacillus populations. No differences were detected with the pathological evaluation. By challenging the pigs with E. coli, jejunum (section of the small intestine) coliform and white blood cell counts were increased ($P<.06$). These results suggest that lactobacillus fermentation product suppresses E. coli counts in the stomach and may improve gain in the artificially reared pig.

Introduction

The value of lactobacillus products (probiotics) in swine diets has been inconclusive. The theoretical concept of probiotics and basic research evaluating microbial cultures appear promising, but the results in the applied studies lack consistency. The proposed modes of action of lactobacillus are: 1) a decrease in pH in the digestive tract, 2) a suppression of intestinal E. coli populations, and 3) production of an antibiotic-like substance that could result in improvement in performance. The difficulty in maintaining a viable lactobacillus culture in swine feed may partially explain the inconsistency in research results. Therefore, the objective of this study was to evaluate the influence of nonviable lactobacillus fermentation (LFP) product (Culbac®) in artificially reared pigs.

^aWe acknowledge the financial support of TransAgra Corp., Memphis, Tn., that made these studies possible.

Experimental Procedures

Pigs were removed from sows at 24-36 hours of age and placed in an environmentally controlled room with temperatures maintained at 90-93°F. Pigs were placed in individual cages (1' x 2' x 1') made of welded wire, with $\frac{1}{4}$ " netting on the floor of the cages. Each cage was equipped with a plastic feeding cup with a 200 ml capacity. Pigs were allotted to the treatments by litter. Pigs were fed four times on day one, three times on day two, and twice a day throughout the trials. Pigs were fed a non-medicated milk replacer¹ mixed at the rate of four parts water to one part dried milk.

In Trial I five levels (0, .25, .5, 1.0, and 2.0 ml per day) of LFP were used to determine the dosage rate on growth, feed efficiency, mortality rate, white blood cell count, and hematocrit. Using eight pigs per treatment, they were bled at Day 10 and 21 of the trial. Urine samples were collected for analysis of para-cresol, which has been suggested to have an inverse relationship with gain and urinary para-cresol levels.

Trial II was conducted to determine the effect of LFP on lactobacillus and coliform counts, histopathology of the small intestine, growth and blood parameters. When pigs were 14 days old, they received inoculum either in a broth containing E. coli (strain K-88, 91; approximately a billion organisms for two days) or broth without E. coli. Pigs were fed three levels of LFP at 0, .5, and 1.0 ml per day. Six pigs from 8 litters were used in this experiment. Four pigs per treatment were sacrificed five days post-inoculation and seven sections of the gastrointestinal tract and feces were excised to enumerate lactobacillus and coliform populations. Two sites of the stomach were cardiac (non-glandular) and fundic (glandular) regions. Three sections of the small intestine were duodenum (150 mm from the pylorus posterior to bile and pancreatic ducts), the jejunum (1 m from the pylorus) and ileum (300 mm from the ileo-cecal junction). The entire cecum and a portion of the colon (100 mm anterior and posterior to the apex) were selected in the large intestine. In addition, a fecal sample was removed from the posterior colon.

A sample (approximately 1 gram) of tissue and ingesta were placed into 100 ml of sterile phosphate buffer in a dilution bottle. Samples were homogenized and diluted in 100-fold steps for enumeration of organisms using pour plate method. MRS agar and red bile agar for lactobacillus and coliforms, respectively, were used. Duplicate samples were incubated anaerobically at 37°C, 48 hours for lactobacillus and aerobically for 24 hours for coliforms. All petri dishes that had 30-300 colonies were counted. Data were expressed as log colony-forming unit (CFU) per gram of wet tissue. Sections of the small intestine were preserved to evaluate the effect of the treatments on histopathological criteria (crypt to villus ratio).

Pigs were bled for hematocrit and white blood cell count at 21 days post-treatment. Urine samples were collected to determine para-cresol concentration.

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Land O' Lakes product

Results and Discussion

The effect of LFP on mortality and period performance of artificially reared pigs is shown in Table 1. In this trial seven pigs died and all deaths were diagnosed as enterotoxemia (toxins produced by E. coli in the intestine). The level of LFP did not affect average daily gain nor feed conversion (dry matter intake basis). The effect of the treatments on hematocrit, white blood cell (WBC) count, and urinary para-cresol levels are shown in Table 2. Pigs receiving .25 ml of LFP had higher $P<.05$ hematocrit at ten days but at 21 days the level was lower ($P<.05$). The WBC count data closely parallels the hematocrit results. Para-cresol levels were the lowest with .5 ml level. Therefore, from Trial I, no detectable dosage rate was observed.

In the second trial, two pigs died and death confirmation was also enterotoxemia. A quadratic affect ($P<.08$) was observed on average daily gain with pigs receiving .5 ml level having the best gain (Table 3). The E. coli challenge did not affect average daily gain nor feed efficiency.

The hematocrit level was not effected by E. coli challenge or level of LFP (Table 4). Since WBC count was higher ($P<.06$) for the pigs challenged with E. coli, this suggests that the pigs are attempting to reduce the invasion of the foreign organism. Urinary para-cresol level was non-detectable for pigs on 1.0 ml in the unchallenged group.

The effect of the LFP on gastrointestinal lactobacillus populations are shown in Table 5. By challenging the pigs with E. coli, an increase ($P<.05$) in coliform counts was detectable. Pigs receiving the E. coli challenge had higher lactobacillus populations ($P<.05$) in the jejunum. The LFP did not affect lactobacillus populations.

By challenging the pigs with E. coli, jejunum coliform counts (Table 6) were increased ($P<.06$). The LFP tended to suppress E. coli populations in the stomach at .5 ml level. Therefore, a dose rate of .5 ml per day increased gessed ($P<.06$) E. coli count in the stomach area without affecting lactobacillus population. These results suggest that lactobacillus fermentation products suppresses E. coli count in the stomach and may improve gain in the artificially reared pigs.

Table 1. Effect of nonviable Lactobacillus Fermentation Product in Artificially Reared Pigs on Mortality and Performance (Trial I)^a

Item	Level, ml/day				
	0	.25	.5	1.0	2.0
No. died	1	0	2	1	3
21-day wt, lbs ^b	9.55	9.17	9.46	8.78	8.56
Avg. daily gain, lbs ^b	.31	.29	.30	.28	.27
Feed/gain ^c	.87	.87	.84	.91	1.00

^a8 pigs/treatment; removed at 24-36 hrs. of age; avg. birth wt., 3.0 lbs.

^bLitter difference ($P<.05$).

^cDry matter intake basis.

Table 2. Effect of Nonviable *Lactobacillus* Fermentation Product in Artificially Reared Pigs on Blood Parameters and Urine Para-cresol. (Trial I)

Item	Level, ml/day				
	0	.25	.5	1.0	2.0
Hematocrit, % ^a					
day 10 ^{bc}	41.1	45.4	42.2	38.4	41.4
day 21 ^{bc}	39.2	36.1	37.0	37.0	39.2
WBC, x 10 ^{3a}					
day 10 ^{bc}	20.8	27.9	22.8	12.5	19.4
day 21	15.5	14.7	17.2	15.2	13.2
P-cresol, µg/ml urine					
Free	14.7	4.0	1.9	3.7	21.1
Conjugated	26.3	17.5	11.1	15.8	47.8

^aPeriod difference (P<.05)^bLitter difference (P<.05)^cTreatment difference (P<.05)Table 3. Effect of Nonviable *Lactobacillus* Fermentation Product in Artificially Reared Pigs Challenged with *E. coli* on Mortality and Performance (Trial II)^a

Level, ml/day	Unchallenged			E.coli challenged ^b		
	0	.5	1.0	0	.5	1.0
No. died	1	0	0	0	1	0
Avg daily gain, lbs ^{cd}	.21	.24	.22	.22	.26	.20
Feed/gain ^e	1.07	1.06	1.02	1.20	.98	1.23

^a8 pigs/treatment; removed at 24-36 hrs. of age; avg. birth wt., 3.32 lbs.^bStrain K88, 91; appr. 10⁹ organisms for 2 days.^cLitter difference (P<.05).^dDose quadratic effect (P<.08).^eDry matter intake basis.

Table 4. Effect of Nonviable Lactobacillus Product in Artificially Reared Pigs Challenged with *E. coli* on Hematocrit, White Blood Cell Count, and Para-cresol Concentration (Trial II).

Level, ml/day	Unchallenged			E.coli challenged ^b		
	0	.5	1.0	0	.5	1.0
Hematocrit, %	37.4	39.6	38.6	40.2	37.2	37.6
WBC, $\times 10^3$ ^a	19.4	16.7	20.0	22.0	22.6	21.4
P-cresol, $\mu\text{g/ml}$ urine						
Free	12.5	12.1	0	1.35	30.2	40.8
Conjugated	28.9	20.9	9.15	24.4	51.2	34.8

^aChallenge effect ($P < .06$)

Table 5. Effect of Nonviable Lactobacillus Fermentation Product on Gastrointestinal Lactobacillus Population (Trial II)^a

Area	Level, ml/day	Unchallenged			E.coli challenged ^b		
		0	.5	1.0	0	.5	1.0
Stomach	Cardiac	6.61	7.46	8.08	7.52	6.56	8.04
	Fundic ^{bc}	6.90	6.29	6.53	6.92	7.45	7.52
Small intestine	Jejunum ^c	6.49	5.78	5.65	6.50	6.19	6.91
	Duodenum	5.29	5.82	5.78	5.81	6.87	6.16
	Ileum	7.31	7.06	7.77	8.04	7.09	7.06
Large intestine	Cecum ^b	8.85	9.13	9.54	7.62	8.02	8.57
	Colon ^{bc}	9.04	9.44	8.72	9.56	9.42	10.67
Feces		9.53	9.08	8.24	10.01	9.35	9.47

^aLog CFU/g wet material

^bChallenge difference ($P < .05$)

^cLitter difference ($P < .05$)

Table 6. Effect of Nonviable *Lactobacillus* Fermentation Product on Gastrointestinal Coliform Populations (Trial II)^a

Area	Level, ml/day	Unchallenged			E.coli challenged ^b		
		0	.5	1.0	0	.5	1.0
Stomach	Cardiac ^{bed}	4.37	3.50	4.67	4.89	3.7	4.58
	Fundic ^b	4.39	3.70	4.49	4.60	4.25	4.65
Small intestine	Jejunum ^c	3.60	3.61	4.02	5.52	4.48	5.49
	Duodenum	3.69	3.56	4.25	4.00	4.3	4.02
	Ileum	6.31	5.36	5.83	6.81	5.80	6.68
Large intestine	Cecum	7.07	5.46	4.48	6.09	7.00	6.05
	Colon	7.19	6.42	8.40	6.76	7.64	9.32
Feces		8.80	6.41	7.44	7.70	8.05	8.42

^aLog CFU/g of wet material^bLitter difference (P<.05)^cDose quadratic effect (P<.06)^dDose effect 0 vs 5ml (P<.05)^eChallenge effect (P<.05)