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Effects of liquid delactosed permeate in nursery piglet diets: Improvements in feed conversion with increased post-weaning diarrhea occurrence

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ABSTRACT - The objective of this study was to assess the effects of liquid delactosed permeate (DLP) alone or mixed as baby feed with a conventional lactose-containing diet for nursery piglets on growth performance, diarrhea occurrence (DO), blood profile, gastrointestinal tract traits, and bacterial population counts. Ninety male hybrid piglets (7.59±0.63 kg) weaned at 25 days old (day 0) were assigned for 35 days in a complete block design based on body weight (BW) to one of three treatments: control diet (CD) containing lactose via whey permeate powder, supplied in mash and dry form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio, 10 replicates, and three piglets per pen. Nursery phases were defined as pre-starter I and II (days 0 to 7, and 8 to 21) and starter (day 22 to 35). Piglets fed MD diet had better feed conversion (FCR) compared with those fed CD in the pre-starter I and II and total period. Piglets in pre-starter I fed MD diet had a higher DO compared with those fed CD, whereas piglets fed SD had intermediate results. Piglets in pre-starter II fed SD had a higher total protein concentration than those fed MD, and CD provided intermediate results. Piglets on MD had higher leukocyte concentrations compared with those fed CD, but piglets fed SD had intermediate results. Piglets fed CD or SD diets had higher colonic content pH than those fed MD. Piglets fed SD or MD diets had longer large intestine lengths than animals fed CD. Delactosed permeate improves FCR in piglets in the pre-starter phases and promotes minor changes in other variables; however, a greater DO is observed in the first few days post-weaning.

Keywords: bacterial population, blood profile, dairy co-products, diarrhea prevalence, growth performance, nursery pig

1. Introduction

Co-products from the dairy industry can be strategic in diets for newly weaned piglets by maintaining a healthier intestinal environment (Yoo et al., 2018). Due to the considerable increase in the use of liquid feed and fermented liquids in pig diets (Zhang et al., 2020), these co-products are alternatives to

expensive and high-quality ingredients (Sutera et al., 2023). Delactosed permeate (DLP) is a co-product of the lactose manufacturing process. It is considered an economic burden for processing companies, and its disposal poses environmental concerns due to its high organic matter content and treatment costs; besides, it is a product of negligible value and, therefore, there is an opportunity to use this dairy co-product in animal feed (Oliveira et al., 2019).

Delactosed permeate has a high concentration of minerals (ADPI, 2015) and oligosaccharides (3′sialyl-lactose, 6′-sialyl-lactose, and lactosamine) (Dallas et al., 2014), which have functional properties for the gastrointestinal tract (GIT) health (Barile et al., 2009). For example, the effects include inhibiting binding of pathogenic bacteria (e.g., Enterobacteriaceae) and improving growth of beneficial bacteria (e.g., Lactobacillaceae) in the small intestine (Yu et al., 2013), stimulating the immunity of the intestinal mucosa and enterocyte proliferation (Jang et al., 2021a), and producing short-chain fatty acids as an energy source for intestinal cells (Venema, 2012). In addition, diets moistened with dairy co-products (e.g., baby feed) can improve piglet performance by increasing feed intake and reducing the dehydration risk (Russell et al., 1996) and sudden dietary changes (Lepine et al., 1991).

Jang et al. (2021a) obtained better results in feed efficiency, crypt cell proliferation rate, and bacterial modulation when 45.9 g of lactose day^{-1} was given to piglets weaned at 21 days old, while Jang et al. (2021b) observed that reducing the daily intake of lactose from 63 to 43 g day^{-1} in diets for piglets weaned at 25 days old was positive. Pierce et al. (2006) observed that 150 g of lactose kg^{-1} diet increased villus height and reduced intestinal pH, but 330 g of lactose kg^{-1} diet increased *Lactobacillus* in piglets weaned at 21 days old.

The form of supply, the inclusion level of these co-products, and the response to lactose content according to weaning age for optimal piglet performance are a theme of debate (Fondevila et al., 2021). The ingestion and excessive lactose fermentation can cause intolerance symptoms, resulting in diarrhea (Forsgård, 2019). This study hypothesizes that weaned piglets fed DLP would improve growth performance through benefits in intestinal health. Therefore, the present study assessed the effects of DLP fed alone or mixed as baby feed with a conventional lactose-containing diet for nursery piglets on performance, diarrhea occurrence (DO), blood profile, GIT traits, and bacterial population counts.

2. Material and methods

2.1. Local and ethical information

The study was conducted in an experimental unit located in Marechal Cândido Rondon, PR, Brazil (24°31'52" S and 54°01'03" W). Research on animals was conducted according to the institutional committee on animal use (protocol no. P026/22).

2.2. Animals, experimental design, housing, and dietary treatments

The experiment lasted 35 days, with no adaptation period. A total of 90 entire male hybrid piglets of commercial lineage (Landrace × Large White), weaned at 25 days old (day 0) and weighing 7.59±0.63 kg, were allotted to one of three treatments in a completely block design based on the initial body weight (BW) categorized into light (up to 7.57 kg BW) or heavy (over 7.57 kg BW) piglets. Each treatment had 10 replicates pens (n = five replicate pens per block) of three piglets of the same BW category per pen as experimental unit. The blocks (n = 2) consisted of 15 pens (experimental unit) and 45 piglets in each block (15 piglets/treatment), totaling 30 pens.

On arrival at the facility, the piglets were weighed and individually identified with numbered earrings and housed in a masonry installation with ceramic tiles and a plastic tarpaulin lining. The elevated pens (1.5 × 1.03 m, 1.54 m^2) were equipped with plastic slatted floor, trough-type feeders, and pacifier-type drinkers, arranged in two rows divided by a central aisle. The pens and the facility were cleaned daily

in the afternoon. The ambient temperature and relative humidity were recorded daily in the morning (08:00 and 10:30 h) and afternoon (14:00 and 16:30 h) using a datalogger (UT330B digital USB; UNI-T, Vketech, Beijing, China) installed in the central part of the facility.

Fans, exhaust fans, and side-hinged glass windows controlled ventilation inside the facility. Individual incandescent and infrared lamps located at the top of each pen were used to heat the environment. An automation system for the heating lamps and camera monitoring controlled via Wi-Fi was used to help control temperature and humidity and observe the animals during the experimental period. A combination of daylight and artificial light was used, with a 12-h light-dark cycle.

The dietary treatments (Table 1) consisted of: control diet (CD) containing lactose via whey permeate powder, supplied in mash and dry form, without liquid DLP; supplemented diet (SD): CD + DLP provided in specific feeders (e.g., polyethylene plastic piglet feeder attached to the floor) and separated from the mash diet; and moist diet (MD): CD moistened with DLP in a 1:1 ratio. All the diets were corn and soybean meal-based with industrial amino acids, and mixed for 15 min in a vertical mixer. The CD was formulated to meet the nutritional and energy recommendations for piglets in the nursery phase, being pre-starter I (0 to 7 days) and II (8 to 21 days), and starter (22 to 35 days) (Rostagno et al., 2017).

Table 1 - Calculated and chemical composition of the experimental diets offered to piglets in the nursery phase (as-fed basis)

Item	Experimental phase		
	Pre-starter I	Pre-starter II	Starter
Ingredient (%)			
Ground corn (7.8%)	40.34	44.26	61.09
Soybean meal (45.4%)	21.69	20.65	24.21
Whole deactivated soybean (37.3%)	10.00	10.00	6.00
Whey powder permeate (3.0%)	14.02	9.41	-
Cooking sugar	5.00	5.00	-
Fish meal (53.0%)	3.00	3.00	3.00
Soybean oil	1.63	3.00	1.62
Dicalcium phosphate	1.48	1.57	1.54
Calcitic limestone	0.89	0.69	0.67
Common salt	0.11	0.37	0.42
Mineral-vitamin premix[1]	0.30	0.30	0.30
Lysine sulphate (54.6%)	0.71	0.89	0.57
DL-methionine (99.5%)	0.36	0.27	0.23
L-threonine (96.8%)	0.42	0.34	0.27
L-tryptophan (99.0%)	0.09	0.05	0.05
L-valine (95.5%)	-	0.18	-
Halquinol (60.0%)	0.01	0.01	0.01
Calculated chemical composition			
Crude protein (%)	21.42	19.87	20.55
Metabolizable energy (kcal kg^{-1})	3,400	3,380	3,350
Total calcium (%)	1.07	0.97	0.91
Available phosphorus (%)	0.52	0.48	0.45
Lactose (%)	11.00	8.00	-
Total sodium (%)	0.22	0.21	0.20
Digestible lysine (%)	1.45	1.35	1.28
Digestible methionine + cysteine (%)	0.81	0.75	0.73
Digestible threonine (%)	0.97	0.90	0.83
Digestible tryptophan (%)	0.27	0.25	0.24
Digestible valine (%)	1.00	0.92	0.88

[1] Mineral premix (content per kg of product): zinc oxide, 26.66 g; iron sulphate, 10 g; copper sulphate, 2,708 mg; iodine, 333 mg; biotin, 26.66 mg; manganese sulphate, 10 g; selenium (sodium selenite), 100 mg. Vitamin premix (content per kg of product): vitamin A, 2,000,000 IU; vitamin D3, 533,330 IU; vitamin E, 6,583 IU; vitamin K3, 1,066 mg; vitamin B1, 334 mg; vitamin B2, 1,333 mg; vitamin B6, 466 mg; vitamin B12, 5,333 mcg; niacin, 9,372 mg; pantothenic acid, 5,022 mg; folic acid, 167 mg.

2.3. Chemical composition and supply of delactosed permeate

The DLP (Table 2) was manufactured by Sooro Renner Nutrição S.A. (Marechal Cândido Rondon, PR, Brazil). The co-product was packed in plastic bottles to facilitate handling and kept refrigerated (4 °C) for a maximum of seven days. It was given to the animals in the SD or MD treatment in the morning (08:00 h) and afternoon (14:00 h) periods. The amount offered to the piglets in the SD treatment was altered in each nursery phase: pre-starter I (morning: 200 mL; afternoon: 250 mL), pre-starter II (morning: 275 mL; afternoon: 275 mL), and starter (morning: 400 mL; afternoon: 400 mL). The ratio (1:1) of CD to DLP given to the piglets in the MD treatment was altered based on the total daily feed intake of the animals in each phase. Before feeding, the CD was weighed and mixed with the DLP to ensure the animals' total intake in each of the two periods.

Table 2 - Chemical composition of liquid delactosed permeate

Item	Content
Dry matter (%)	37.00
Total solids (%)	28.57 (68.25% lactose)
Ash (%)	4.46
Sodium (mg 100 g^{-1})	371.18
Potassium (mg 100 g^{-1})	499.06
Chlorides (%)	0.80
Crude protein (%)	2.44
Ethereal extract (%)	0.00
Carbohydrates (%)	21.67
Lactose (%)	19.50
Galactose (%)	1.08
Glucose (%)	1.08
pH	5.56
Acidity (°D)	105.88

2.4. Growth performance and diarrhea occurrence

The animals were fed *ad libitum* and had free access to water throughout the experiment. The piglets were weighed at the beginning and end of each phase using a digital scale (Model UL-50, Brand DIGI-TRON, Curitiba, PR, Brazil) to monitor final BW (FBW) and calculate average daily BW gain (ADG, kg day^{-1}). The diets and leftovers in the feeder and on the floor were collected manually, weighed daily, and deducted from the total supply to calculate the average daily feed intake (ADFI, kg day^{-1}) and feed conversion ratio (FCR, kg kg^{-1}). In the case of leftovers from the MD treatment at the change of each phase, these were dried in a forced-air circulation oven (Tecnal, SF-325 NM; Piracicaba, SP, Brazil) and then weighed. The intake of DLP provided alone was not considered in the ADFI of piglets fed the SD.

Diarrhea occurrence was assessed throughout the experimental period. The fecal consistency of the animals was observed daily (08:00 h) at pen level by a single trained person through visual analysis, assigning scores from 0 to 3 according to the physical appearance of the feces, in which 0 = normal feces, 1 = soft feces, 2 = watery/pasty feces, and 3 = liquid feces (Pérez-Calvo et al., 2019). Scores 2 and 3 were considered diarrheal feces.

2.5. Sampling and analysis of blood metabolites

Blood samples (≅ 10 mL, 08:30 h) were taken at the end of the pre-starter II (day 21) and starter (day 35) phases. All the piglets were fasted for 6 h, and 10 piglets per treatment (one animal per

experimental unit) were chosen at the end of each phase for blood collection based on the BW closest to the average BW of their respective pen. The collection procedure was carried out via puncture of the anterior cranial vena cava, using 20 mL plastic syringes and 0.70 × 30 mm gauge needles. The same animals were used for both blood samples. The blood was then transferred to four sterile glass tubes containing a type of anticoagulant (heparin) for analysis of urea (heparin), complete blood count (EDTA), glucose (potassium fluoride), and albumin, total cholesterol, triacylglycerols, and total protein (serum).

The tubes containing the collected samples were placed in a thermal box containing ice (4 °C) and sent to the laboratory for analysis. The blood samples were centrifuged (analog centrifuge, model 80-2B, Centrilab, Maringá, PR, Brazil) at 3,000 rpm for 10 min at room temperature. Approximately 3 mL of the supernatant was then transferred to Eppendorf-type polyethylene microtubes, duly labeled, and stored in a freezer at −20 °C.

The analytical procedures for the analysis of albumin (enzymatic-colorimetric method), glucose (enzymatic-colorimetric method, Trinder), total protein (colorimetric-biuret method), and total cholesterol, triacylglycerols, and urea (enzymatic-colorimetric method) were carried out in technical duplicate by spectrophotometry (Bel SPECTRO S05, Ramos, RJ, Brazil), using specific kits from the Gold Analisa Diagnostic® brand (Belo Horizonte, MG, Brazil).

To assess the complete blood count, the blood samples were sent to the Lotus Veterinary Laboratory (Cascavel, PR, Brazil) for quantification of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, red blood cell distribution width, mean corpuscular hemoglobin concentration, plasma proteins, platelets, total leukocytes, segmented neutrophils, eosinophils, lymphocytes, and monocytes.

2.6. Slaughter procedures and gastrointestinal tract traits

At the end of the experimental period (day 35), seven animals from each treatment were slaughtered based on the BW closest to the average BW of their respective pen. The animals were not the same blood donors. They were fasted for 8 h and electrically stunned (240 volts for 3 s), followed by bleeding. Slaughter was carried out in a commercial slaughterhouse (Marechal Cândido Rondon, PR, Brazil).

2.7. pH of digestive tract contents, relative weight, and length of organs

The viscera were exposed through a central incision. The GIT portions were aseptically isolated with a double ligature, and the pH was measured in the homogenized contents of the medial region of the stomach, jejunum (150 cm from the ileocecal junction), ileum (15 cm from the ileocecal junction), cecum, and medial colon using a digital pH meter (HI 99163, Hanna Instruments Inc., Rhodes Island, USA). After measuring the pH, the organs of the GIT (stomach, small intestine, cecum, empty large intestine, and liver with gallbladder) were weighed (digital scale, model 9094, Toledo, Brazil) to calculate the relative weight of the organs based on the BW of the animals at slaughter. A common tape measure was used to measure the length of the small and large intestines.

2.8. Intestinal morphometry

The fragments (≅ 3 cm long) of the jejunum and ileum of all the slaughtered animals were collected, washed with distilled water and physiological solution (0.9% sodium chloride), and then stored in 50-mL sterile plastic containers containing a prepared solution of 10.0% neutral buffered formalin (100 mL of 37.5% formaldehyde, 900 mL of distilled water, 4 g of monobasic sodium phosphate, and 6.5 g of dibasic sodium phosphate) for 48 h (Genova et al., 2021). The samples were then placed in a 70.0% alcohol solution and sent to a commercial laboratory (Mercolab, Cascavel, PR, Brazil). The samples were washed in saline solution, fixed in Bouin's solution, dehydrated in a gradual series of ethanol, and cleaned with xylene. The tissues were embedded in paraffin blocks to prepare the slides. Five-micrometer sections were cut from the paraffin blocks using a rotary microtome (Leica RM2245, Leica Biosystems, São Paulo, SP, Brazil), for subsequent staining with hematoxylin and eosin.

The images of the slides were captured by a digital camera (Pro Series, Silver Spring, MD, USA) coupled to an Olympus BX40 microscope (Olympus, Tokyo, Japan). The morphometric examination of the images was carried out using imaging software (Image-Pro plus 4.1, Media Cybernetics®, Silver Spring, Maryland). Ten measurements of villus height (V) and their respective crypt depths (C) in each intestinal fragment (jejunum and ileum) were evaluated to calculate the average value for each animal and then obtain the V:C ratio.

2.9. Bacterial population counts

After evisceration, the jejunal and ileal mucosa were exposed using a scalpel. Samples (≅ 5 g) of the contents of the jejunum and ileum of the piglets were collected using the technique of bacterial isolation, cultivation, counting, and characterization by the traditional plating method. The samples were analyzed according to the procedures of ISO 16649-2:2001 (ISO, 2006). Then, they were immediately packed in sterile plastic containers, identified, and transported under refrigeration (4 °C) for laboratory analysis (Mercolab, Cascavel, PR, Brazil).

In the commercial laboratory, 1 g of each sample was serially diluted in 1.0% peptone water. Each dilution was vortexed (Phoenix, model AP 56; Araraquara, SP, Brazil) for 30 s. An aliquot of 100 µL of each diluted sample was spread evenly on the surface of the plates containing specific agar for each bacterium. The microbial populations evaluated were *Escherichia coli* (eosin methylene blue levine agar, Kasvi brand; São José dos Pinhais, PR, Brazil) and *Lactobacillus* (Man, Rogosa and Sharpe agar, Acumedia brand; São Paulo, SP, Brazil). The plates were incubated at 37 °C (EL 202; Eletrolab, São Paulo, SP, Brazil) aerobically for 24 h to count *E. coli* and anaerobically for 48 h to count *Lactobacillus* (Genova et al., 2022). The bacterial colonies were counted, and the data multiplied by the respective dilution and transformed into log ($\log_{10}$). The results were expressed in log of colony forming units (CFU) g^{-1}.

2.10. Statistical procedures

Statistical analyses were carried out using the procedures of SAS (Statistical Analysis System, version 9.2). Before the analysis of variance (ANOVA) or covariance (ANCOVA), the data was evaluated for outliers (values greater than or equal to 3.0 standard deviations in absolute value were considered influential). The pen was considered the experimental unit, except for slaughter and blood data, in which the animal was considered the experimental unit. The data was analyzed considering treatment and BW block as fixed and random effects, respectively. Initial BW was included in the model as a covariate when $P<0.05$, therefore, using ANCOVA. The following overall model was used for the growth performance data:

$$Y_{ijk} = \mu + T_i + b_j + \beta\,(X_{ijk} - \bar{X}...) + \varepsilon_{ijk}, \quad (1)$$

in which Y_{ijk} = average observation of the dependent variable in each plot measured in the i-th treatment, in the j-th block, and in the k-th replicate; μ = overall average; T_i = fixed effect of treatment (i = 1, 2, and 3); b_j = random effect of block (j = 1 and 2); β = regression coefficient of Y over X; X_{ijk} = average observation of the covariate (initial BW) in each plot, measured in the i-th treatment, in the j-th block, and in the k-th replicate; $\bar{X}...$ = overall average for the covariate X; ε_{ijk} = random error of the plot associated with each Y_{ijk} observation. Other variables were analyzed using the model mentioned above without the covariate effect.

Bacterial count data was transformed into $\log_{10}$ CFU g^{-1} for analysis. The treatment effect on the dependent variables was assessed using ANOVA or ANCOVA. Duncan's post hoc test was used to compare means between dietary treatments; it was used because the present study required that type-II error be avoided and possible effects be not lost, when $P\leq0.05$. In addition, it had lower values of the minimum significant difference, implying that it was easier to detect differences between treatments if they existed, that is, Duncan's test has higher sensitivity to detect smaller differences between group averages. All data were reported as means with pooled standard error of the mean.

A generalized linear model was fitted for DO (binomial distribution and logit link function). Diarrhea occurrence results were presented as observed proportions (relative frequency in %). Treatment and time were considered as fixed effects and BW block as a random factor. The treatment effect was verified using type III analysis. Significant differences were defined as P≤0.05, and DO estimates were compared using a difference between averages test, by the χ^2 statistic.

3. Results

3.1. Growth performance and diarrhea occurrence

The ambient temperature and relative humidity recorded during the experimental period were 24±2.48 °C and 65±7.73%, respectively. Piglets fed MD dietary treatment had better (P≤0.05) FCR compared with those fed the CD in the pre-starter I, pre-starter II, and total period (Table 3). In addition, piglets fed the SD diet in the pre-starter I also had better (P≤0.05) FCR than those fed the CD diet, but showed intermediate results in the pre-starter II and in the total period. There was no effect of dietary treatment on growth performance in the starter phase.

Piglets fed the MD diet in pre-starter I had higher (P≤0.05) DO compared with those fed the CD diet, while the SD diet provided in intermediate results (Table 3). There was no treatment effect on DO in the pre-starter II and starter phases.

Table 3 - Effects of liquid delactosed permeate (DLP) on growth performance and diarrhea occurrence (DO) of nursery piglets[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
	Pre-starter I (day 0 to 7)				
IBW (kg)	7.69	7.53	7.57	0.075	-
FBW (kg)	9.42	9.53	9.34	0.113	0.173
ADG (kg)	0.23	0.28	0.25	0.011	0.172
ADFI (kg)	0.31	0.31	0.26	0.011	0.139
FCR (kg kg^{-1})	1.35a	1.10b	1.06b	0.042	0.004
DO (%)	16.00B	14.00AB	18.00A	0.111	0.022
	Pre-starter II (day 8 to 21)				
FBW (kg)	15.68	16.04	16.36	0.313	0.255
ADG (kg)	0.44	0.47	0.50	0.022	0.244
ADFI (kg)	0.60	0.59	0.60	0.016	0.981
FCR (kg kg^{-1})	1.37a	1.25ab	1.20b	0.013	0.003
DO (%)	9.29	8.57	7.14	0.051	0.237
	Starter (day 22 to 35)				
FBW (kg)	24.91	25.69	25.14	0.427	0.289
ADG (kg)	0.65	0.67	0.63	0.010	0.295
ADFI (kg)	1.02	1.04	0.97	0.017	0.210
FCR (kg kg^{-1})	1.57	1.56	1.54	0.024	0.769
DO (%)	1.33	2.00	2.67	0.038	0.176
	Total period (day 0 to 35)				
ADG (kg)	0.49	0.51	0.49	0.011	0.211
ADFI (kg)	0.72	0.72	0.67	0.014	0.098
FCR (kg kg^{-1})	1.48a	1.40ab	1.37b	0.024	0.044

IBW - initial body weight; FBW - final body weight; ADFI - average daily feed intake; ADG - average daily weight gain; FCR - feed conversion ratio; SEM - pooled standard error of the mean.
[1] Data are means of 10 pen replicates per treatment and three animals per pen as the experimental unit.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.
a,b - Averages followed by different lowercase letters in the row differ according to Duncan's post hoc test (P≤0.05).
A,B - Observed proportions followed by different capital letters in the same row differ using a difference between means test, by the χ^2 statistic.

3.2. Blood metabolites

Regarding the biochemical profile, the piglets in pre-starter II phase that consumed the SD diet had a higher (P≤0.05) concentration of total protein than the animals fed the MD diet, and the animals fed CD diet showed intermediate results (Table 4). There was no treatment effect on the blood biochemical profile in the starter phase.

Regarding the hematological profile, piglets fed MD diet had higher (P≤0.05) leukocyte concentrations compared with the animals fed the CD diet, and the animals that received the SD diet had intermediate results (Table 5).

Table 4 - Effects of liquid delactosed permeate (DLP) on the blood biochemical profile of piglets in the nursery phase[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
	Pre-starter II (day 21)				
Albumin (g dL^{-1})	2.02	2.05	1.97	0.035	0.577
Glucose (mg dL^{-1})	115.11	114.21	114.15	3.213	0.975
Total protein (g dL^{-1})	4.70ab	4.81a	4.51b	0.045	0.019
Total cholesterol (mg dL^{-1})	73.16	69.02	66.00	1.801	0.271
Triacylglycerols (mg dL^{-1})	57.88	61.42	64.21	2.988	0.694
Urea (mg dL^{-1})	13.16	11.56	9.67	0.898	0.245
	Starter (day 35)				
Albumin (g dL^{-1})	2.24	2.35	2.39	0.046	0.150
Glucose (mg dL^{-1})	114.53	114.44	114.77	2.680	0.998
Total protein (g dL^{-1})	4.84	4.79	4.91	0.099	0.712
Total cholesterol (mg dL^{-1})	54.94	55.67	55.09	2.418	0.991
Triacylglycerols (mg dL^{-1})	38.42	39.60	35.05	1.438	0.365
Urea (mg dL^{-1})	22.46	20.37	23.19	1.185	0.577

SEM - pooled standard error of the mean.
[1] Data are means of 10 piglets per treatment.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.
a,b - Averages followed by different lowercase letters in the row differ according to Duncan's post hoc test (P≤0.05).

Table 5 - Effects of liquid delactosed permeate (DLP) on the hematological profile of nursery piglets on day 35[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
Red blood cells (×10^6 μL^{-1})	5.93	5.90	5.92	0.073	0.991
Hemoglobin (g dL^{-1})	12.01	11.76	11.68	0.122	0.534
Hematocrit (%)	35.15	34.95	34.95	0.375	0.972
MCV (fL)	60.12	60.08	60.08	0.060	0.534
RDW (%)	20.22	19.95	19.25	0.646	0.704
MCHC (g dL^{-1})	33.33	33.31	33.29	0.012	0.377
Plasma proteins (g dL^{-1})	5.36	5.30	5.35	0.042	0.834
Platelets (×10^3 μL^{-1})	347.44	376.75	447.75	700.400	0.153
Leukocytes (×10^3 μL^{-1})	19.51b	21.81ab	23.04a	19.803	0.052
Segmented (%)	45.50	45.55	44.50	1.065	0.853
Eosinophils (%)	1.10	1.15	1.40	0.184	0.793
Lymphocytes (%)	51.30	52.70	52.25	1.038	0.805
Monocytes (%)	1.60	1.45	1.10	0.182	0.527

MCV - mean corpuscular volume; RDW - red blood cell distribution width; MCHC - mean corpuscular hemoglobin concentration; SEM - pooled standard error of the mean.
[1] Data are means of 10 piglets per treatment.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.
a,b - Averages followed by different lowercase letters in the row differ according to Duncan's post hoc test (P≤0.05).

3.3. pH of digestive tract contents, length, and relative weight of organs

The piglets that consumed the CD or SD diets had higher (P≤0.05) pH values of the colonic content compared with animals fed the MD diet (Table 6). Piglets fed the SD or MD diets had longer (P≤0.05) large intestine lengths than the animals fed the CD diet. There was no treatment effect on relative organ weight.

Table 6 - Effects of liquid delactosed permeate (DLP) on the pH of digestive tract contents and length and relative weight of organs in nursery piglets on day 35[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
pH					
Stomach	3.85	3.38	3.41	0.138	0.106
Jejunum	5.77	5.62	5.52	0.154	0.790
ileum	5.18	5.70	5.24	0.179	0.503
Cecum	5.38	5.44	5.35	0.035	0.370
Colon	5.80a	5.89a	5.55b	0.063	0.001
Length (cm)					
Small intestine	52.35	50.56	52.23	1.508	0.938
Large intestine	12.98b	14.02a	14.17a	0.249	0.057
Relative weight (% of body weight)					
Empty stomach	0.76	0.74	0.75	0.017	0.266
Empty small intestine	3.66	3.88	3.92	0.093	0.157
Empty cecum	0.27	0.28	0.25	0.013	0.970
Empty large intestine	1.68	1.74	1.72	0.041	0.497
Liver with gallbladder	2.78	3.07	2.87	0.067	0.158

SEM - pooled standard error of the mean.
[1] Data are means of seven piglets per treatment.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.
a,b - Averages followed by different lowercase letters in the row differ according to Duncan's post hoc test (P≤0.05).

3.4. Intestinal morphometry

There was no difference between treatments in intestinal morphometry on day 35 of the experiment (Table 7).

Table 7 - Effects of liquid delactosed permeate (DLP) on the intestinal morphometry of nursery piglets on day 35[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
Jejunum					
Villus height (µm)	468	509	499	18.87	0.717
Crypt depth (µm)	200	204	214	11.37	0.785
Villus height:crypt depth	2.83	2.66	2.49	0.141	0.582
Ileum					
Villus height (µm)	416	477	498	17.77	0.144
Crypt depth (µm)	211	195	205	12.16	0.845
Villus height:crypt depth	2.30	2.78	2.75	0.177	0.526

SEM - pooled standard error of the mean.
[1] Data are means of seven piglets per treatment.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.

3.5. Bacterial population counts

There were no differences between treatments in bacterial populations or in the *Lactobacillus*:*E. coli* ratio in the jejunum and ileum (Table 8).

Table 8 - Effects of liquid delactosed permeate (DLP) on the count of the number of colony- forming units (log_{10} g^{-1}) of *Escherichia coli*, *Lactobacillus* and their ratios in the small intestine of nursery piglets on day 35[1]

Item	Treatment[2]			SEM	P-value
	CD	SD	MD		
Jejunum					
Escherichia coli	4.93	5.50	4.67	0.272	0.499
Lactobacillus	7.83	8.13	8.46	0.187	0.459
Lactobacillus:E. coli ratio	1.54	1.50	1.94	0.094	0.134
Ileum					
Escherichia coli	5.49	6.36	4.76	0.351	0.120
Lactobacillus	8.31	8.31	8.49	0.124	0.742
Lactobacillus:E. coli ratio	1.63	1.34	1.93	0.114	0.064

SEM - pooled standard error of the mean.
[1] Data are means of seven piglets per treatment.
[2] Control diet (CD) supplied in dry, mash form, without DLP; supplemented diet (SD): CD + DLP provided in specific feeders and separated from the mash diet; moist diet (MD): CD moistened with DLP in a 1:1 ratio.

4. Discussion

In the current study, we tested DLP (19.5% lactose, and 2.4% crude protein) fed separately in specific feeders (in liquid form) or mixed with a diet containing lactose (11.0 and 8.0% lactose in pre-starter I and II phases, respectively) in the form of baby feed, with the hypothesis that DLP supplementation would improve growth performance by positively influencing the intestinal health of piglets weaned at 25 days old. Delactosed permeate is a coproduct with a low crude protein concentration (Oliveira et al., 2019), but it is a source of oligosaccharides, minerals (Barile et al., 2009), and lactose (although with a lower content than conventional permeate) (Oliveira et al., 2019). In the present study, positive effects were observed on feed conversion in piglets in the pre-starter phases fed DLP in both forms of supply. This is attributed to the fact that the nutrients and energy present in DLP help with growth rate, stimulation of immunity, and development of the intestinal mucosa (Jang et al., 2021a), positively affecting the absorption cells (e.g. enterocytes) that line the intestinal mucosa (Li et al., 2018).

On the other hand, piglets weaned at 23 days old and 5.8 kg BW, fed a diet containing 35.5% lactose, had lower ADG compared with those given a diet with 22.5% lactose in the first two weeks post-weaning (Mahan and Newton, 1993). This finding suggested that weaning age alters the growth response to dairy co-product intake and influences dietary lactose requirements for optimal growth performance, as also observed in a study conducted by Jang et al. (2021b), who evaluated weaning age groups (21 and 25 days old) and whey permeate contents (7.5, 11.2, 15.0, and 18.7%, that is, 5.3, 7.9, 11.1, and 13.6% of lactose, respectively) in piglet diets. According to the same authors, the daily intake of 14.4% whey permeate (10.3% of lactose, 43.1 g of lactose day^{-1}) increased the feed efficiency of piglets weaned at 25 days old, weighing from 7 kg to 11 kg BW.

The lactose present in DLP plays an important role in post-weaning piglet performance due to its sweetness, which improves the palatability of the diet (Zhao et al., 2021), although this characteristic did not alter the animals' feed intake in the present study. As it is also a carbohydrate that is easily digested by lactase, lactose is converted into glucose and galactose, providing readily available energy (Vente-Spreeuwenberg et al., 2003). This additional energy content provided by the supply of DLP may reflect in improvements in the growth of nursery piglets due to a satiety effect, as also reported by

Jang et al. (2021a,b). However, there is a decline in lactase activity in piglets between the third and fifth week of birth, reflecting a lack of response in growth performance in piglets in the starter phase (seven to nine weeks) (Pluske et al., 2003), as observed in the present study.

In addition, the weaning period can cause stress to the animal due to environmental changes and abrupt dietary changes, modifying the environment and intestinal morphometry (Hu et al., 2014). Diets moistened with DLP can promote improvements in nutrient and energy digestibility (although not evaluated in this study) and positively influence piglet performance responses (e.g., better feed conversion in animals fed moist diet). Liquid delactosed permeate can provide a greater supply of energy, nutrients, and water to the enterocytes, and this promotes greater development of the intestinal villi (although we did not observe such alterations), improving digestive and absorption capacity (Vasa et al., 2024).

The digestibility of dietary lactose can provide more digestible energy or a suitable microenvironment for the development of intestinal epithelial cells, favoring the ability to absorb nutrients in weaned piglets (Zhao et al., 2021) and, therefore, DLP can contribute to better nutrient absorption. Zhang et al. (2020) obtained a trend of better energy digestibility in the group of piglets fed diet moistened with water (1:2.5 ratio). Therefore, the composition of water present in DLP (60.0 to 75.0%), a nutrient that plays a crucial role in muscle growth and allows for a better transition from milk feeding (Zhang et al., 2020) associated with the type of diet supply (e.g., moistened or liquid), may be an alternative to reduce cases of dehydration in the post-weaning period that compromise the performance of nursery piglets (Russell et al., 1996).

However, by stimulating feed intake using a diet supplemented with DLP, there is also an increase in lactose content, and the increase in this carbohydrate can cause disorders in the GIT (Zhao et al., 2021). Excessive lactose fermentation results in diarrhea because it leads to intestinal barrier dysfunction (Venema, 2012), as observed in the present study in pre-starter I phase (days 0 to 7) in piglets given the MD diet. Similar results were obtained by Pierce et al. (2005), in which piglets fed a diet consisting of 29.5% lactose had a higher DO in the second and third weeks post-weaning compared with piglets fed a diet containing 17.5% lactose.

This supports the results observed in the present study because excess lactose also causes an osmotic imbalance in the intestinal epithelium (Zhao et al., 2021), signs similar to those of lactose intolerance in humans (Forsgård, 2019). This hypothesis needs to be further investigated because it has previously been shown that lactase activity decreased by 60.0 to 80.0% throughout the small intestine in nursery piglets during the first eight days post-weaning (Pié et al., 2004). This fact might explain the occurrence of post-weaning diarrhea in piglets fed higher lactose content due to lower lactase enzyme activity, causing damage to intestinal barrier functions (e.g., inflammation) (Peace et al., 2011).

The reduction in lactase activity with DLP supplementation or excessive intake of lactose may be associated with a reduction in pH in the intestine due to microbial fermentation that produces short-chain fatty acids (SCFA) from lactose and milk oligosaccharides (Montalto et al., 2006). For the results of better FCR but higher DO, we hypothesized that: first, although piglets can digest lactose, excessive lactose intake may cause undigested lactose to reach the large intestine. As a result, it is fermented by intestinal bacteria, which produce gas and organic acids. This can lead to osmotic diarrhea, as water is fluid into the intestine. Second, a sudden increase in the lactose content of DLP can cause intestinal dysbiosis. For example, some pathogenic bacteria other than *E. coli* may show increased growth, causing digestive disorders, which can manifest as diarrhea. Third, due to its high sugar and mineral content, DLP can increase the osmolarity of the intestinal content, promoting water retention in the intestine and contributing to diarrhea, without negatively affecting the animals' performance.

With regard to the blood profile, the use of dairy co-products in diets can promote changes in blood concentration, immune system (D'Alessandro et al., 2022), and GIT in newly weaned piglets (Xiao et al., 2016). Total protein (e.g., globulin, albumin) show marked changes in piglets as they age (Tóthová et al., 2021). Marder et al. (1990) reported that total protein concentrations are affected by factors such as dehydration, disease, age, and even the diet provided (e.g., crude protein content and intake).

Although a change in total protein concentration was observed in the pre-starter phase (on day 21 of the experiment), these results were within the average (values of 5.5 g dL^{-1} in piglets) reported by Ekert Kabalin et al. (2017). It has been previously shown that total protein concentrations are affected by the enhanced activity of metabolic changes (e.g., high growth rate of pigs) (Chmielowiec-Korzeniowska et al., 2012) and are associated with the physiological maturation of the liver (París-Oller et al., 2022).

When the complete blood count was evaluated, there was a change only in the concentration of leukocytes, although this increase was kept within the limits considered normal for the species (between 8.7 and 37.7×10^9) (Friendship and Henry, 1992). Hematological changes (e.g., number of leukocytes) in newborn piglets may be associated with the maturation and development of organs (e.g., large intestine) (Ekert Kabalin et al., 2008), as well as with protection of the organism against infections (Czech et al., 2017), because the immune system tries to compensate for a functional immaturity through an increased number of leukocytes (Sipos et al., 2011). Therefore, the blood profile results found were positive for the health and development of the animals in this study. However, more studies are needed to investigate the interference of co-products such as DLP on the blood metabolites of piglets in the nursery phase.

Lactose can be fermented by intestinal bacterial populations (e.g., *Lactobacillus* and *Bifidobacteria*), which produce lactic and acetic acids (Kim et al., 2005). These compounds reduce intestinal pH (although in the present study, there was no effect on the small intestine) and inhibit the growth of pathogenic bacteria such as *E. coli* (Zhao et al., 2021). In the present study, the animals fed the moist diet treatment had lower pH values of the colonic contents. The fermentative processes of lactose or its monosaccharides to generate lactic acid or SCFA (Zhao et al., 2021) promote colonic acidity (Bach Knudsen, 2012). However, piglets fed DLP alone had higher pH values of the colonic content compared with those given the moist diet, which could be related to the form in which the diets were given and the fermentation process of the nutrients.

Short-chain fatty acids can also influence intestinal morphometry, because the colon easily absorbs these compounds and uses them as a source of energy for the growth of colonocytes (Comalada et al., 2006). This fact explains the greater length of the large intestine in piglets from both dietary treatments containing DLP. In a previous study, Zhou et al. (2019) evaluated the concentration of SCFA and the development of the GIT in piglets weaned at 21 days old up to 14 days post-weaning, and observed an increase in the concentration of SCFA and, consequently, a greater length of the large intestine, especially in the colonic portion.

Although no significant results were obtained in the count of intestinal bacterial populations, a previous study (Tran et al., 2012) observed a minor change in the rectal microbial composition of some *Lactobacillus* species in post-weaning piglets fed diets containing 20.0, 15.0, and 5.0% lactose in the pre-starter I and II and starter phases, respectively. In the current study, the *E. coli* count was markedly lower in the ileum of piglets fed the moist diet, influencing the *Lactobacillus*:*E. coli* ratio. The oligosaccharides present in DLP are responsible for improvements in GIT health because these compounds are complex glycans that act as prebiotics and antimicrobials responsible for immune responses in the intestine (Bode, 2012) and for inhibiting the binding of pathogens to intestinal epithelial cells (Smilowitz et al., 2014).

Although we did not observe changes in intestinal morphometry in any of the dietary treatments tested, Hu et al. (2013) reported that the adverse impacts of weaning on intestinal morphometry tend to intensify during the first week post-weaning, reaching a complete recovery by the fourteenth day post-weaning. In the present study, the intestinal morphometry of piglets was evaluated at 35 days post-weaning, when the control diet did not contain lactose, except in animals fed DLP. This may explain the lack of significant results because the animals' intestines had possibly already recovered from injuries or were not affected as expected. Intestinal villi have a quick recovery rate (Amdi et al., 2021), and it is plausible that the piglets had in fact regenerated their intestinal villi, as well as the lactose content ingested at this phase was insufficient to significantly alter the jejunal and ileal morphometry in starter piglets.

Further studies in the nursery phase on the implications of this dairy co-product in the feeding of nursery piglets are crucial.

5. Conclusions

The use of liquid delactosed permeate given alone or mixed as baby feed with a conventional diet containing lactose improves feed conversion in piglets in the pre-starter phases, although a greater DO is observed in these animals in the first few days post-weaning. In addition, the use of DLP in both forms of supply promotes minor changes in other variables (blood profile and GIT traits) in the study, but without negatively affecting the growth rate of piglets in the nursery phase.

Data availability

The entire dataset supporting the results of this study was published in the article itself.

Author contributions

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Conflict of interest

The authors declare no conflict of interest.

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