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INFLUENCE OF *LACTOBACILLUS ACIDOPHILUS* INOCULUM ON GNOTOBIOTIC AND CONVENTIONAL PIGS[1]

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Summary

Two trials were conducted to determine the effect of *L. acidophilus* (bacteria commonly found in probiotics) inoculum on gnotobiotic and conventional pigs. The localization and population levels of *L. acidophilus* (LA) in the gastrointestinal (GI) tract of 24 gnotobiotic pigs (12 treated and 12 control) were determined by microbiological techniques. Each of the treated pigs received a 10-ml inoculum (2×10^{11} viable cells/ml) of acidophilus culture (strain DDS 1) isolated from milk. The control pigs received a sterile deionized water inoculum. The inocula (10 ml) were given in milk daily for 3 days starting when the pigs were approximately 5 days old. At 3, 5, 7 and 9 days postinoculation, pigs were killed, and tissues were removed from seven locations in the GI tract. A 1-g sample of tissue and ingesta (contents) was homogenized and diluted for plate counts on MRS Agar. The treated group had higher LA populations ($P<.001$) than did the controls. The LA population remained relatively constant over the four periods for the treated group. The colonization did not appear to influence serum metabolites but did increase white blood cell counts ($P<.06$). In the treated group, the large intestine (cecum and colon) had higher LA populations ($P<.001$) than the stomach (cardiac and fundic regions) and small intestine (duodenum, jejunum and ileum). Fecal samples were cultured for determination of the correlation between fecal and tissue lactobacillus populations. The correlation coefficients were not significant, suggesting that fecal flora count is a poor indicator of GI flora. A second trial was conducted to determine the effects of LA inoculum on growth, *E. coli* and lactobacillus counts, hematology and serum protein levels in nursing, naturally farrowed pigs. When the pigs were 2 days of age, all litters (two control and two treated) were inoculated daily with either the LA culture (10 ml/day) or sterile water via stomach tubes for 3 days. The pigs were killed and the tissues excised and processed in the same way as in the trial with the gnotobiotic pigs. As the pigs grew older, lactobacillus populations increased linearly ($P<.05$) and *E. coli* populations decreased linearly ($P<.01$), regardless of treatment. The LA inoculum significantly increased the lactobacillus and coliform populations of the cardiac (nonglandular) region of the stomach. As with the gnotobiotic pigs, correlation coefficients between fecal and tissue flora were not significant for either lactobacilli or coliforms. Average daily gain was slightly ($P<.10$) reduced by the LA inoculum, and a slightly higher ($P<.10$) white blood cell count was observed for the conventional pigs.

(**Key Words:** Gnotobiotic Pigs, Probiotics, *L. acidophilus*, Colonization, Intestinal Flora.)

Introduction

In the establishment and maintenance of microbial populations in the digestive tract, some form of adhesion between the organism and the tissue wall is necessary. This association is commonly called colonization or implantation. It has been theorized (Parker, 1975) that for probiotics to be beneficial, colonization must occur.

Fuller and Brooker (1974) and Tortuero (1973) observed that lactobacillus colonization in the crop epithelium of newly hatched chicks

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TABLE 1. INFLUENCE OF ACIDOPHILUS CULTURE ON POPULATION AND LOCATION OF *L. ACIDOPHILUS* IN GNOTOBIOTIC PIGS[a]

Tissue[d]	Treatment	Days postinoculation[bc] 3	5	7	9	Avg
		Log colony forming units/g				
Cardiac	Control	3.95	4.73	4.09	4.51	4.29
	Treated	7.32	7.00	7.10	7.24	7.15
Fundic	Control	3.50	4.45	3.99	3.32	3.82
	Treated	5.63	6.41	7.17	6.04	6.31
Duodenum	Control	2.74	4.47	4.29	2.65	3.54
	Treated	6.31	6.42	7.18	6.28	6.54
Jejunum	Control	2.85	4.85	4.06	3.05	3.92
	Treated	6.54	6.64	7.14	6.68	6.75
Ileum	Control	2.87	4.46	3.55	3.01	3.47
	Treated	8.38	8.10	8.32	7.33	8.03
Cecum	Control	3.08	4.51	3.91	2.82	3.53
	Treated	10.38	10.09	9.23	10.24	9.99
Colon	Control	2.71	4.58	4.55	2.76	3.74
	Treated	11.41	10.01	11.30	11.41	11.03
Feces	Control	4.73	5.25	2.83	4.76	3.89
	Treated	11.46	10.18	10.73	11.65	11.01
Avg	Control	3.21	4.66	3.91	3.36	3.79
	Treated[e]	8.48	8.11	8.52	8.36	8.37

[a]Each observation represents three pigs.

[b]Time effect ($P<.001$).

[c]Quadratic time response ($P<.001$).

[d]Tissue effect ($P<.001$).

[e]Treatment effect ($P<.001$).

improved gains and feed efficiency. Morotomi *et al.* (1975) and Savage (1972), using germ-free rats, observed that lactobacilli were effective in suppressing other bacterial species. They noted that lactobacillus colonization occurred in the nonglandular portion of the stomach. Muralidhara *et al.* (1977) showed that *L. lactis* (isolated from human intestinal contents, concentrated and frozen) suppressed *E. coli* and suggested that lactobacillus colonization occurred in conventional pigs.

The objective of this study was to determine the effect of *L. acidophilus* inoculum on gnotobiotic and conventional pigs.

Experimental Procedure

In the first trial, gnotobiotic pigs were used to determine the extent and location of colonization of *L. acidophilus* (LA) in the gastrointestinal (GI) tract. The gnotobiotic pigs were derived by the closed hysterotomy (without general anesthesia) method described by Miniats and Jol (1978). After completion of the surgery, eight randomly selected piglets were placed in plastic germ-free isolators. The two isolators were constructed of stainless steel and surmounted with polyvinyl canopies, and were maintained under positive pressure. The isolators were divided into four compartments by removable stainless steel partitions. Each compartment had a wire mesh floor designed to facilitate separation of waste and prevent coprophagy. Air was sterilized by passage through a fiberglass filter and temperature was maintained at 32 C for the entire period. The piglets were fed about 50 ml of a canned milk diet[6] twice a day starting at about 12 hr after birth; the milk was fed in shallow, sterile, stainless steel troughs. At 5

[6]SPF Lab, Borden Co., 350 Madison Ave., New York.

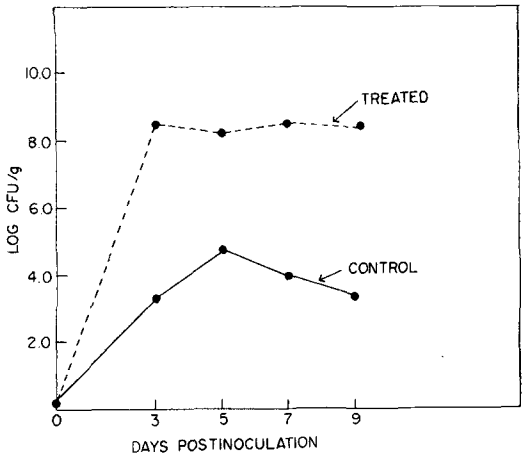

Figure 1. Influence of acidophilus culture on colonization of *L. Acidophilus* in gnotobiotic pigs.

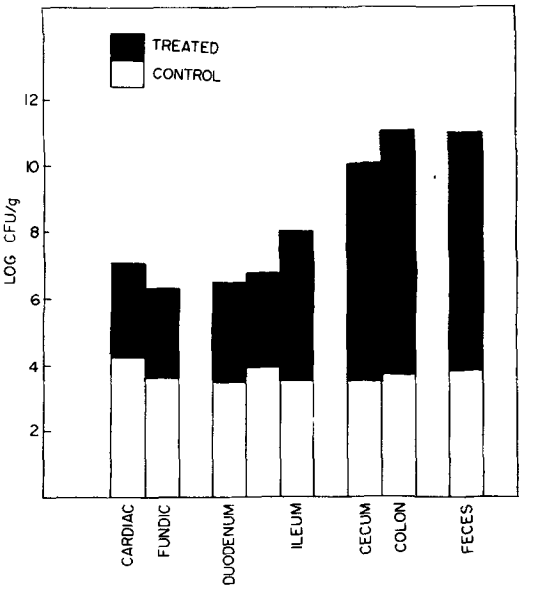

Figure 2. Influence of acidophilus culture on location and population of *L. Acidophilus* in gnotobiotic pigs.

days of age, one group of pigs (individually housed in one isolator) received a 10-ml inoculum (2×10^{11} viable cells/ml) of LA culture[7] (strain DDS 1), a frozen concentration that had been isolated from milk. The other four pigs, in the control isolator, received a sterile deionized water inoculum. The inocula were given in the milk once a day for 3 days. To reduce possible contamination of the isolators, the inocula, in capped test tubes, and the canned milk were transferred into the decontamination area and disinfected with 2% solution of peracetic acid. After 30 min, the containers were transferred from the decontamination area to the isolators.

At 3, 5, 7 and 9 days postinoculation, one pig was removed from each isolator. Approximately 12 hr after feeding, blood samples for cell counts and hematocrit were collected into evacuated glass tubes[8] that contained sodium fluoride to inhibit glycolysis and EDTA to prevent coagulation. Blood for serum protein and urea analyses were collected into evacuated glass tubes that contained sodium heparin. Cell counts and hematocrits were performed by conventional methods. After centrifugation, the sera were transferred to sterile plastic culture tubes, sealed and frozen (−10 C) until analyzed. Total serum protein was determined by the Lowry method with Folin-Ciocalteau reagent (Lowry *et al.*, 1951). Serum albumin was determined with a kit[9] by a bromcresol green colorimetric determination. Serum globulin was estimated as the difference between total protein and albumin. Urea nitrogen determinations were completed with an automated system as described by Frankel (1970).

After blood sampling, the pigs were killed

TABLE 2. FECAL-TISSUE CORRELATION COEFFICIENTS FOR GNOTOBIOTIC PIGS THAT RECEIVED *L. ACIDOPHILUS* CULTURE[a]

Independent variable	Correlation with feces
Cardiac	.29
Fundic	−.37
Duodenum	−.25
Jejunum	−.23
Ileum	.21
Cecum	−.02
Colon	.51

[a]Represents partial or residual correlation coefficients.

[7]Acidophilus Culture, Great Lakes Biochemical Co., Milwaukee, WI.

[8]Vacutainer tube, Becton-Dickinson, Rutherford, NJ.

[9]Sigma Technical Bull. No. 630, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO.

TABLE 3. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON HEMATOLOGY IN GNOTOBIOTIC PIGS

Parameter	Treatment	Days postinoculation 3	5	7	9	Avg
Hematocrit, %	Control	28.5	27.1	34.6	30.6	30.2
	Treated	30.8	31.2	34.8	32.8	32.4
Red blood cells, million/mm^3	Control	5.60	4.75	6.33	5.74	5.60
	Treated	5.06	5.70	6.25	5.99	5.75
White blood cells, thousand/mm^3	Control	4.42	4.67	5.07	4.00	4.64
	Treated	6.40	6.43	5.29	5.93	6.03*

*$P<.06$.

by electrocution, and an autopsy was performed to excise samples from seven predetermined locations along the GI tract for microbiological evaluation. The two sites of the stomach were the cardiac (nonglandular) and fundic (glandular) regions. The three sites of the small intestine were the duodenum (150 mm from the pylorus posterior to bile and pancreatic ducts), the jejunum (1 m from the pylorus) and the ileum (300 mm from ileocecal junction). The entire cecum and a portion of the colon (100 mm anterior and posterior to apex) were selected in the large intestine. In addition, a fecal sample was removed from the posterior colon for determination of the correlation between fecal and tissue flora.

A sample (approximately 1 g) of tissue and ingesta was placed in 100 ml of sterile phosphate buffer in a dilution bottle. The samples were homogenized with a Polytron[10] homogenator. The blade of the homogenator was rinsed twice, and rinse solution was poured into the dilution bottle. The blade of the homogenator was cleaned and flamed between each sample. The samples were diluted in 100-fold steps for enumeration of the organisms on pour plates with MRS agar. Duplicate samples were incubated anaerobically with a gas mixture[11] (10% H_2 and 90% CO_2) in an anaerobic incubator[12] for 48 hr at 37 C. All

[10] Polytron, Brinkmann Instruments, Westbury, New York.

[11] Anaerobic gas mixture, Union Carbide Corp., Linde Division, New York.

[12] Forma Scientific Anaerobic Incubator (Model 3159), Forma Scientific, Inc., Marietta, OH.

TABLE 4. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON BLOOD PROTEIN LEVELS IN GNOTOBIOTIC PIGS

Criterion[a]	Treatment	Days postinoculation 3	5	7	9	Avg
Total protein	Control	2.91	2.55	2.94	3.13	2.88
	Treated	2.65	2.79	2.58	3.15	2.79
Albumin	Control	1.42	1.10	1.88	2.23	1.66
	Treated	.99	1.53	1.77	2.00	1.57
Globulin	Control	1.49	1.45	1.06	.89	1.22
	Treated	1.67	1.25	.80	1.14	1.22
A:G ratio	Control	1.09	.78	2.28	2.36	1.63
	Treated	.60	1.56	2.52	1.87	1.64
Urea nitrogen	Control	21.7	28.7	12.6	16.8	19.9
	Treated	19.2	37.2	8.4	17.1	20.5

[a] Values for all criteria are millgrams/100 ml.

TABLE 5. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON LACTOBACILLUS COUNTS IN CONVENTIONAL PIGS[a]

Tissue[c]	Treatment	Days postinoculation[b] 0	3	5	7	9	Avg
		Log colony forming units/g					
Cardiac[d]	Control	8.78	8.57	8.26	8.41	9.04	8.61
	Treated	7.05	9.21	8.94	9.15	10.06	8.95
Fundic	Control	6.98	7.99	7.92	8.84	8.59	8.06
	Treated	6.88	7.07	8.01	7.95	9.38	7.86
Duodenum	Control	7.33	8.26	8.35	7.79	8.03	7.95
	Treated	6.48	8.35	8.28	8.65	8.12	7.98
Jejunum[e]	Control	7.12	6.94	7.95	7.96	5.56	7.10
	Treated	5.98	7.90	8.33	8.96	8.85	8.00
Ileum	Control	8.72	8.62	7.08	9.80	7.76	8.27
	Treated	6.82	8.84	7.96	8.19	8.35	8.03
Cecum	Control	9.73	10.35	10.54	11.40	11.42	10.69
	Treated	9.25	10.02	11.06	11.19	11.13	10.53
Colon	Control	10.24	10.68	11.27	11.32	11.37	10.97
	Treated	10.38	10.79	10.71	10.96	10.97	10.76
Feces	Control	9.86	10.30	10.43	9.96	10.44	10.20
	Treated	9.59	10.55	10.25	10.51	9.86	10.15
Avg	Control	8.52	8.96	8.97	9.44	9.03	8.98
	Treated	7.85	9.09	9.19	9.45	9.60	9.03

[a]Each observation represents two pigs.

[b]Linear effect ($P<.05$).

[c]Tissue effect ($P<.001$).

[d]Treatment effect ($P<.05$).

[e]Treatment effect ($P<.10$).

petri dishes that had 30 to 300 colonies were counted.

The experimental procedures were repeated for two more sows, with an additional pig from each litter killed without receiving the canned milk diet.

In the second trial, conventional pigs from four sows (two treated and two control) were placed in an environmentally regulated room with a cement wall divider designed to cross-contamination between the treatment groups. When the pigs reached 2 days of age, each litter was inoculated daily with either the LA culture (10 ml/pig/day) or sterile water via stomach tubes for 3 days. All pigs were weighed on the first inoculation date and at the time of sacrifice for determination of average daily gains. The procedure used for bleeding, killing and lactobacilli count was the same as that described for the trial with the gnotobiotic pigs. Coliforms were also counted, using violet red bile agar[13].

Data were analyzed by standard analysis procedures, and correlation coefficients were calculated by procedures and methods of Barr *et al.* (1976).

Results and Discussion

Gnotobiotic Pigs. The localization and population levels of *L. acidophilus* in the gnotobiotic pigs are summarized in table 1. The treated pigs had higher ($P<.001$) LA populations than the controls, and the population remained fairly constant over the four treatment periods for the treated group (figure 1). The control group showed an increase in lactobacilli after receiving the milk diet, but the levels were

[13] Difco Laboratories, Detroit, MI.

lower than those observed for the treated pigs. Microbiological counts were made on the canned milk product. MRS agar, which is selective for lactobacillus but not specific for *L. acidophilus*, was used. It is possible that contaminates from the milk caused a low level persistence of the organism but were insufficient for colonization. It is believed that the lactobacillus species in the milk may have been a thermophilic organism that is common in canned milk products. A few bulged milk cans were observed.

In the treated group, the large intestine had higher ($P<.001$) LA populations than the stomach and small intestine (figure 2), whereas in the control group, the populations were similar at all the locations. Since the ingesta were not separated from the tissue, the area of colonization could not be accurately defined. Morotomi *et al.* (1975) and Savage (1972) have reported that lactobacilli colonize in the keratinized cells of the nonglandular portion of the stomach. The microbial counts in the large intestine may have been confounded with organisms that had been sloughed off the epithelium of the stomach.

Many researchers have used fecal lactobacillus count as an indicator of lactobacillus population in the GI tract. The correlation coefficients between tissue and fecal flora are presented in table 2. From these data, it appears that fecal lactobacillus count is a poor indicator of tissue lactobacillus population.

L. acidophilus inoculum did not significantly affect the hematocrit and red blood cell counts

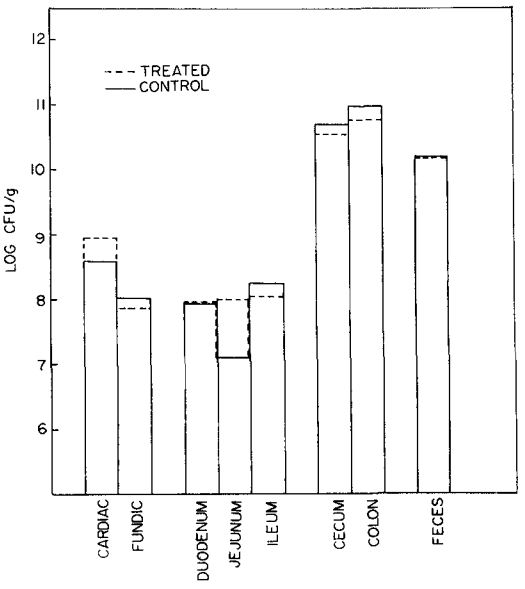

Figure 4. Influence of *L. Acidophilus* inoculum on location and population of lactobacilli in conventional pigs.

but did increase ($P<.06$) white blood cell counts (table 3). It is well recognized that normal microbial flora contributes to the defense of the hose. Abrams and Bishop (1965) showed that flora inocula given to germ-free mice increased leukocytic exudation, a process of great defensive importance to the host. Wagner (1959) inoculated monoflora rats with lactobacilli, which resulted in increased antibody formation. Since an increase in leukocytes was observed, lactobacilli may have a role in the immume response. As an additional

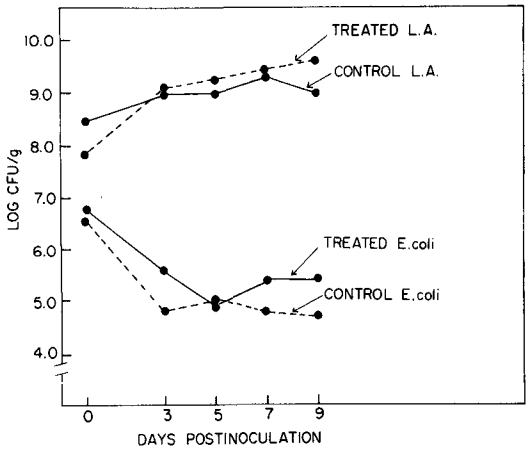

Figure 3. Influence of *L. Acidophilus* on lactobacillus and coliform counts in conventional pigs.

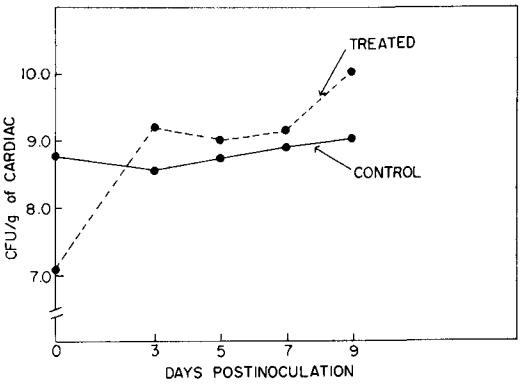

Figure 5. Influence of *L. Acidophilus* inoculum on lactobacillus populations in the cardiac portion of conventional pigs.

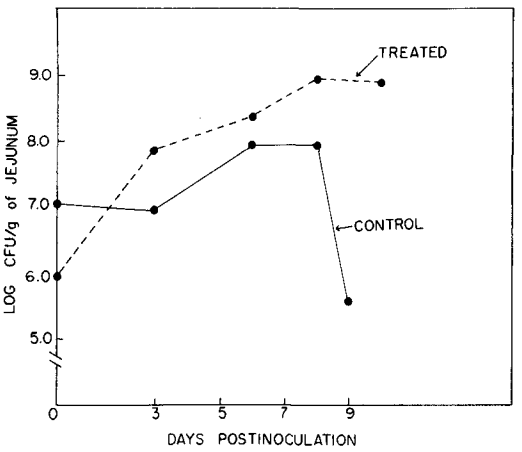

Figure 6. Influence of *L. Acidophilus* inoculum on lactobacillus populations in jejunum of conventional pigs.

indicator of the possible role of lactobacilli, serum proteins (albumin and globulin) and urea nitrogen were determined (table 4). The LA inoculum did not significantly affect the serum metabolites.

Conventional Pigs. It is evident from the gnotobiotic work that LA populations were maintained without significant change for up to 9 days postinoculation. These results raise the question of whether LA inoculum would colonize in the conventional pig. The data in table 5 support the contention that LA will, in fact, colonize in the gut of contaminated pigs. As the pigs grew older, lactobacillus populations increased ($P<.05$) linearly. Although the effect of LA inoculum averaged over the tissue locations was not significant, the log of colony-forming units was con-

TABLE 6. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON COLIFORM COUNTS IN CONVENTIONAL PIGS[a]

Tissue[d]	Treatment	Days postinoculation[bc] 0	3	5	7	9	Avg
		Log colony forming units/g					
Cardiac[e]	Control	4.09	2.38	3.22	3.30	2.98	3.19
	Treated	4.98	4.96	3.81	4.36	4.69	4.56
Fundic	Control	3.52	2.95	3.04	2.82	3.22	3.11
	Treated	4.72	3.80	3.73	3.58	3.92	3.95
Duodenum	Control	5.04	4.53	4.00	3.18	3.53	4.05
	Treated	4.15	4.82	3.80	4.37	3.38	4.10
Jejunum	Control	4.13	3.27	4.30	4.54	2.78	3.81
	Treated	4.79	4.13	4.59	4.61	4.97	4.62
Ileum	Control	7.00	5.93	4.35	6.20	5.64	5.82
	Treated	6.62	5.64	4.36	4.32	4.40	5.07
Cecum	Control	9.52	6.85	6.86	6.35	6.71	7.26
	Treated	9.53	7.05	5.92	8.01	6.95	7.49
Colon	Control	9.73	5.99	6.86	5.68	6.67	6.99
	Treated	9.58	6.52	5.79	6.61	6.86	7.07
Feces	Control	9.80	6.58	7.54	6.46	6.29	7.34
	Treated	9.77	7.49	7.17	7.25	8.06	7.50
Avg	Control	6.60	4.81	5.02	4.82	4.73	5.20
	Treated	6.78	5.55	4.90	5.39	5.40	5.60

[a]Each observation represents two pigs.

[b]Linear effect ($P<.01$).

[c]Quadratic effect ($P<.01$).

[d]Tissue effect ($P<.001$).

[e]Treatment effect ($P<.05$).

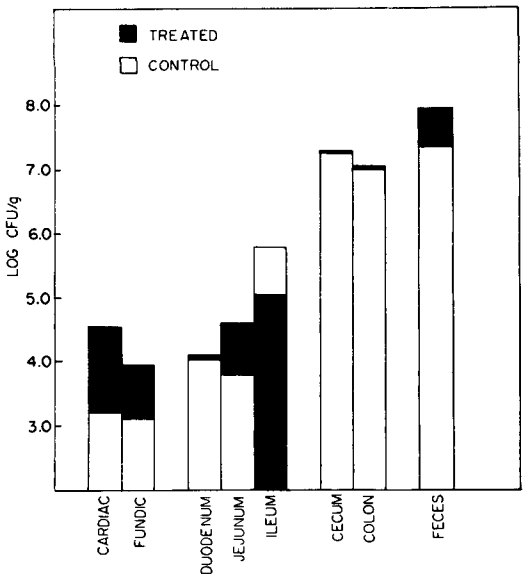

Figure 7. Influence of *L. Acidophilus* inoculum on location and population of coliforms in conventional pigs.

TABLE 7. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON PARTIAL CORRELATION COEFFICIENTS OF FECAL COUNTS WITH TISSUE COUNTS IN CONVENTIONAL PIGS

Organism	Independent variable	Correlation coefficient with feces[a]
Lactobacilli	Cardiac	.55
	Fundic	.07
	Duodenum	−.14
	Jejunum	.15
	Ileum	−.04
	Cecum	.52
	Colon	.50
Coliforms	Cardiac	−.39
	Fundic	−.46
	Duodenum	−.22
	Jejunum	.12
	Ileum	.47
	Cecum	.60
	Colon	.25

[a]Residual or partial correlation coefficients.

sistently higher for the treated group (figure 3). There was a difference ($P<.001$) in populations at the various locations along the tract (figure 4). As with the gnotobiotic pigs, the large intestine had the greatest population of lactobacilli. The inoculum significantly increased the lactobacillus populations of the cardiac (nonglandular) region of the stomach (figure 5). These results are in agreement with those reported by several researchers (Savage, 1969, 1971; Kunstyr, 1974; Kunstyr *et al.*, 1976; Morotomi *et al.*, 1975), who observed colonization in the nonglandular region of the stomach in rodents. The lactobacillus populations of the jejunum were also slightly affected ($P<.10$) by the inoculum (figure 6).

The effect of LA inoculum on *E. coli* populations is shown in table 6. As the pigs grew older, a linear and quadratic decrease ($P<.01$) in the coliform counts was observed. Several researchers (Muralidhara *et al.*, 1977; Sandine *et al.*, 1972; Mitchell and Kenworthy, 1976) have reported that the administration of lactobacilli suppressed *E. coli*, a normal and abundant bacterial inhabitant of the digestive tract. It has been suggested that *E. coli* are the major cause of scouring in swine (Sandine *et al.*, 1972). In this study, both lactobacillus and coliform counts were increased ($P<.05$) in the cardiac region of the stomach (figure 7), with no evidence of diarrhea.

As with the gnotobiotic pigs, the correlation coefficients between fecal and tissue flora were

TABLE 8. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON AVERAGE DAILY GAIN AND HEMATOLOGY IN CONVENTIONAL PIGS[a]

Item	Treatment	
	Control	Treated
ADG, g[b]	151	124
Hematocrit, %[c]	35.7	31.4
RBC, million/mm^3	5.14	4.76
WBC, thousand/mm^3[b]	13.3	17.6
Nucleated RBC, no./100 WBC	11.7	7.4
Smudge cells, no./100 WBC	22.3	26.4
Differential count, %		
Neutrophils	30.1	36.6
Bands[b]	19.2	16.4
Lymphocytes	49.2	46.4
Monocytes	.3	.1
Eosinophils	.7	.4
Basophils	.2	.1

[a]Each observation represents 10 pigs.

[b]$P<.10$.

[c]$P<.05$.

TABLE 9. INFLUENCE OF *L. ACIDOPHILUS* INOCULUM ON SERUM PROTEINS IN CONVENTIONAL PIGS[a]

Item[b]	Treatment	Days postinoculation					Avg
		0	3	5	7	9	
Urea nitrogen	Control	14.25	9.37	9.04	11.69	11.99	11.27
	Treated	34.15	16.06	15.07	13.01	13.34	18.32
Total protein	Control	3.69	3.29	3.28	3.55	4.38	3.64
	Treated	3.91	4.29	3.81	4.02	3.62	3.93
Albumin[c]	Control	1.58	2.13	2.49	2.75	3.11	2.41
	Treated	1.58	1.89	2.28	2.68	2.79	2.24
Globulin[cd]	Control	2.11	1.17	.80	.80	1.27	1.23
	Treated	2.34	2.40	1.54	1.35	.82	1.69
A:G ratio[d]	Control	.75	1.96	3.14	4.36	2.46	1.96
	Treated	.72	.79	1.33	2.01	3.77	1.33

[a]Each observation represents two pigs.

[b]All values listed are milligrams/100 ml.

[c]Quadratic effect ($P<.05$).

[d]Linear effect ($P<.05$).

not significant for lactobacilli and coliforms (table 7). Therefore, the validity of fecal flora as an indicator of microbial population of the intestinal tract is questionable.

Average daily gains were slightly reduced ($P<.10$) by the lactobacillus inoculum (table 8). The LA-inoculated group had a lower ($P<.10$) hematocrit with slightly higher white blood cell counts. The other hematological parameters, serum proteins and urea nitrogen (table 9) were not significantly affected by the LA inoculum.

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