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Effects of Lactose and Co-dried Milk-Yeast on Growth Performance and Gastrointestinal Health of Nursery Pigs

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Effects of Lactose and Co-dried Milk-Yeast on Growth Performance and Gastrointestinal Health of Nursery Pigs

Lactose may affect the immune response mediated from the gut and interact with gastrointestinal microbiota; however no effects of milk-yeast on growth performance were detected.

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Summary

An experiment was conducted to evaluate the effects of dietary lactose alone or in combination with dried milk-yeast product on growth performance, gastrointestinal microbiota, and immune parameters in weanling pigs. Pigs fed lactose and lactose with milk-yeast tended (P = 0.07) to have greater BW compared to control pigs (19.56 and 19.60 vs. 18.55 lb) at the end of phase 1 (week 1 to 2); however, no differences in BW were observed during phase 2 (week 3 to 4), phase 3 (week 5), or overall (week 1 to 5). With respect to growth performance, pigs fed lactose and lactose plus milk-yeast had greater (P = 0.05) ADG, and tended (P = 0.07) to have greater ADFI compared to control pigs during phase 1. There were no differences observed for ADG or ADFI during phase 2, 3, or the overall experimental period. With respect to immune parameters, a main effect of treatment was observed for circulating immunoglobulin (Ig)A where control pigs had greater (P < 0.01) concentrations of IgA compared to pigs fed lactose with or without milk-yeast; however, no effects of dietary treatment were observed for circulating IgG or tumor necrosis factor alpha. Lastly, fecal microbiota of control pigs had a greater microbial diversity index (Shannon's, P = 0.03) compared to pigs fed lactose plus milk-yeast on day 0; however, no differences in microbial diversity indices were observed on days 7 or 14 among dietary treatments. In addition, a shift in microbial composition, limited to a small number of microbial groups, was observed on day 7 with lactose fed pigs having greater (P = 0.05) putative L. jonhsonii *staining intensity compared to control pigs and pigs fed lactose plus milk-yeast. On day 14,* L. reuteri *tended (P = 0.15) to be enhanced, and* L. delbrueckii *was virtually eliminated (P = 0.04) by feeding lactose with or without milk-yeast. This research indicates that growth performance, immune parameters, and composition of the fecal microbiota may be affected by dietary inclusion of lactose alone or in combination with milk-yeast.*

Introduction

Stressors at weaning (including dietary, environmental, and social stressors) lead to the reduction of feed intake, nutrient absorption (due to villous atrophy in conjunction with a greater incidence of diarrhea), and consequently, decrease the overall growth performance of weaned pigs. Feeding lactose has been shown to increase feed intake and feed efficiency in weanling pigs. Although inconsistent results exist, there is evidence that dietary lactose may be a potential prebiotic for nursery pigs and may have a positive effect on modulating gut microbiota. In the gastrointestinal tract (GIT), lactose is fermented to lactic acid by lactic acid-producing bacteria (resulting in a decrease in pH) which is not favorable for pathogenic bacteria but may promote the proliferation of commensal bacteria. Furthermore, the inclusion of lactose may increase the production of short-chain fatty acids, particularly butyric acid, which are important sources of energy for gut epithelial cells. In addition, supplementation of yeast culture or live yeast in nursery diets has resulted in positive effects on growth performance and gut health of pigs. Previous research showed that pigs fed yeast had increased beneficial bacteria (i.e., lactobacilli) and reduced coliform bacteria in the small intestine. Yeast mixtures contain a variety of active components such as enzymes, hormones, nucleic acids, and cell wall products (e.g., mannanoligosaccharides and beta glucans) that may be beneficial to the host.

There has been considerable focus on research evaluating the effects of lactose and yeast individually; however, the effect of feeding lactose in combination with milk-yeast on the growth performance and gastrointestinal health of nursery pigs has not been investigated. Therefore, the objective of the current study was to determine effect of lactose and lactose in combination with milk-yeast on growth performance, gastrointestinal microbiota, and immune parameters of nursery pigs.

Table 1. Composition of experimental diets (as-fed basis).

	Phase 1			Phase 2			Phase 3		
Treatments[1]	A	B	C	A	B	C	A	B	C
Lactose, %	0	20	20	0	15	15	0	5	5
Milk-yeast, %	0	0	5	0	0	5	0	0	5
Ingredient, %									
Corn	61.500	37.085	33.408	61.460	43.140	39.510	58.760	52.640	48.968
Soybean meal, 46.5% CP	20.5	20.5	20.5	22	22	22	28.75	28.75	28.75
Spray-dried porcine plasma	5	5	5	2.5	2.5	2.5	0	0	0
Select menhaden fish meal	6	6	6	7.5	7.5	7.5	6	6	6
DairyLac 80	0	25	23.913	0	18.750	17.600	0	6.250	5.163
Dicalcium phosphate, 18.5% P	1.325	0.500	0.295	0.750	0.150	0.000	0.900	0.700	0.500
Limestone	0.250	0.400	0.475	0.300	0.390	0.430	0.300	0.350	0.420
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4
Zinc oxide	0.3	0.3	0.3	0.3	0.3	0.3	0	0	0
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine•HCl	0.210	0.210	0.080	0.265	0.275	0.145	0.255	0.260	0.130
DL-Methionine	0.120	0.180	0.195	0.115	0.160	0.170	0.125	0.130	0.145
L-Threonine	0.095	0.125	0.135	0.110	0.135	0.145	0.110	0.120	0.125
Mecadox 2.5	1	1	1	1	1	1	1	1	1
Corn oil	3	3	3	3	3	3	3	3	3
Milk-yeast	0	0	5	0	0	5	0	0	5

[1]Dietary treatments included: control (A; no Dairylac 80 and milk-yeast); Dairylac 80 (B); and Dairylac 80 supplemented with 5% milk-yeast (C).
[2]Vitamin premix containing: vitamin A as retinyl acetate, 5,500 IU; vitamin D_3 as cholecalciferol, 550 IU; vitamin E as alpha-tocopherol acetate, 30 IU; vitamin K as menadione dimethylpyrimidinol bisulfide, 4.4 mg/kg; niacin, 33 mg/kg, pantothenic acid as d-Calcium pantothenate, 22.05 mg/kg; riboflavin, 11 mg/kg; vitamin B12 as cyanocobalamin, 33 mg/kg.
[3] Trace mineral premix containing: copper (as $CuSO_4 \bullet 5H_2O$), 10 mg/kg; iodine (as Ca $(IO_3) \bullet H_2O$), 0.25 mg/kg; Iron ($FeSO_4 \bullet 2H_2O$), 125 mg/kg; manganese (MnO), 15 mg/kg; Selenium (Na_2SeO_3), 0.3 mg/kg; Zinc ($ZnSO_4 \bullet H_2O$), 125 mg/kg.

Materials and Methods

Animals and Experimental Design

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska–Lincoln. One hundred eight weaned pigs (20 $\pm$ 1 day of age) were sorted by initial body weight (BW) and sex, and randomly allotted to dietary treatment (three treatments; six pigs/pen). The average initial body weight was 13.33 $\pm$ 0.07 lb and there were six replicates/treatment with three barrows and three gilts per pen. Pigs were housed in a temperature-controlled room and each pen had a single nipple waterer and a single self-feeder for ad libitum access to water and feed. The study consisted of a five-week feeding experiment divided into three phases: phase 1 (week 1 to 2), phase 2 (week 3 to 4), and phase 3 (week 5).

Dietary Treatments

The ingredient composition and calculated analysis of experimental diets are presented in Tables 1 and 2, respectively. Dietary treatments included the following: A) Control (CTL; no Dairylac 80 or milk-yeast); B) Dairylac 80; and C) Dairylac 80 supplemented with milk-yeast (5%). Phase 1, 2 and 3 diets were formulated to contain 1.47, 1.42, and 1.37 true ileal digestible Lys, respectively. Total Lys was 1.56% in phase 1 and 2, and 1.51% in Phase 3 diets. Except for the control diet, dietary treatments in phase 1, 2, and 3 contained a total of 20, 15 and 5% lactose, respectively. Diets were formulated to meet or exceed NRC (1998) requirements. Dairylac 80 and co-dried milk-yeast 5050 (International Ingredient Corp., St. Louis, Mo.) were the sources of lactose and milk-yeast used in experimental diets. Dairylac 80 produced from sweet and dried whey soluble was a granular and nonhygroscopic product, and contained 3.2% CP, and 0.06% Lys (analyzed composition) and 80% lactose. Co-dried milk-yeast was produced from 50% dried near-dated-milk and 50% dried brewer's yeast containing 17.4% lactose, 33% CP and 1.82% Lys (analyzed composition). This milk-yeast product was included in treatment C during all three feeding phases.

Data and Sample Collection

Individual pig weights and feed disappearance were recorded on days 0, 7, 14, 21, 28, and 35 to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG:ADFI). Blood samples were collected from all pigs via jugular venipuncture. Serum was harvested following centrifugation (20 min at

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1,500 × *g*). Two pigs (one gilt and one barrow) in each pen were randomly selected for collection of fecal samples. Blood and fecal samples were collected at timepoints that coincided with pig BW measurements. Serum and fecal samples were frozen at -20°C for subsequent analyses.

Laboratory Analysis

A porcine specific, enzyme-linked immunosorbent assay (ELISA) was used to quantify circulating immunoglobulin (Ig) G and A (Bethyl Laboratories, Inc., Montgomery, Tex.), and tumor necrosis factor (TNF)-α (R&D Systems, Minneapolis, Minn.).

Isolation of fecal DNA was conducted as described by Martinez et al (2009). The resultant DNA was utilized for subsequent polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analyses. Briefly, for investigation of the entire microbe population in fecal samples, PCR was performed by using universal primers to amplify the V3 region of 16S rRNA gene. In addition, to analyze specific *Lactobacillus* populations in fecal samples, PCR was performed by using the lactic acid bacteria specific primers. Images obtained from DGGE were evaluated using the BioNumerics software and staining intensities of individual bands were determined as proportion of peak surface area relative to the surface area of the entire molecular fingerprint of the sample. Diversity of microbiota was calculated by Shannon's and Simpson's indices using the following formulas:

$$\text{Shannon's index} = \sum_{i=1}^{n} - pi \ln (pi)$$

$$\text{Simpson's index} = \sum_{i=1}^{n} \frac{- ni(ni-1)}{N(N-1)}$$

In which, n*i* was the number of organisms belonging to species *i* (as proportion of band intensity in respect to entire intensity of fingerprint); N was the total number of organisms in microbial population; p*i* was the proportion of a species i present in a sample. The larger Shannon's index, the more diverse the microbial population. Conversely, the smaller the Simpson's index, the more diverse the microbial population.

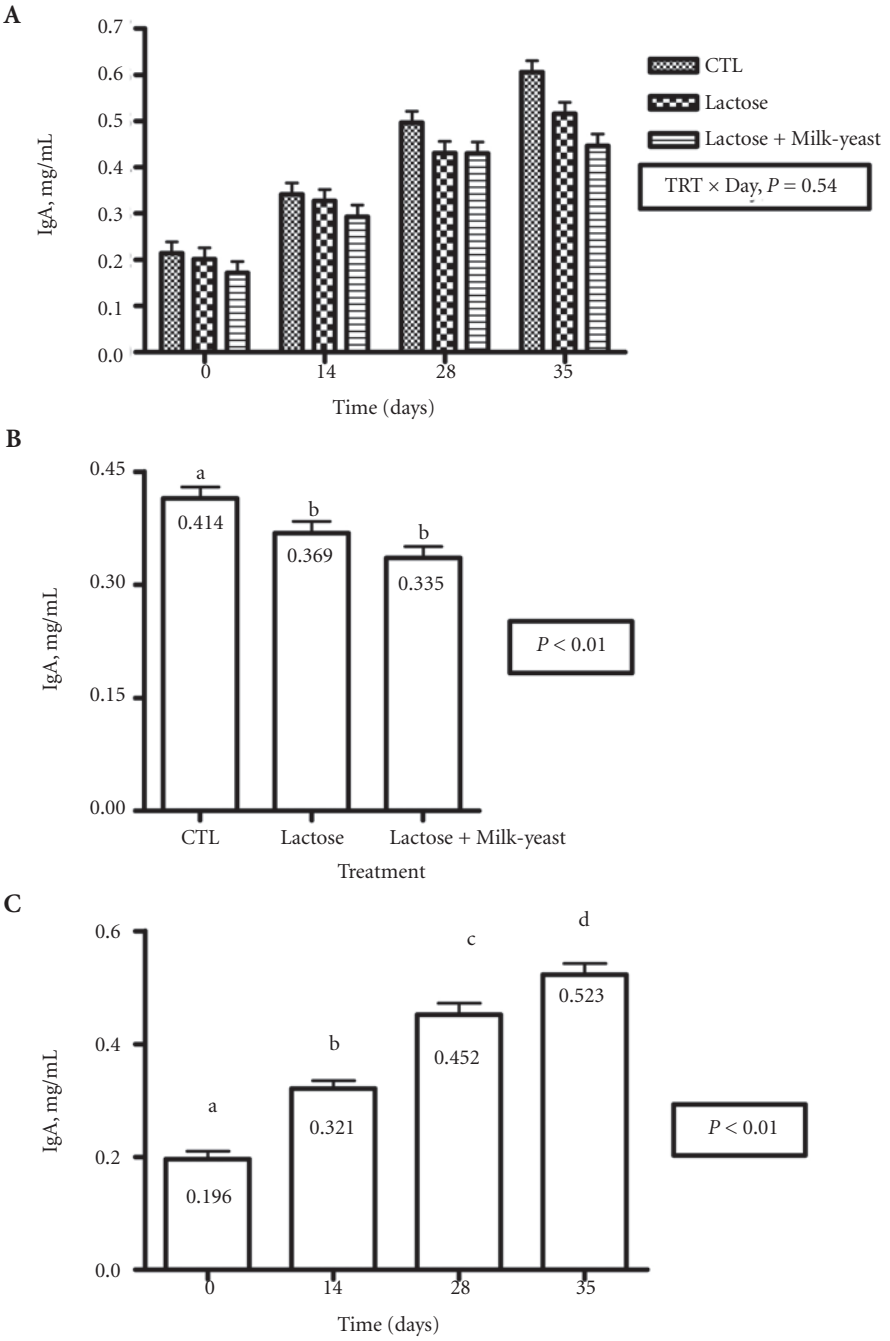

Figure 1. Effects of feeding lactose and milk-yeast on circulating concentrations of Immunoglobulin (Ig) A in weanling pigs. Each bar represents the least-squares mean (± SEM) of 36 (for days 0 and 14) and 18 (for days 28 and 35) observations. Bars with different superscripts differ at $P < 0.05$; panel A represents the interactive treatment × day means, panel B represents treatment means, and panel C represents day means.

Statistical Analysis

Each pen was considered as an experimental unit. Data were analyzed as a completely randomized design using the MIXED procedure of SAS. Pen was considered a random effect. All means are presented as least-squares means.

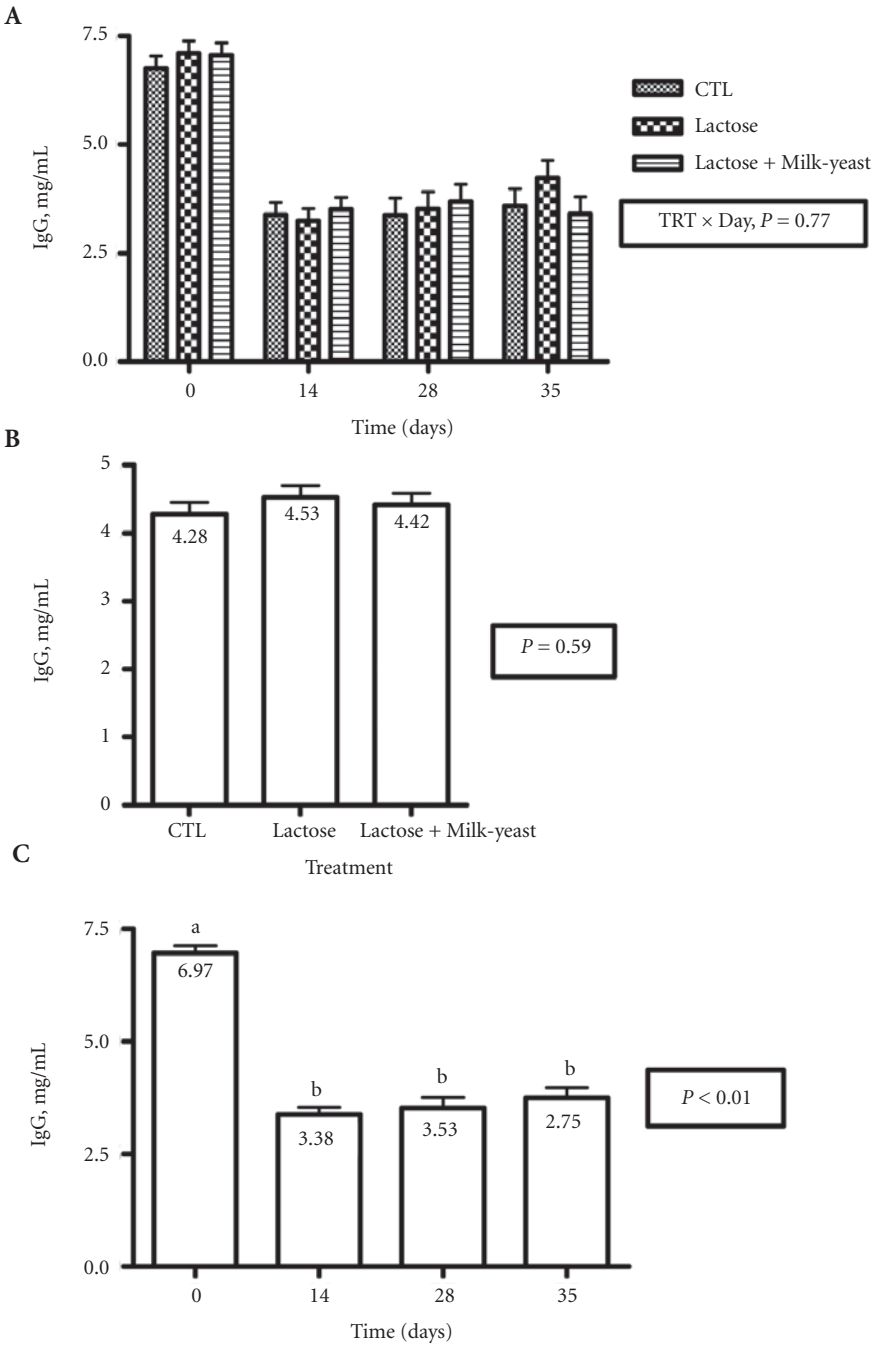

Figure 2. Effects of feeding lactose and milk-yeast on circulating concentrations of Immunoglobulin (Ig) G in weanling pigs. Each bar represents the least-squares mean (± SEM) of 36 (for days 0 and 14) and 18 (for days 28 and 35) observations. Panel A represents interactive treatment × day means, panel B represents treatment means, and panel C represents day means.

Results and Discussion

Growth Performance

Pigs fed lactose with or without milk-yeast tended to have greater BW at the end of week 1 ($P = 0.09$) and 2 ($P = 0.07$) compared to the CTL pigs. There were no effects of dietary treatment on pig BW during week 3, 4, 5, or overall (week 1 to 5).

Compared to CTL pigs, pigs fed lactose and milk-yeast had greater ($P = 0.01$) ADG and ADG:ADFI during week 1 and increased ($P = 0.05$) ADFI during week 2. During phase 1 (week 1 to 2), pigs fed lactose supplemented with milk-yeast had greater ($P = 0.05$) ADG and tended ($P = 0.07$) to have greater ADFI compared to CTL pigs. No differences in ADG, ADFI, or ADG:ADFI were observed between pigs fed lactose supplemented with and without milk-yeast during phase 1 (week 1 to 2).

During phase 2, there were no differences among treatments for ADG, ADFI and ADG:ADFI; however, CTL pigs tended ($P = 0.08$) to have greater ADG:ADFI compared to lactose fed pigs with or without milk-yeast during phase 2 (week 3 to 4). There were no effects of treatment on growth performance during phase 3 (week 5). Overall (week 1 to 5), there were no effects of dietary treatment on growth performance. These results agree with previous research that reported the inclusion of lactose had positive effects on growth performance during the early nursery phases. In summary, the traditional effects of lactose on phase-1 nursery performance were observed. However, there were no effects of milk-yeast product on growth performance.

Immune parameters: IgA, IgG, and tumor necrosis factor (TNF) α

There were no observed treatment × time (day) interactions observed for any of the immune parameters evaluated (Figures 1A, 2A, and 3A). However, significant main effects of treatment (IgA; Figure 1B) and time (IgA, IgG, and TNF-α; Figures 1C, 2B, and 3B, respectively) were observed. Specifically, when means were averaged among all timepoints, CTL pigs had greater ($P < 0.01$) circulating IgA compared to pigs fed lactose with or without milk-yeast. Dietary lactose is considered as a prebiotic for pigs and may improve gut barrier function by facilitating mucus secretion and tight junction formation, by providing immunologic factors, and by lowering GIT pH which is unfavorable for pathogenic bacteria. Thus, there is a

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possibility that a decreased pathogenic load on the GIT exists in pigs fed diets containing lactose with or without milk-yeast compared to the pigs fed no lactose or milk-yeast. In addition, circulating IgA increased ($P < 0.01$) with time throughout the duration of the experiment. This result may be explained by the maturation of the piglet immune system. Previous research indicates that maternal IgA predominates in piglet serum until approximately 5 weeks of age, at which time the first IgA-bearing B cells are evident in the lamina propria of the GIT wall and IgA becomes the predominant Ig isotype in the GIT.

With respect to IgG, pigs were raised in a clean research environment with no clinical signs of disease which may explain the lack of any effects among dietary treatments. However, a main effect of time ($P < 0.01$) was observed with IgG present at the greatest concentration at weaning (day 0) and lower concentrations present throughout the duration of the experiment. Again, as with IgA, this may be explained by the residual concentrations of IgG present in the piglets as a result of passive transfer from the dam and the inability for young pigs to synthesize their own IgG until they are more mature.

Tumor necrosis factor-α is a proinflammatory cytokine which is synthesized and secreted during stress, endotoxemia, or disease. The concentration of TNF-α in piglets in the current experiment was only measured on days 0 and 14. There were no main effects of treatment on TNF-α; however, when means were averaged among treatments, pigs had greater ($P < 0.05$) concentrations of TNF-α at weaning (day 0) compared to day 14. It is speculated that stress due to weaning may be responsible for the increase in this proinflammatory cytokine at day 0.

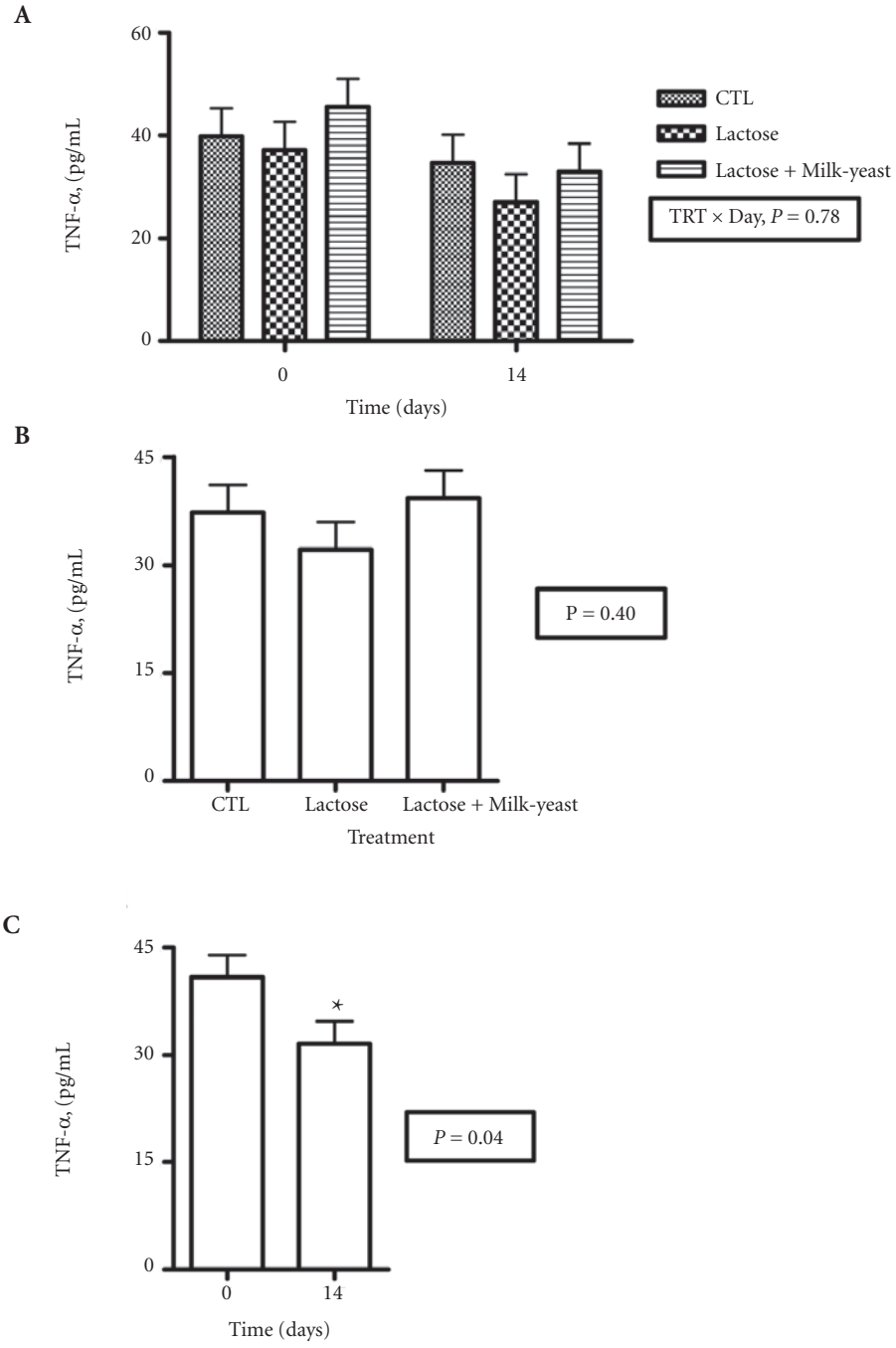

Figure 3. Effects of feeding lactose and milk-yeast on circulating concentration of tumor necrosis factor (TNF)-α. Each bar represents the least-squares mean (+ SEM) of 24 observations. Panel A represents interactive treatment × day means; Panel B represents treatment effects; And panel C represents day effects.

DGGE Analysis of the Lactobacillus Biota in Fecal Samples

The ratio of staining intensity of dominant bands as a proportion of total fingerprint intensity using *Lactobacillus*-targeted primers is summarized in Table 4. At weaning (day 0), there were no differences among treatments on fecal *Lactobacillus* staining intensity. Bands corresponding to *L. johnsonii* (range: 17.68 to 20.68%), *L. sobrius/amylovorus* (range: 6.15 to 11.39%), *and L. mucosae* (range: 11.74 to 17.93%) in the marker are the most dominant Lactobacilli present in pigs at weaning. Bands corresponding to *L. reuteri* appeared at a lower proportion at weaning (5.16 to 9.7%).

At day 7 postweaning, pigs fed

Table 2. Calculated and determined diet composition (as-fed basis).

	Phase 1			Phase 2			Phase 3		
Treatment[1]	A	B	C	A	B	C	A	B	C
Lactose, %	0	20	20	0	15	15	0	5	5
Milk-yeast, %	0	0	5	0	0	5	0	0	5
Calculated analysis									
CP,[2] %	22.8	22	23.1	22.5	22	23.1	22.5	22.3	23.5
Total lys, %	1.57	1.56	1.56	1.56	1.56	1.56	1.51	1.51	1.51
Tid[3] lys, %	1.47	1.47	1.47	1.42	1.42	1.42	1.37	1.37	1.37
Ca, %	0.85	0.85	0.85	0.82	0.82	0.82	0.8	0.8	0.8
P, %	0.81	0.75	0.74	0.73	0.68	0.69	0.71	0.7	0.69
Available P, %	0.53	0.53	0.53	0.44	0.44	0.45	0.4	0.4	0.4
ME[4], kcal[5]/lb	1,532	1,529	1,543	1,534	1,532	1,547	1,529	1,528	1,543
Determined analysis									
CP, %	21.68	21.05	22.14	21.75	20.78	22.36	22.10	21.70	22.86
Ether extract, %	6.30	5.36	5.71	6.32	5.79	6.13	6.32	6.02	6.32
Lys, %	1.43	1.46	1.37	1.42	1.39	1.44	1.40	1.42	1.41
Met, %	0.46	0.52	0.49	0.48	0.50	0.52	0.48	0.48	0.52
Thr, %	0.92	0.94	1.0	0.94	0.94	1.01	0.89	0.89	0.94

[1]Dietary treatments included: control (A, no Dairylac 80 and milk-yeast); Dairylac 80 (B); Dairylac 80 supplemented with 5% dried milk-yeast (C).
[2]Crude protein.
[3]True ileal digestible.
[4]Metabolizable energy.

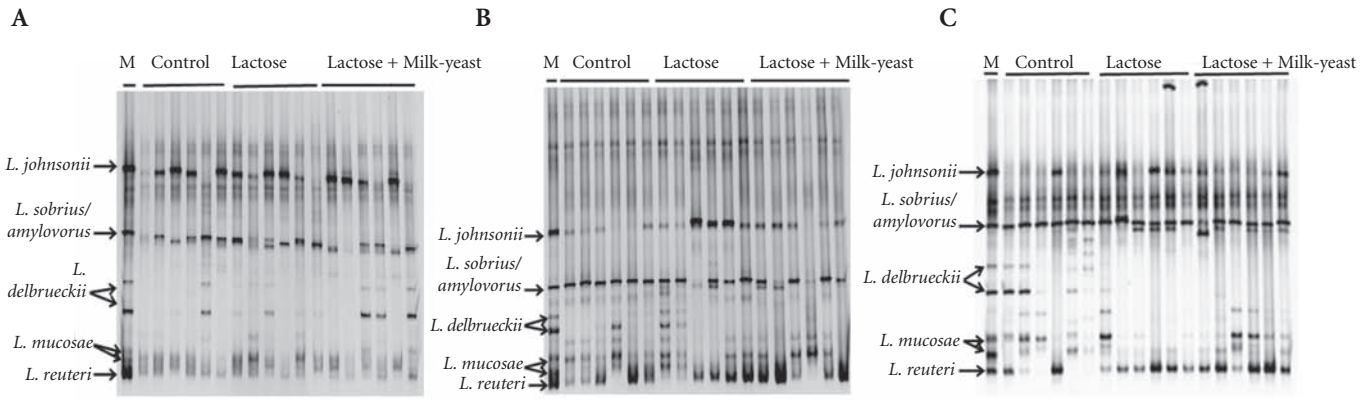

Figure 4. Effects of feeding lactose and milk-yeast on *Lactobacillus* diversity determined by DGGE using *Lactobacillus*-targeted primers. Each lane contains DNA isolated from fecal samples obtained from individual pigs (n = 18); Lane *M is* a marker containing reference *Lactobacillus* species commonly present in pigs. Panel A represents fecal samples of pigs at weaning. Panel B represents fecal samples of pigs at 7 days after weaning. Panel C represent fecal samples of pigs at 14 days after weaning.

lactose with (18.17%) or without milk-yeast (7.63%) had greater (P = 0.05) staining intensity of the putative *L. johnsonii* when compared to the CTL pigs (4.64%). Previous data suggest that 16 to 89% of *L. johnsonii* can utilize lactose and this may explain the increase of this species in pigs fed lactose for 7 days. Interestingly, *L. johnsonii* is believed to be a probiotic species with ability to produce hydrogen peroxide *in vitro* and contribute to the defense against pathogen infection. There were no differences among other *Lactobacillus* species at day 7; however, the tendency of one unknown species (P = 0.08) did appear in pigs fed lactose with or without yeast, but were absent in CTL pigs. There were changes in *Lactobacillus* composition at day 7 where *L. sobrius/amylovorus* (range: 14.11 to 26.33%) and *L. reuteri*

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(range: 17.52 to 20.65%) became the most dominant species compared to day 0 (range: 6 to 10% and 5 to 9%, respectively for *L. sobrius/amylovorus* and *L. reuteri*). The increasing abundance of these two species may be a reason for the reduction of average staining intensity of other species such as *L. johnsonii* at day 7 (10.15%) compared to day 0 (19.51%). In addition, *L. johnsonii* is known as a casein-utilizing species; therefore, the change of protein supply from sow's milk to protein in the nursery diet may result in decreasing casein available and reduction of species utilizing this protein at day 7.

At day 14 postweaning, the CTL pigs had greater putative *L. sobrius/amylovorus* ($P = 0.10$) and *L. delbrueckii* ($P = 0.04$). Otherwise, there were no differences among treatments on other *Lactobacillus* species. However, putative *L. reuteri* was numerically greater in pigs fed lactose with yeast (16.56%) or without milk-yeast (12.5%) compared to the control pigs (7.82%). This is a significant finding because *L. reuteri* has been reported to confer health benefits to humans and animals and is commonly used as a probiotic bacteria for pigs. In summary, feeding lactose with or without milk-yeast did affect the maintenance of some *Lactobacillus* species which are probiotic species (*L. reuteri* and *L. johnsonii*) in pigs after weaning.

DGGE Analysis of the Total Fecal Microbiota by DGGE

The effects of feeding lactose and milk-yeast on total microbial composition were determined by the staining intensity and diversity indexes (Shannon's and Simpson's) using PCR-DGGE in combination with universal primers (Table 6). At days 0, 7, and 14, there were no differences among treatments on microbial population based on Shannon's and Simpson's diversity indices. However, a greater ($P = 0.03$) Shannon's index was identified for control pigs at weaning, compared lactose-fed pigs with and

Table 3. Effects of feeding lactose and milk-yeast on pig performance.

	Treatment[1]				
Item	A	B	C	SEM[2]	*P*-value
BW, lb					
Week 0	13.38	13.38	13.31	0.07	0.75
Week 1	14.15	14.45	14.63	0.15	**0.09**
Week 2	18.55	19.56	19.60	0.33	**0.07**
Week 3	26.11	27.63	27.10	0.53	0.15
Week 4	35.31	36.98	36.37	0.66	0.22
Week 5	46.35	48.05	47.85	0.84	0.33
Phase 1 (week 1)					
ADG, lb	0.11[a]	0.16[b]	0.19[b]	0.02	**0.01**
ADFI, lb	0.25	0.26	0.28	0.01	0.2
G:F, lb/lb	0.44[a]	0.61[b]	0.68[b]	0.05	**0.01**
Phase 1 (week 2)					
ADG, lb	0.63	0.70	0.71	0.04	0.24
ADFI, lb	0.75[a]	0.86[b]	0.87[b]	0.03	**0.05**
G:F, lb/lb	0.84	0.82	0.82	0.02	0.87
Phase 1 (week 1,2)					
ADG, lb	0.37[a]	0.43[b]	0.45[b]	0.02	**0.05**
ADFI, lb	0.50	0.56	0.57	0.02	**0.07**
G:F, lb/lb	0.74	0.77	0.79	0.02	0.34
Phase 2 (week 3)					
ADG, lb	1.08	1.15	1.07	0.04	0.35
ADFI, lb	1.44	1.60	1.52	0.05	0.14
G:F, lb/lb	0.76	0.72	0.70	0.01	**0.07**
Phase 2 (week 4)					
ADG, lb	1.31	1.34	1.32	0.03	0.87
ADFI, lb	1.93	2.04	1.96	0.05	0.3
G:F, lb/lb	0.68	0.65	0.67	0.01	0.14
Phase 2 (week 3,4)					
ADG, lb	1.20	1.25	1.20	0.03	0.48
ADFI, lb	1.68	1.82	1.74	0.05	0.18
G:F, lb/lb	0.71	0.68	0.69	0.01	**0.08**
Phase 3 (week 5)					
ADG, lb	1.58	1.58	1.64	0.05	0.57
ADFI, lb	2.30	2.36	2.38	0.05	0.49
G:F, lb/lb	0.69	0.67	0.69	0.02	0.63
Overall (week 1 to 5)					
ADG, lb	0.94	0.98	0.99	0.02	0.42
ADFI, lb	1.33	1.41	1.40	0.04	0.25
G:F, lb/lb	0.71	0.69	0.70	0.01	0.51

[1]Dietary treatments included: control (A; no Dairylac 80 and milk-yeast); Dairylac 80 (B); and Dairylac 80 supplemented with 5% milk-yeast (C).
[2]Standard error of the mean.
[ab]Means in the same row with different superscript differ ($P < 0.05$).

Table 4. Ratio of staining intensities[1] of dominant bands as a proportion of total fingerprint intensity using *Lactobacillus* primer specific DGGE.

Day	Band	Putative species	Control	Lactose	Lactose + Milk-yeast	SEM	*P*
0	1	*L. johnsonii*	20.2	17.7	20.7	6.6	0.94
	2	*L. sobrius/amylovorus*	10.6	11.4	6.15	3.4	0.52
	3	*L. delbrueckii*	2.08	1.50	8.91	2.7	0.12
	4	*L. mucosae*	15.6	17.9	11.7	3.9	0.55
	5	*L. reuteri*	9.7	5.16	5.95	2.4	0.37
7	1	*L. johnsonii*	4.64[a]	18.17[b]	7.63[a]	3.8	**0.05**
	2	*L. sobrius/amylovorus*	26.3	16.2	14.1	4.2	0.12
	3	unknown	0.00[a]	3.39[b]	2.20[ab]	1.0	**0.08**
	4	*L. delbrueckii*	1.82	2.47	2.07	1.8	0.97
	5	*L. mucosae*	14.3	7.00	15.2	4.6	0.40
	6	*L. reuteri*	20.7	18.7	17.5	5.4	0.92
14	1	*L. johnsonii*	14.0	17.2	12.3	2.5	0.38
	2	*L. sobrius/amylovorus*	20.5[a]	17.9[ab]	14.4[b]	1.8	**0.10**
	3	*L. delbrueckii*	7.77[a]	0.10[b]	0.00[b]	2.2	**0.04**
	4	*L. mucosae*	8.33	3.87	9.32	2.7	0.33
	5	*L. reuteri*	7.82	12.5	16.6	3.6	0.26
	6	unknown	2.70	5.90	5.51	1.9	0.44

[1]Intensity of individual bands as determined as percentage of the peak surface area relative to the surface area of the entire molecular fingerprint of the sample; pigs were at weaning (day 0), 7 and 14 days after weaning. Each value represents a least squares mean of 6 pigs per treatments.
[ab]Means in the same row with different superscript differ ($P < 0.1$)

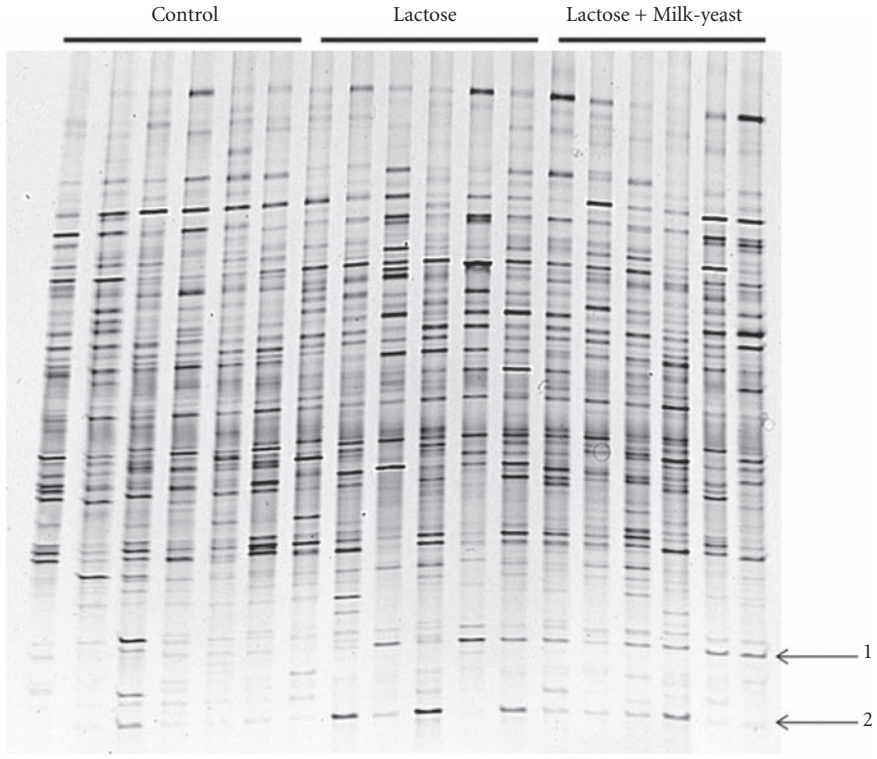

Figure 5. Effects of feeding lactose and milk-yeast on diversity of total microbiota population determined by DGGE using universal PCR primers. Each lane contains DNA isolated from fecal samples obtained from individual pigs (n = 18) at 7 days post weaning. The arrows indicated the bands whose staining intensity was increased (Table 5).

Table 5. Ratio of staining intensities[1] of dominant bands as a proportion of total fingerprint intensity using universal primer DGGE.

Day	Band	Control	Lactose	Lactose + Milk-yeast	SEM[1]	*P*
7	1	0.27[a]	1.31[b]	1.58[b]	0.33	**0.04**
	2	0.31	1.81	0.88	0.56	0.19

[1]Standard error of the mean.
[ab]Means in the same row with different superscript differ ($P < 0.05$).

Table 6. Effects of feeding lactose and milk-yeast on diversity of fecal microbiota.

Day	Treatment	Shannon's[1] index (mean ± SEM[3])	Simpson's[2] index (mean ± SEM)	*P*-value Shannon	*P*-value Simpson
	Control	3.44 ± 0.04[a]	0.045 ± 0.003		
0	Lactose	3.33 ± 0.04[ab]	0.051 ± 0.003	0.03	0.14
	Lactose + Milk-yeast	3.24 ± 0.04[b]	0.055 ± 0.003		
	Control	3.49 ± 0.58	0.039 ± 0.002		
7	Lactose	3.50 ± 0.58	0.404 ± 0.002	0.97	0.88
	Lactose + Milk-yeast	3.57 ± 0.58	0.039 ± 0.002		
	Control	2.93 ± 0.096	0.084 ± 0.013		
14	Lactose	2.91 ± 0.096	0.091 ± 0.013	0.69	0.52
	Lactose + Milk-yeast	3.02 ± 0.096	0.069 ± 0.013		

[1]The larger the Shannon's index, the more diverse the microbial population.
[2]The smaller the Simpson's index, the more diverse the microbial population.
[3]Standard error of the mean.
[ab]Means in the same column with different superscript differ ($P < 0.05$). Each value represents a least squares mean of 6 observations.

without milk-yeast. In addition, DGGE analysis revealed changes in the composition of the fecal microbiota in as much as two bands were significantly more common in animals fed lactose and lactose with yeast at day 7 (Figure 5, bands 1 and 2). Pigs fed lactose with or without yeast had greater ($P = 0.04$) staining intensities of a bacteria type aligned at band 1 in comparison with CTL pigs (Table 5). This dietary effect may relate to the change of *Lactobacillus* composition in pigs at the same age as presented in Table 6.

Conclusions

During phase 1, pigs fed lactose with and without milk-yeast had increased growth performance compared to CTL pigs; however, this effect of lactose and milk-yeast did not continue into the latter phases of the nursery stage. In addition, lactose and milk-yeast may have positive effects on the health status of weanling pigs; however, more research is needed to examine the interplay among growth performance, health, and microbial populations of young pigs fed prebiotics such as lactose and milk-yeast.

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