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# EFFECT OF *LACTOBACILLUS ACIDOPHILUS* ON STARTER PIGS FED A DIET SUPPLEMENTED WITH LACTOSE[1]

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## Summary

Seventy-two crossbred pigs (average initial weight 6 kg) were used to study the effect of *L. acidophilus* (lactic acid-producing bacteria common in probiotics) in starter diets on gain, feed conversion, fecal lactobacillus and coliform counts, hematology and serum proteins. The nonmedicated experimental diets were corn-soybean meal (18% crude protein) diets with two levels of lactose (0 and 10%). It has been theorized that lactose is necessary for lactobacilli to colonize in the digestive tract. One-half of the pigs received a 10-ml inoculum daily for 2 weeks via stomach tubes. Two inocula were used: water (control) and *L. acidophilus* (LA) culture strain DDS 1 ($2 \times 10^{11}$ viable cells/ml). The pigs that did not receive an inoculum were assigned to a diet with or without Probios ($4 \times 10^6$ viable cells/g), a LA feed additive. The experimental treatments were: (1) control, (2) Probios, (3) water inoculum, (4) acidophilus inoculum, (5) lactose + Probios, (7) lactose + water inoculum and (8) lactose + acidophilus inoculum. Gain and feed conversion were not significantly affected by treatments. There was a trend toward improved average daily gain (ADG) with lactose and Probios. The water inoculation reduced ADG by 14.4% in relation to the performance of pigs receiving the other diets. This reduction in ADG appeared to be related to the extrinsic stress caused by the inoculation process. Also, the stress of the inoculation affected fecal flora by reducing lactobacilli ($P<.05$) and increasing coliforms ($P<.05$). *L. acidophilus* inoculum improved ADG (11.0%) and feed conversion (1.5%). Although the probiotic products tended to improve ADG (7.2%), LA therapy may have been a function of the dietary carbohydrate source. The LA inoculum increased ($P<.05$) fecal lactobacillus counts. After the daily inocula were discontinued, an increase in lactobacilli was observed for the water-inoculated group, and a decrease in lactobacilli was noted for the microbe-inoculated group. Pigs receiving lactose in combination with the lactobacillus inoculum had the highest lactobacillus counts and the best ADG. Probios did not affect ($P>.05$) fecal lactobacillus counts. Neither *L. acidophilus* inoculum nor Probios was effective ($P>.05$) in suppressing *E. coli*. Lactobacillus treatments did not affect ($P>.05$) red or white blood cell counts, serum proteins (albumin and globulin) or blood urea nitrogen.

**(Key Words:** Swine, Probiotics, *L. acidophilus*, Probios, Lactose.)

## Introduction

It has been shown that weaning can cause changes in gastric function that accelerate the growth of *E. coli*, a normal bacterial inhabitant of the digestive tract that increases in numbers during diarrhea (Schulman, 1973). Several researchers (Muralidhara *et al.*, 1977; Mitchell and Kenworthy, 1976; Hill *et al.*, 1970a,b; Porter and Kenworthy, 1969; Moon, 1975) have shown that lactobacilli suppress hemolytic coliforms, which may be an integral part of postweaning lag, commonly observed in nurseries in swine production.

Lactobacillus therapy has been shown to help improve gain and feed efficiency of poultry (Tortuero, 1973; Fuller and Brooker, 1974) and swine (Parker, 1975; Baird, 1977; Hale and Newton, 1979). Other researchers (Hines and Koch, 1971; Mahan and Newland, 1976; Cline *et al.*, 1976; Holden, 1976) have observed no response in swine, and the value of lactobacillus products has not been fully elucidated. Gordon *et al.* (1957) sugggested

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that the efficacy of the product may be related to the extent of viability of concentrate or dried preparation of lactobacilli. Donaldson (1964) and Hawley *et al.* (1959) suggested that large quantities of lactose are necessary for lactobacilli to establish themselves in the gut.

The objective of this study was to determine the effect of viable cultures of LA in lactose-supplemented starter diets on performance, fecal lactobacillus and coliform counts, hematology and serum proteins of young pigs.

#### Experimental Procedure

Seventy-two crossbred pigs (average initial weight 6 kg) were blocked on the basis of initial weight and then allotted at random to eight dietary treatments in a 2 × 4 factorial arrangement of treatments. The pigs were housed in 24 pens (three animals per pen) in an environmentally regulated nursery. Three pens were assigned to each dietary treatment.

The nonmedicated experimental diets (table 1) were corn-soybean meal (18% crude protein) diets with two levels of lactose (0 and 10%). Lactose was substituted in the diet for an equal weight of starch. One-half of the pigs received a 10-ml inoculum daily for 2 weeks via stomach tubes. Two inocula were used: water and a commercial LA culture[3] (strain DDS 1), which was isolated originally from milk products. The frozen acidophilus culture was thawed in lukewarm water for 1 hr prior to inoculation. The lactobacillus population of the culture was determined to have approximately $2 \times 10^{11}$ viable cells/ml. The water inoculation was given to equalize across treatments the amount of stress due to the inoculation procedure. The pigs that did not receive an inoculum were assigned to a diet with or without Probios[4]. The lactobacillus population of Probios, as determined with LBS agar[5], a medium selective for *L. acidophilus*, was $4 \times 10^6$ viable cells/g of Probios. Probios was stored in a refrigerator before being added to the basal diets on a biweekly basis. The experimental treatments were: (1) control, (2) Probios, (3) water inoculum, (4) acidophilus inoculum, (5) lactose, (6) lactose + Probios, (7) lactose + water inoculum and (8) lactose + acidophilus inoculum.

TABLE 1. COMPOSITION OF DIETS

| Ingredient, % | Internat'l. Ref. No. | % Lactose[a] 0 | % Lactose[a] 10 |
|---|---|---|---|
| Corn, yellow, ground | 4-02-931 | 44.65 | 44.65 |
| Soybean meal | 5-04-604 | 29.02 | 29.02 |
| Oats, ground | 4-03-309 | 10.00 | 10.00 |
| Starch | | 10.00 | . . . |
| Lactose | | . . . | 10.00 |
| Lard | 4-00-409 | 2.50 | 2.50 |
| Dicalcium phosphate | 6-01-080 | 1.63 | 1.63 |
| Limestone, ground | 6-02-632 | .60 | .60 |
| Premix[b] | | 1.60 | 1.60 |

[a]Calculated analysis: crude protein, 18%; lysine, .99%; Ca, .70%; P, .65%.

[b]Premix consisted of .5% iodized salt, .1% trace mineral mix and 1.0% vitamin mix in a finely ground corn carrier. Vitamin mix provided (per kg) diet: vitamin A, 3,307 IU; vitamin $D_3$, 441 IU; vitamin E, 22 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 2.2 mg; d-pantothenic acid, 13.2 mg; niacin, 17.6 mg; choline chloride, 110.2 mg; vitamin $B_{12}$, 22 mcg; ethoxyquin, 4.4 mg. Trace mineral mix provided (milligrams/kilogram) of diet: Zn, 100; Fe, 50; Mn, 27.5; Cu, 5; Co, .5; I, .75.

Pigs were weighed and feed intake was recorded weekly for 28 days. Blood samples were taken from each pig 0, 4, 7 and 14 days after the initiation of the trial for red and white blood cell counts. Blood was collected into evacuated glass tubes[6] containing sodium fluoride and EDTA. Blood cell counts were performed by conventional methods. Blood samples were also obtained 7 and 21 days after initiation of the study for determination of urea nitrogen and serum proteins (albumin and globulin). After centrifugation, the serum was transferred to sterile plastic culture tubes, sealed and frozen at −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 by a colorimetric procedure[7] available commercially. Serum globulin was estimated as the difference between total protein and albumin. Urea nitrogen determinations were completed with an automated system described by Frankel (1970).

[3]Acidophilus culture, Great Lakes Biochemical Co., Milwaukee, WI.

[4]NuLabs Division, Pioneer Hi-Bred International, Portland, OR.

[5]LBS agar, BBL, Division of Becton-Dickinson Co., Cockeysville, MD.

[6]Vacutainer tubes, Becton-Dickinson, Rutherford, NJ.

[7]Sigma Tech. Bull. No. 630, Sigma Chemical Co., St. Louis, MO.

At the end of each week, a fresh fecal sample was taken from each pig and placed in a sterile plastic bag. The samples were pooled by pen and an aliquot of approximately 1 g was placed in 100 ml of sterile phosphate buffer. The samples were diluted in 100-fold steps for enumeration of LA (LBS agar) and coliforms (VRBA[8]) by the pour plate method. LBS plates were incubated for 72 hr at 35 C in an anaerobic incubator with $CO_2$, and VRBA plates were incubated aerobically for 24 hr at 35 C using duplicate samples. Petri dishes that had 30 to 300 colonies were counted.

Data were analyzed by standard analysis of variance procedures (Barr *et al.*, 1976). Orthogonal contrasts were used to compare treatment means, and correlation coefficients were calculated to compare parameters (Snedecor and Cochran, 1967).

#### Results and Discussion

Gains and feed conversion were not significantly affected by treatments (table 2). There was a trend toward improved average daily gain (ADG) with lactose and Probios. The water inoculation reduced ADG by 14.4%. Reduction in ADG appeared to be related to the extrinsic stress caused by handling of the pigs for the daily inoculation. The LA inoculum improved ADG (11.0%) and feed conversion (FC) (1.5%). The dried probiotic (Probios) appeared to increase ADG slightly over that for the acidophilus-inoculated group (.214 *vs* .201 kg). However, the greatest gain was observed (.225 kg) when acidophilus inoculum was given in combination with the 10% lactose diet. It appeared that the LA activity was dependent upon the dietary carbohydrate source.

The effect of LA products on fecal lactobacilli and coliforms is summarized in table 3. The water inoculum decreased ($P<.05$) lactobacillus counts and increased ($P<.05$) coliform counts. Several researchers (Tannock and Savage, 1974; Kenworthy and Crabb, 1963; Schulman, 1973) have observed decreased lactobacilli and increased coliforms when animals have been subjected to adverse dietary and environmental conditions, such as weaning, diet change and transporting. The alternating of fecal flora may explain the 14% decrease in gain due to the inoculation procedure.

The LA inoculum increased ($P<.05$) fecal lactobacillus counts. While the pigs were receiving the daily acidophilus inoculum, their lactobacillus counts were substantially higher than those for the water-inoculated group (figure 1). When the daily inocula were terminated, an increase in lactobacilli was observed for the control group, while lactobacillus counts for the treated animals decreased.

[8] Violet Red Bile Agar, Difco Laboratories, Detroit, MI.

TABLE 2. EFFECT OF *L. ACIDOPHILUS* ON GAINS AND FEED CONVERSION OF BABY PIGS FED A STARTER DIET SUPPLEMENTED WITH LACTOSE[a]

| Item | Lactose, % | | Treatment: No inoculum None | Treatment: No inoculum Probios[b] | Treatment: Inoculated Water | Treatment: Inoculated Acidophilus[c] | Avg |
|---|---|---|---|---|---|---|---|
| ADG, kg | 0 | | .199 | .215 | .195 | .176 | .196 |
| | 10 | | .214 | .213 | .166 | .225 | .205 |
| | | Avg | .207 | .214 | .181 | .201 | |
| FC | 0 | | 2.16 | 2.09 | 1.88 | 1.92 | 2.01 |
| | 10 | | 1.91 | 2.04 | 2.07 | 1.98 | 2.00 |
| | | Avg | 2.04 | 2.07 | 1.98 | 1.95 | |

[a] 72 crossbred pigs (three pigs per treatment; three replications). Average initial weight, 6 kilograms. Length of study, 28 days.

[b] Lactobacillus product (NuLabs); use rate = 1 g/kg diet; $4 \times 10^6$ organisms/g.

[c] *L. acidophilus* culture (Great Lakes Biochemicals) strain DDS 1; received 10 ml daily for 2 weeks ($2 \times 10^{11}$ organisms/ml).

TABLE 3. EFFECT OF *L. ACIDOPHILUS* ON FECAL COUNTS OF BABY PIGS FED A STARTER DIET SUPPLEMENTED WITH LACTOSE[a]

| Item | Lactose, % | | Treatment: No inoculum None | No inoculum Probios[b] | Inoculated Water | Inoculated Acidophilus[c] | Avg |
|---|---|---|---|---|---|---|---|
| | | | Log CFU/g of feces[d] | | | | |
| *L. acidophilus*[efg] | 0 | | 8.08 | 8.05 | 7.43 | 7.83 | 7.85 |
| | 10 | | 8.04 | 8.31 | 7.60 | 8.16 | 8.03 |
| | | Avg | 8.06 | 8.18 | 7.52 | 8.00 | |
| Coliforms[e] | 0 | | 7.46 | 7.74 | 8.03 | 7.97 | 7.80 |
| | 10 | | 7.38 | 7.79 | 7.80 | 8.10 | 7.77 |
| | | Avg | 7.42 | 7.77 | 7.92 | 8.04 | |

[a]Each value represents 36 observations (four time periods, three pens, three pigs per pen).

[b]Lactobacillus product (NuLabs); use rate = 1 g/kg diet; fed culture continuously.

[c]*L. acidophilus* (Great Lakes Biochemicals) strain DDS 1; received 10 ml daily for 2 weeks.

[d]CFU = colony forming units.

[e]No inoculum versus water inoculum ($P<.05$).

[f]Acidophilus inoculum versus water inoculum ($P<.05$).

[g]Probios + acidophilus inoculum versus no probios + water inoculum ($P<.05$).

After termination of daily inocula, the treated group had a slightly higher LA population, which might suggest possible colonization in the digestive tract.

The validity of fecal lactobacillus counts as an indicator of colonization in the tract is questionable. Pollmann *et al.* (1980), in a study of both gnotobiotic and conventional pigs, found no significant correlations between seven digestive tract locations (stomach, small and

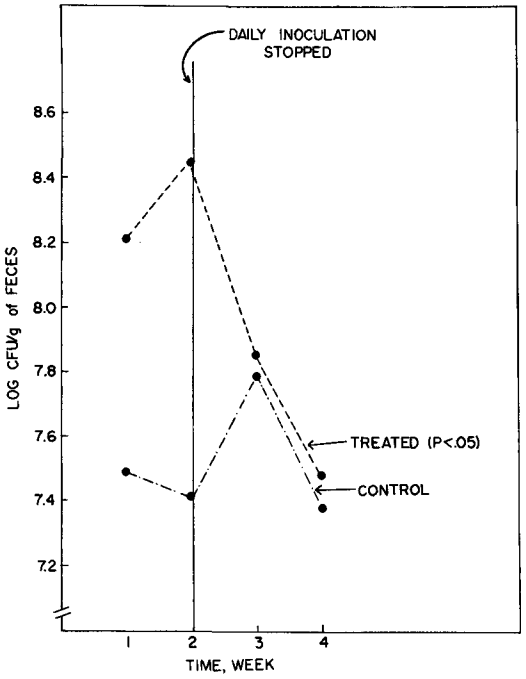


Figure 1. Effect of *L. Acidophilus* inoculum on lactobacillus fecal counts of starter pigs.

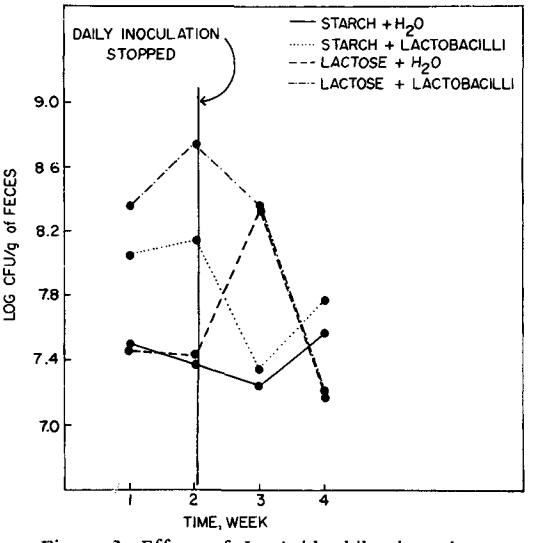


Figure 2. Effect of *L. Acidophilus* inoculum on fecal lactobacillus counts of starter pigs fed a diet supplemented with lactose.

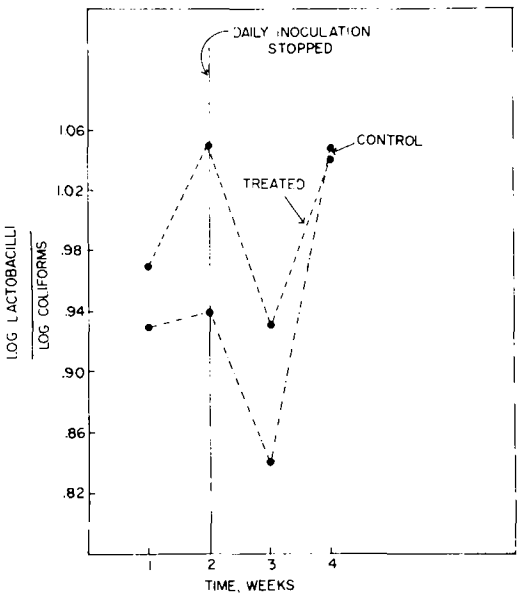


Figure 3. Effect of *L. Acidophilus* inoculum in starter pigs on coliform fecal counts.

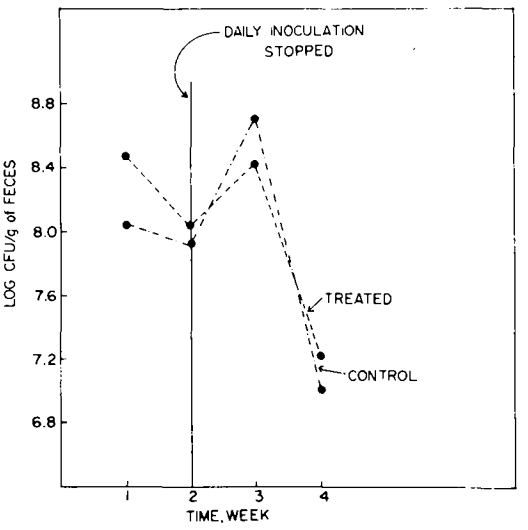


Figure 4. Effect of *L. Acidophilus* inoculum in starter pigs on lactobacillus to coliform ratio.

large intestine) and fecal lactobacilli or coliforms.

The possible effect of lactose on fecal lactobacillus counts is illustrated in figure 2. Donaldson (1964) and Hawley *et al.* (1959) suggested that large quantities of lactose are necessary for lactobacilli to become established in the gut. It is evident (figure 2) that pigs receiving lactose in combination with the lactobacillus inoculum had the highest lactobacillus counts. After the daily inocula were discontinued, both groups of animals receiving lactose treatments had similar lactobacillus counts.

The LA inoculum was not effective ($P>.05$) in suppressing *E. coli* (figure 3), contrary to results reported by several researchers (Muralidhara *et al.*, 1977; Mitchell and Kenworthy, 1976; Hill *et al.*, 1970a,b; Porter and Kenworthy, 1969). A negative correlation ($r = -.51$) between ADG and coliform counts ($P<.05$) was observed, which suggests possible hazards of an increase in coliform population. Muralidhara *et al.* (1977) proposed a relationship between lactobacilli (L) and coliforms (C) by expressing the data as a lactobacilli to coliform ratio (L:C). He suggested that the higher the L:C ratio is, the better the microflora contribution to growth performance of host. The L:C ratio (figure 4) was higher for

TABLE 4. EFFECT OF *L. ACIDOPHILUS* ON HEMATOLOGY OF BABY PIGS FED A STARTER DIET SUPPLEMENTED WITH LACTOSE[a]

| Item | Lactose, % | | Treatment: No inoculum None | Treatment: No inoculum Probios | Treatment: Inoculated Water | Treatment: Inoculated Acidophilus | Avg |
|---|---|---|---|---|---|---|---|
| RBC[b], $10^6/mm^3$ | 0 | | 5.75 | 5.71 | 5.98 | 5.89 | 5.83 |
| | 10 | | 6.02 | 5.73 | 5.56 | 5.71 | 5.76 |
| | | Avg | 5.89 | 5.72 | 5.77 | 5.80 | |
| WBC, $10^3/mm^3$ | 0 | | 15.79 | 16.67 | 15.44 | 19.69 | 16.90 |
| | 10 | | 17.69 | 20.56 | 20.89 | 14.92 | 18.03 |
| | | Avg | 16.74 | 18.62 | 18.17 | 17.31 | |

[a]Each value represents 36 observations (four time periods, three pens, three pigs per pen).

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

TABLE 5. EFFECT OF *L. ACIDOPHILUS* ON SERUM PROTEINS OF BABY PIGS FED A STARTER DIET SUPPLEMENTED WITH LACTOSE[a]

| Item[b] | Lactose, % | | Treatment | | | | Avg |
|---|---|---|---|---|---|---|---|
| | | | No inoculum | | Inoculated | | |
| | | | None | Probios | Water | Acidophilus | |
| Urea nitrogen | 0 | | 12.78 | 13.13 | 12.57 | 12.58 | 12.77 |
| | 10 | | 12.70 | 12.10 | 11.72 | 11.92 | 12.11 |
| | | Avg | 12.74 | 12.62 | 12.15 | 12.25 | |
| Total protein | 0 | | 5.69 | 5.34 | 5.48 | 4.95 | 5.37 |
| | 10 | | 4.47 | 4.58 | 5.53 | 5.10 | 4.92 |
| | | Avg | 5.08 | 4.96 | 5.51 | 5.03 | |
| Albumin[c] | 0 | | 2.55 | 2.66 | 2.78 | 2.66 | 2.66 |
| | 10 | | 2.69 | 2.60 | 2.73 | 2.81 | 2.71 |
| | | Avg | 2.62 | 2.63 | 2.76 | 2.74 | |
| Globulin[c] | 0 | | 3.14 | 2.68 | 2.70 | 2.29 | 2.70 |
| | 10 | | 2.07 | 1.99 | 2.81 | 2.29 | 2.29 |
| | | Avg | 2.61 | 2.34 | 2.76 | 2.29 | |

[a]Each value represents 18 observations (two time periods, three pens, three pigs per pen).

[b]Milligrams/100 milliliters.

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

the acidophilus-inoculated group than for the water-inoculated group. Two weeks after termination of the daily inocula, the L:C ratios were similar for the treated and control groups. These data suggest that a daily source of lactobacilli is necessary to maintain increased fecal lactobacilli and higher L:C ratios.

Although the Probios did not significantly affect fecal lactobacillus or coliform counts, a slight increase in lactobacilli (8.05 *vs* 8.31) was observed in the lactose-supplemented group. Probios also tended to increase fecal coliforms (7.42 *vs* 7.77). This observation conflicts with the report of Moon (1975), who found that Probios was effective in suppressing *E. coli*.

The lactobacillus treatments did not significantly affect hematology (table 4). As expected, red blood cell counts increased ($P<.001$) linearly as the pigs matured, but no dietary effects were observed. White blood cells were not significantly affected. Wagner (1959) and Pollmann *et al.* (1980) observed increased leukocytic activity in gnotobiotic animals inoculated with lactobacilli, which suggests that lactobacilli may be involved in the immune response of conventional animals as well as gnotobiotes.

Serum proteins and urea nitrogen were not affected ($P<.05$) by dietary treatment (table 5). A time effect was observed ($P<.01$) for albumin and globulin regardless of treatment. Albumin levels were higher ($P<.01$) at the first bleeding than at the second, while globulin levels were higher ($P<.01$) at the second bleeding than at the first. Albumin to globulin ratios were near normal. Significant correlations were observed between serum albumin levels and ADG ($r = .54$) and between serum globulin levels and FC ($r = .51$).

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