Addition of *Pichia guilliermondii* yeast to diets for finishing pigs (50-100 kg)

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ABSTRACT
Objective: To determine whether the inclusion of *Pichia guilliermondii* in finishing diets improves productive performance, carcass characteristics, blood metabolites, and modulates the gut microbiota in pigs.
Design/methodology/approach: The study was conducted using a completely randomized design with 28 hybrid pigs (Yorkshire × Landrace × Duroc), with seven replicates per treatment. Four levels of additive inclusion in the diet were evaluated: T0: 0% (control), T1: 0.1%, T2: 0.2%, and T3: 0.3%. The following variables were analyzed: body weight, average daily feed intake (ADFI), average daily gain (ADG), feed gain ratio (FGR), and fat-free lean gain (FFLG). Carcass characteristics included backfat thickness (BFT), Longissimus dorsi muscle area (LMA), and lean meat percentage (LMP). Blood metabolites included total cholesterol (TC) and plasma urea (PU). Fecal bacterial populations of lactic acid bacteria and coliforms were also evaluated.
Results: The inclusion of *Pichia guilliermondii* to finishing pig diets had no effect on most of the variables evaluated, except for an increase in plasma cholesterol levels.
Limitations on study/implications: The physiological stage of pigs is a factor that makes modulation of the gut microbiota difficult.
Findings/conclusions: The inclusion of *Pichia guilliermondii* in finishing diets is not an effective strategy for improving production efficiency or modifying the intestinal microbiota during the finishing stage of fattening pigs, although cholesterol concentration increased in response to supplementation during the Finishing II stage.

Keywords: Yeast, finishing pigs, postbiotic, nutrition, bacteria.

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INTRODUCTION

The swine industry is continuously searching for nutritional technologies that improve growth performance and animal health. Among these strategies are the inclusion of inorganic minerals such as copper, recognized for its effects on growth and immunity

(Espinosa and Stein, 2021); the use of phytobiotic additives such as hydrolyzable tannins with antioxidant and antimicrobial properties (Aguirre-Meza *et al.*, 2016); the incorporation of enzymes such as lysozyme, which acts as a natural antimicrobial agent (CIAP, 2015); and the application of probiotics (Ayala *et al.*, 2022). Among the most commonly used probiotics are lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (Morales-Oñate and Morales-Oñate, 2020), as well as yeasts such as *Saccharomyces* and Pichia guilliermondii (Coda *et al.*, 2013; Sampath *et al.*, 2023).

In response to the growing problem of antimicrobial resistance, the use of yeast-derived probiotics has been proposed as a strategy to promote growth, improve digestion, and enhance the immune system. Specifically, *Saccharomyces cerevisiae* has demonstrated the ability to modulate the intestinal microbiota, promote nutrient absorption, and improve the overall health status of animals, resulting in better feed conversion, greater weight gain, and a reduction in enteric diseases (Pang *et al.*, 2022). The effectiveness of these effects depends on factors such as the strain used, the method of administration, and environmental conditions (Sampath *et al.*, 2023).

On the other hand, *Pichia guilliermondii* has been identified as a yeast with antimicrobial, antioxidant, and antifungal potential, capable of surviving in environments with low nutrient availability and low pH, it also shows immunomodulatory properties and the ability to improve intestinal health and productive performance in monogastric animals (Cardozo *et al.*, 2018). *Pichia guilliermondii* is an inactivated whole-cell yeast with the capacity to chelate enteric pathogens and modulate the host immune system. The cell wall of *P. guilliermondii* is rich in β-glucans and mannans; activation of immune receptors by these components leads to modulation of the immune response. Due to its smaller size, the surface area per unit weight of *P. guilliermondii* is four times greater than that of *Saccharomyces cerevisiae*, allowing a greater binding capacity to pathogens such as *Salmonella enterica* and *Escherichia coli* (Cardozo *et al.*, 2018).

Supplementation of *P. guilliermondii* to sows during gestation and lactation has been shown to improve sow performance at farrowing, including a greater number of piglets born and higher birth weight, as well as improved offspring performance after weaning, reflected in higher weaning weights and better performance during the starter phase. However, the beneficial effects of dietary supplementation with *P. guilliermondii* at different production stages may be inconsistent, as some studies in piglets from supplemented sows have reported no differences in weight gain or feed intake during the lactation and weaning phases, and even adverse effects on feed efficiency have been observed (Oguey and Thayer, 2024).

Because there is evidence that supplementation with *Pichia guilliermondii* in finishing pigs may improve the modulation of intestinal immune homeostasis, reduce systemic inflammation, and decrease the metabolic diversion of energy and amino acids toward immune maintenance, we hypothesized that these effects could be reflected in improvements in intestinal microbiota and growth performance. Therefore, the objective of the present study was to evaluate the use of *Pichia guilliermondii* postbiotic in diets for finishing pigs (50-100 kg BW) and its effects on growth performance, intestinal microbiota, and blood metabolite levels.

MATERIALS AND METHODS

The study was conducted at the Swine Unit of the Experimental Farm of the Colegio de Postgraduados, Montecillo Campus, located in the municipality of Texcoco, State of Mexico (98° 48′′27" W and 19° 48′′23" N, at an altitude of 2,245 m above sea level). The climate is temperate with summer rainfall, with a mean annual temperature of 15.9 °C and an average annual precipitation of 644.8 mm (García de Miranda, 2004).

Animal management was carried out in strict compliance with the Regulations for the Use and Care of Animals for Research of the Colegio de Postgraduados (COBIAN, 2023), under protocol number COBIAN/010/24. A total of 28 hybrid pigs (Yorkshire × Landrace × Duroc), including both sexes (gilts and barrows), were used in the experiment. The animals entered the study with an average initial body weight of 50.51 ± 1.89 kg. The pigs were housed in individual pens measuring 1.2 × 1.5 m with the following characteristics: flooring consisted of solid concrete partially covered with plastic slats for liquid drainage. Water was provided *ad libitum* through a nipple drinker, and feed was offered *ad libitum* using a hopper feeder.

Cleaning of the facility and inspection of the pigs' health status were carried out daily throughout the experimental period to ensure animals welfare.

Treatments and diets

An experiment with four treatments was designed, consisting of a basal diet (with a standard protein level) supplemented with three increasing levels of inactivated *Pichia guilliermondii* yeast (considered a postbiotic). The inactivated *P. guilliermondii* yeast was obtained from the commercial product CitriStim® (Pancosma, ADM). CitriStim® is an inactivated whole-cell yeast primarily obtained as a by-product of citric acid production. The inclusion levels were selected according to the manufacturer's recommendations. The treatments were as follows: T0: control; T1: 0.1%; T2: 0.2%; and T3: 0.3%. The ingredient composition of each experimental diet is detailed in Tables 1 and 2. The diets were formulated using common ingredients in swine nutrition, including corn, soybean meal, synthetic amino acids (L-lysine, DL-methionine, L-threonine, and L-tryptophan), supplemented with vitamin and mineral premixes. Nutrient levels were established according to the requirements recommended by the National Research Council (NRC, 2012). Diet formulations for all treatments were calculated to be isoproteic and isoenergetic using the Solver tool in Excel (version 365, Microsoft Corporation, 2019).

Variables and samples collection

To validate the nutritional composition of the experimental diets (control and treatments supplemented with *Pichia guilliermondii*), a proximate chemical analysis of the Finishing I and Finishing II diets was performed. The analyzed parameters were determined according to AOAC methods (2012; 2023) and the system proposed by Van Soest *et al.* (1991).

Growth performance: The body weight of each pig was individually recorded at the beginning and end of each stage (Finishing I and II) to determine average daily gain (ADG) and final body weight (FBW). Average daily feed intake (ADFI) was also recorded. Feed:gain ratio (FGR) was calculated using ADG and ADFI values.

Table 1. Ingredients and calculated nutritional composition of experimental diets for pigs in the Finishing I stage (50-75 kg) supplemented with *Pichia guilliermondii* yeast.

Ingredient	T0 (0%)	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)
Corn grain	82.39	82.31	82.23	82.15
Soybean meal	15.31	15.23	15.15	15.07
Soybean oil	0.09	0.14	0.20	0.25
L-Lysine	0.25	0.26	0.26	0.26
DL-Methionine	0.00	0.00	0.00	0.00
L-Threonine	0.00	0.01	0.01	0.01
L-Tryptophan	0.01	0.01	0.01	0.01
Calcium carbonate	1.12	1.12	1.11	1.11
Ortophosphate	0.01	0.01	0.01	0.01
Mycotoxins sequestrant	0.40	0.40	0.40	0.40
Vitamins premix	0.03	0.03	0.03	0.03
Minerals premix	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Yeast	0.00	0.10	0.20	0.30
Estimated Nutritional Content (%)				
ME (Mcal kg^{-1})	3.27	3.27	3.27	3.27
Crude Protein	13.00	13.00	13.00	13.00
Calcium	0.50	0.50	0.50	0.50
Phosphorus	0.30	0.30	0.30	0.30
Lysine	0.61	0.61	0.61	0.61
Threonine	0.37	0.37	0.37	0.37
Tryptophan	0.10	0.10	0.10	0.10
Arginine	0.65	0.65	0.65	0.64
Histidine	0.37	0.37	0.37	0.37
Isoleucine	0.41	0.41	0.41	0.41
Leucine	1.34	1.34	1.33	1.33
Valine	0.49	0.49	0.49	0.48
Methionine + Cystine	0.36	0.36	0.35	0.35
Determined Nutritional Content (%)				
Dry Mater	87.13	86.89	86.96	86.96
Ashes	2.27	2.23	2.77	2.76
Crude Protein	12.92	14.63	13.96	14.23

[A] Provided per kg of feed: vitamin A, 15,000 IU; vitamin D3, 2,500 IU; vitamin E, 37.5 IU; vitamin K, 2.5 mg; thiamine, 2.25 mg; riboflavin, 6.25 mg; niacin, 50 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.0375 mg; biotin, 0.13 mg; choline chloride, 563 mg; pantothenic acid, 20 mg; folic acid, 1.25 mg.
[B] Provided per kg of feed: Fe, 150 mg; Zn, 150 mg; Mn, 150 mg; Cu, 10 mg; Se, 0.15 mg; I, 0.9 mg; Cr, 0.2 mg.

Carcass Characteristics: Body composition variables were measured using the real-time ultrasound device Sonovet 600 equipped with a 3.5 MHz convex transducer (Medison Inc., Cypress, CA, USA). Measurements taken at the level of the tenth rib included backfat thickness (BFT) and *Longissimus dorsi* muscle area (LMA) at the beginning and end of

Table 2. Ingredients and calculated nutritional composition of experimental diets for pigs in the Finishing II stage (75-110 kg) supplemented with *Pichia guilliermondii* yeast.

Ingredient	T0 (0%)	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)
Corn grain	85.57	85.555	85.54	85.52
Soybean meal	12.71	12.621	12.53	12.44
Soybean oil	0.00	0.000	0.00	0.00
L-Lysine	0.17	0.173	0.18	0.20
DL-Methionine	0.00	0.00	0.00	0.00
L-Threonine	0.00	0.00	0.00	0.00
L-Tryptophan	0.00	0.00	0.00	0.00
Calcium carbonate	0.99	0.99	0.99	0.99
Ortophosphate	0.04	0.04	0.04	0.05
Mycotoxins sequestrant	0.10	0.10	0.10	0.10
Vitamins premix	0.03	0.03	0.03	0.03
Minerals premix	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Yeast	0.00	0.10	0.20	0.30
Estimated Nutritional Content (%)				
ME (Mcal kg^{-1})	3.28	3.28	3.27	3.27
Crude Protein	12.00	12.00	12.00	12.00
Calcium	0.45	0.45	0.45	0.45
Phosphorus	0.30	0.30	0.30	0.30
Lysine	0.55	0.55	0.55	0.55
Threonine	0.33	0.33	0.33	0.33
Tryptophan	0.09	0.09	0.09	0.09
Arginine	0.59	0.58	0.58	0.58
Histidine	0.35	0.34	0.34	0.34
Isoleucine	0.37	0.37	0.37	0.37
Leucine	1.28	1.28	1.28	1.27
Valine	0.45	0.45	0.45	0.45
Methionine + Cystine	0.34	0.34	0.33	0.33
Determined Nutritional Content (%)				
Dry Mater	87.14	86.70	87.13	87.25
Ashes	4.37	4.55	4.40	3.37
Crude Protein	15.30	13.54	13.05	12.53

[A] Provided per kg of feed: vitamin A, 15,000 IU; vitamin D3, 2,500 IU; vitamin E, 37.5 IU; vitamin K, 2.5 mg; thiamine, 2.25 mg; riboflavin, 6.25 mg; niacin, 50 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.0375 mg; biotin, 0.13 mg; choline chloride, 563 mg; pantothenic acid, 20 mg; folic acid, 1.25 mg.
[B] Provided per kg of feed: Fe, 150 mg; Zn, 150 mg; Mn, 150 mg; Cu, 10 mg; Se, 0.15 mg; I, 0.9 mg; Cr, 0.2 mg.

each stage. These data were used to calculate fat free lean gain (FFLG) and lean meat percentage (LMP) using procedure 5 proposed by Burson and Berg (2001).

Blood metabolites: For the determination of blood metabolites, blood samples were collected from each pig on the last day of each experimental stage. Blood samples were

obtained by puncture of the anterior vena cava using heparinized Vacutainer® tubes (BD Vacutainer Systems, NJ, USA). The tubes were identified and stored on ice for transport to the laboratory. Once in the laboratory, the samples were centrifuged using a Sigma 2-16k centrifuge (Germany) at 1,788 × g for 20 min to separate the plasma from the cellular fraction. The plasma from each sample was transferred into properly labeled 2 mL polypropylene Eppendorf tubes. Plasma samples were stored at −20 °C until biochemical analysis. Serum levels of total cholesterol and urea were analyzed as indicators of metabolic status. Determinations were performed using commercial enzymatic kits specific for each metabolite (SPINREACT®: Cholesterol-LQ and Urea 37). Absorbance readings were obtained using a visible-light spectrophotometer (Beckman DU65 spectrophotometer) at the following wavelengths: cholesterol, 505 nm; and urea, 510 nm.

Fecal Microbial Populations: Two samplings were carried out, one at the end of each fattening stage (Finishing I and Finishing II), to evaluate the dynamics of intestinal microbial populations using the viable plate count method. Fecal samples were obtained by direct rectal stimulation using a sterile lubricated glove to collect the feces. Immediately after collection, samples were placed in sterile bags, identified, and stored in a cooler for transport and immediate laboratory analysis. Serial dilutions were prepared to obtain concentrations of 10^{-4}, 10^{-5}, and 10^{-6}, which were used for spread plating. The surface spread plate technique was performed using a glass spreader, inoculating 100 μL of the selected dilutions in duplicate onto Petrifilm™ plates. MacConkey agar was used to quantify total coliforms and *Escherichia coli*, whereas MRS agar was used to quantify lactic acid bacteria. All plates were incubated at 29 °C. Petrifilm™ plates were monitored and quantified for seven days until no further colony growth was observed.

After incubation, colonies were counted on plates containing between 30 and 300 colony-forming units (CFU). To standardize the results, the dry matter (DM) content of each fecal sample was determined by drying an aliquot in an oven at 50 °C until constant weight was achieved. Final bacterial counts were expressed as CFU per gram of dry matter (CFU/g DM), and data were transformed to base-10 logarithms (Log_{10}) for statistical analysis.

Statistical Analysis

The study was conducted under a completely randomized design with four treatments using a total of 28 experimental units during the two fattening periods (Finishing I and Finishing II), with seven replicates per treatment in each stage. The number of replicates was established considering a coefficient of variation (CV%) of 6% for average daily gain (ADG) and a minimum detectable difference of 10% (Johnston *et al.*, 2003). The CV information for ADG in finishing pigs was obtained from previous studies conducted within our research line.

The experimental unit consisted of one pig housed in an individual pen, ensuring the statistical independence of observations for the proper application of analysis of variance and avoiding confounding effects due to competition or social interaction. Statistical analyses were performed using SAS (SAS Institute Inc., 2002).

The effects of treatments on growth performance, carcass characteristics, blood metabolites, and bacterial populations were analyzed using analysis of variance (ANOVA) through the GLM (General Linear Model) procedure. Subsequently, Tukey's honestly significant difference (Tukey's HSD) test was used to compare treatment means at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Finishing I stage

Growth performance

Treatments supplemented with the *P. guilliermondii*-based postbiotic did not induce statistically significant changes ($P > 0.05$) in any of the growth performance variables evaluated during the Finishing I period, including final body weight (FBW), average daily feed intake (ADFI), average daily gain (ADG), and feed:gain ratio (FGR). Average daily gain remained between 0.72 and 0.80 kg d^{-1} across all groups. Lean meat gain was also similar, ranging from 0.265 to 0.280 kg d^{-1} ($P = 0.89$), as shown in Table 1.

This lack of response has previously been reported in finishing pigs, where direct supplementation with *P. guilliermondii* during advanced production phases does not always result in improved growth performance (Bass *et al.*, 2022). This contrasts with studies in piglets, in which the yeast has demonstrated potential to improve feed conversion and weight gain, effects attributed to the modulation of the microbiota and improved nutrient digestibility in the immature intestine (de Paula *et al.*, 2022; Thayer *et al.*, 2023). The absence of effects during the finishing stage may be associated with the greater physiological and microbial stability of adult pigs, or because the inclusion levels used were insufficient to generate significant benefits during a phase in which nutritional demands for growth are high.

Carcass Characteristics

Consistent with the growth performance results, the main carcass parameters measured at the end of the experimental period showed no significant differences among treatments ($P > 0.05$). Specifically, final *Longissimus dorsi* muscle area and final backfat thickness were statistically similar among treatments. Final lean meat percentage also remained unchanged ($P = 0.69$). These results suggest that the postbiotic, at the inclusion levels evaluated, did not alter nutrient partitioning in a manner that favored lean tissue deposition over adipose tissue during this critical growth phase.

Other studies have reported a positive carryover effect following maternal supplementation, indicating greater lean meat gain and improved carcass performance in the offspring (Bass *et al.*, 2019; Thayer *et al.*, 2023). The similarity in carcass results observed in the present study reinforces the idea that the effectiveness of the additive may depend on the production stage or the route of supplementation.

Blood metabolites

Analysis of blood metabolites revealed that plasma urea concentration (PU) remained unchanged among treatments ($P = 0.72$). PU is an indicator of amino acid catabolism;

therefore, the absence of differences suggests that inclusion of the postbiotic did not affect protein utilization efficiency or nitrogen balance in the pigs, which may explain the lack of effect on LMA (Montoya *et al.*, 2018; Bass *et al.*, 2019).

In contrast, a significant effect on total cholesterol concentration (TC) was detected (P=0.0036) in response to dietary supplementation with *P. guilliermondii*. Pigs in T2 reached the highest TC concentration (180.57 mg dL^{-1}), which was significantly higher than those observed in T1 (144.23 mg dL^{-1}) and T3 (135.35 mg dL^{-1}). This result contrasts with the hypocholesterolemic effects commonly reported for probiotic yeasts.

The use of yeasts and yeast cell wall products in pig diets has generally been associated with reductions in total serum cholesterol and LDL cholesterol levels compared with control groups, since *P. guilliermondii* may act as a modulator of the microflora and enhance the binding of pathogenic bacteria, thereby indirectly influencing lipid metabolism through improvements in digestive health (Marrero *et al.*, 2014; Ruiz *et al.*, 2017; Tenea *et al.*, 2021).

The increase in TC observed in T2, in the absence of an increase in final backfat thickness, suggests that the postbiotic did not promote lipid accumulation in subcutaneous adipose tissue, but rather modified cholesterol turnover and circulation in the serum. It is possible that the inclusion of *P. guilliermondii* affected bile salt reabsorption at the intestinal level. Reduced bile salt reabsorption forces the liver to synthesize new bile salts from circulating cholesterol, a process that typically lowers TC levels. If the postbiotic modulated this process, it may have caused a transient accumulation of lipoproteins in the plasma (García *et al.*, 2006; Montoya *et al.*, 2018).

Microbial variables

The results of the microbiological analysis showed no significant differences (P>0.05) among treatments for any of the variables measured during Finishing I, as shown in Figure 1.

Lactic Acid Bacteria (LAB) Counts

LAB counts were not affected by inclusion of the postbiotic (P=0.72), with values ranging from 9.09 to 9.58 Log CFU g DM^{-1}. These results indicate that *P. guilliermondii* yeast did not exert a modulatory effect on the microbiota sufficient to significantly increase LAB populations in the digestive tract of finishing I pigs. The microbial stability of adult pigs, which already possess an established microbiota, may mask the stimulatory effects commonly observed in piglets. Although *P. guilliermondii* strains have demonstrated the ability to stimulate bacterial growth in *in vitro* systems, their *in vivo* performance depends on the specific interaction with the diet and the ecological niche already occupied within the gastrointestinal tract (Ruiz, 2016; Montoya *et al.*, 2018).

Coliforms

Coliform bacteria also showed no significant differences among treatments (P=0.70), with values ranging from 7.46 to 8.39 Log CFU g DM^{-1}. The absence of a reduction in coliform counts in the supplemented groups contrasts with the competitive exclusion

Table 3. Growth performance variables in pigs during the Finishing I stage.

Variable	T0	T1	T2	T3	EEM	P lineal
Initial BW (Kg)	47.25	50.57	51.28	50.42	5.15	-
Final BW (Kg)	79.71	84.57	81.71	82	13.93	0.89
ASFI (Kg d^{-1})	2.48	2.52	2.57	2.47	0.11	0.95
ADG (Kg d^{-1})	0.77	0.80	0.72	0.75	0.02	0.65
FGR	3.25	3.15	3.56	3.31	0.10	0.11
FFLG (Kg d^{-1})	0.28	0.28	0.27	0.27	0.01	0.89
LMAi (cm^2)	20.77	21.51	22.60	21.93	1.39	0.82
LMAf (cm^2)	30.97	31.18	33.48	32.14	2.55	0.77
LMPi	42.13	42.19	42.66	42.57	3.18	0.92
LMPf	39.86	39.22	40.37	40.10	3.43	0.69
BFTi (mm)	0.81	0.77	0.78	0.75	0.01	0.35
BFTf (mm)	1.3	1.21	1.24	1.25	0.02	0.74
Blood Metabolites (mg dL^{-1})						
TC	156.74 ab	144.23 b	180.57 a	135.35 b	4.55	0.0036
PU	20.07	23.29	19.31	20.51	4.7	0.72

T0: Control; T1: Standard protein + 1 kg t^{-1}; T2: Standard protein + 2 kg t^{-1}; T3: Standard protein + 3 kg t^{-1}. BWi: Initial body weight; BWf: Final body weight; ADFI: Average daily feed intake; ADG: Average daily gain; FGR: Feed:gain ratio; FFLG: Fat Free Lean gain; LMAi: Initial *Longissimus dorsi* muscle area; LMAf: Final *Longissimus dorsi* muscle area; LMPi: Initial lean meat percentage; LMPf: Final lean meat percentage; TC: Total cholesterol; PU: Plasma urea nitrogen. a, b, c Different letters indicate statistically significant differences ($P \leq 0.05$).

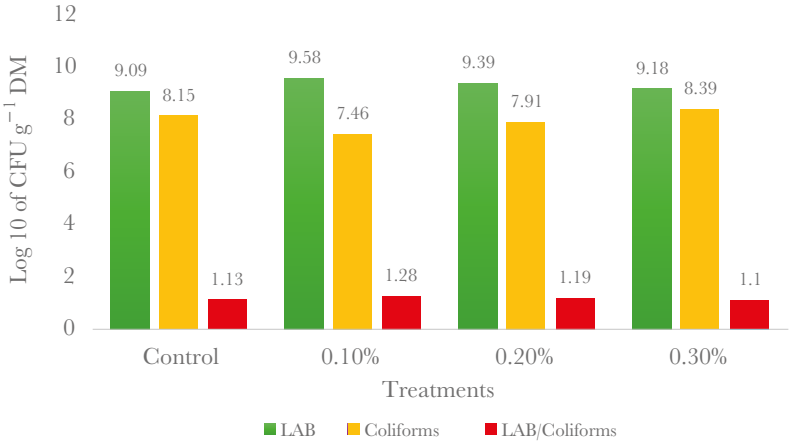

Figure 1. Effect of supplementation with the postbiotic (*P. guilliermondii*) on the concentrations of lactic acid bacteria, coliforms (LAB), and the ratio in the intestine of pigs during the Finishing I stage. Treatment means or main effects with different letters indicate statistical differences ($P<0.05$). CFU g^{-1} DM. Ratio: LAB/Coliforms.

activity expected from yeasts such as *P. guilliermondii* (Tenea *et al.*, 2021). These results indicate that the postbiotic did not exert a direct suppressive effect on pathogens, or that its antimicrobial activity was insufficient to displace the pre-existing coliform populations during this stage. Studies involving *P. guilliermondii* have focused primarily on modulation of the immune response (Bass *et al.*, 2019) and improvement of overall intestinal health (de

Paula *et al.*, 2022); however, the ability to reduce coliform load in the intestine of finishing I pigs was not demonstrated in the present experiment.

LAB/Coliform Ratio

The LAB/coliform ratio was similar among all groups (P=0.61), ranging from 1.10 to 1.28. This ratio is considered a key and sensitive indicator of intestinal microbial balance and mucosal health. A higher ratio (reflecting a greater proportion of lactic acid bacteria relative to coliforms) indicates a more favorable intestinal environment associated with improved nutrient absorption and a lower load of toxic metabolites produced by Gram-negative bacteria. The lack of modulation observed in the present study indicates that the postbiotic was not able of exerting sufficiently strong competitive exclusion to stimulate the growth of LAB populations (Montoya *et al.*, 2018).

Finishing II Stage: growth performance

During the Finishing II phase, inclusion of the *P. guilliermondii* postbiotic did not produce changes in BWf, ADFI, ADG, or FGR (P>0.05). FFLG was similar among all treatments, as shown in Table 4 (P=0.88).

The absence of response in Finishing II pigs agrees with the findings of Bass *et al.* (2022), who reported that the effectiveness of this additive is expressed as a carryover effect when administered during gestation and lactation, resulting in piglets with greater body weight and improved performance during the nursery stage, although these benefits dissipate toward the finishing phase (Bass *et al.*, 2019; Thayer *et al.*, 2023). In finishing pigs, digestive physiology is already mature and, consequently, the microbiome is more stable; therefore, the postbiotic is unable to generate a significant impact on intestinal colonization and modulation.

Carcass characteristics

Carcass variables measured at the end of Period II showed no significant differences among treatments (P>0.05). Both LMAf (37.57 to 39.95 cm^2), BFTf (1.50 to 1.65 mm), and LMPf (38.81 to 39.87%) were statistically similar among treatments. This similarity in carcass composition was expected given the absence of effects on FFLG and ADG. If the additive does not influence nutrient absorption in favor of muscle deposition over adipose tissue, carcass indicators remain unchanged (Bass *et al.*, 2022). Although the mechanism of action is associated with improvements in intestinal health and immunity, the translation of these effects into improvements in carcass quality during the finishing phase is uncommon. Thayer *et al.* (2023) evaluated carcass responses to direct supplementation or carryover effects and found that carcass characteristics are generally not affected by the inclusion of yeast in pig diets. This confirms that, during the final stages of growth, body composition is highly determined by genetics and dietary energy density.

Blood Metabolites

Analysis of blood metabolites during Period II revealed that TC and PU concentrations were similar among all groups. PP remained between 20.31 and 24.78

mg dL^{-1} (P=0.68), whereas TC concentrations ranged from 178.04 to 190.60 mg dL′1 (P = 0.90).

The similarity in plasma urea indicates that protein utilization remained constant, which is consistent with the absence of effects on FFLG and a stable PU value indicates that the postbiotic did not improve absorption efficiency during the final weeks of the fattening period (Montoya *et al.*, 2018).

Regarding TC, the absence of significant differences during this period (P=0.90) contrasts with the modulation observed during Period I (P=0.0036). Although *P. guilliermondii* biomass has the capacity to assimilate cholesterol *in vitro* due to the β-glucan composition of its cell wall (Tenea *et al.*, 2021), the *in vivo* dynamics are more complex. During this finishing phase, pigs have greater body weight and a higher growth rate, which may reflect a mechanism of metabolic adaptation or greater homeostatic stability in pigs with a fully developed physiological system (Marrero *et al.*, 2014). Unlike piglets, finishing pigs have already established a balance between hepatic cholesterol synthesis and biliary excretion.

It is possible that the postbiotic dose was insufficient to increase bile salts binding, since the mechanism involved in cholesterol reduction is the inhibition of bile salts reabsorption, forcing the liver to utilize serum cholesterol for the synthesis of new bile salts. Therefore, in animals with greater metabolic body weight, the effect of the postbiotic on hepatic and serum cholesterol recirculation is minimal, which may explain the lack of a significant response during Finishing II period.

Table 4. Productive performance variables in pigs during the Finishing II stage.

Variable	T0	T1	T2	T3	EEM	P lineal
BWi (Kg)	79.71	84.57	81.71	82	1.39	-
BWf (Kg)	97.85	102.57	101.28	98.57	2.15	0.92
ADFI (Kg d^{-1})	2.97	3.05	3.11	2.99	0.16	0.92
ADG (Kg d^{-1})	0.86	0.85	0.93	0.78	0.03	0.60
FGR	3.75	3.60	3.41	3.88	0.61	0.71
FFLG (Kg d^{-1})	0.29	0.31	0.33	0.31	0.01	0.88
LMAi (cm^2)	30.90	31.18	33.48	32.14	2.55	0.77
LMAf (cm^2)	37.57	38.36	39.35	39.95	3.73	0.89
LMPi	39.86	39.22	40.37	40.10	3.43	0.69
LMPf	38.81	38.85	39.40	39.87	2.32	0.52
BFTi (mm)	1.2	1.22	1.24	1.22	0.03	0.97
BFTf (mm)	1.65	1.58	1.5	1.52	0.07	0.73
Blood Metabolites (mg dL^{-1})						
TC	190.60	188.12	178.04	187.64	11.48	0.90
PU	23.48	20.31	24.78	23.51	5.06	0.68

T0: Control; T1: Standard protein + 1 kg t^{-1}; T2: Standard protein + 2 kg t^{-1}; T3: Standard protein + 3 kg t^{-1}; BWi: initial body weight; BWf: final body weight; ADFI: Average daily feed intake; ADG: Average daily gain; FGR: Feed:gain ratio; FFLG: Fat free lean gain; LMAi: Initial *Longissimus dorsi* muscle area; LMAf: Final *Longissimus dorsi* muscle area; LMPi: Initial lean meat percentage; LMPf: Final lean meat percentage; BFTi: Initial back fat thickness; BFTf: Final back fat thickness; TC: Total cholesterol; PU: Plasma urea nitrogen concentration. a, b, c Different superscript indicates statistical difference (P≤0.05). Letters are shown only when differences were detected.

Lactic acid bacteria counts

The population of lactic acid bacteria quantified on MRS agar did not differ significantly among treatments, as shown in Figure 2 (P=0.48), with values ranging from 8.90 (T2) to 9.66 (T0) CFU g DM^{-1}. The absence of a positive response in LAB populations indicates that *P. guilliermondii* did not act as a trophic agent for the beneficial microbiota. Although *P. guilliermondii* may contain metabolites capable of stimulating fermentation, finishing pigs possess a more stable and consolidated microbiome that is resistant to low-level modulation by non-viable additives (Gutiérrez-Acosta *et al.*, 2016). Fermentation in the large intestine of these animals already generates optimal production of short-chain fatty acids (SCFA), indicating that the impact of inactivated yeast on promoting additional LAB growth is minimal.

Coliforms

Coliform counts on MacConkey agar also showed no significant differences among experimental groups (P=0.69). Values ranged from 6.73 CFU g DM^{-1} (T0) to a maximum of 7.55 CFU g DM^{-1} (T3), without a clear reduction trend attributable to the postbiotic. Yeast in the form of cell wall components or metabolites acts as an immunostimulant through binding to pathogen adhesion sites in the small intestine. However, in the large intestine of finishing pigs, from which the samples were obtained, the microbiota has already reached a high degree of complexity and density.

The stability of coliform populations is consistent with the lack of effects on ADG, since pathogen reduction is a primary pathway for improving performance in young animals (Bass *et al.*, 2019; Zhao and Yang, 2025).

LAB/Coliform Ratio

The LAB/coliform ratio was similar among all treatments, with values of 1.45 in T0 and 1.22 in T3. This ratio confirms that the *P. guilliermondii* postbiotic did not alter the

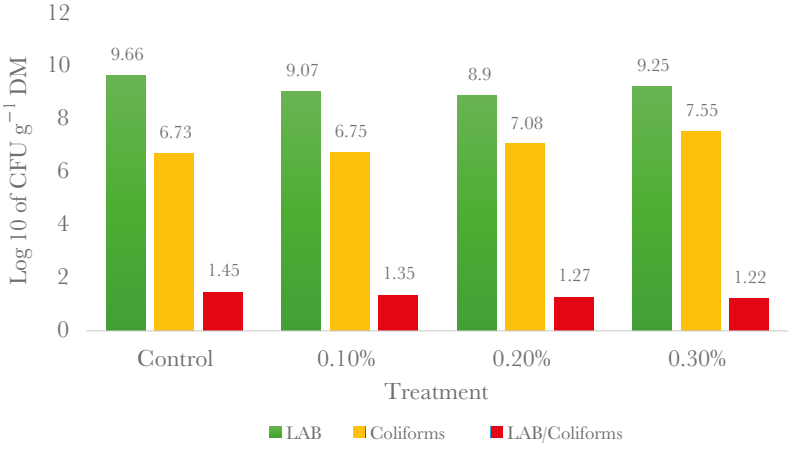

Figure 2. Effect of supplementation with the postbiotic (*P. guilliermondii*) on the concentrations of lactic acid bacteria (LAB), coliforms, and their ratio in the intestine of pigs during the Finishing II stage. Treatment means or main effects with different letters indicate statistical differences (P<0.05). CFU g^{-1} DM. Ratio: LAB/Coliforms.

overall ecological balance of the microbiota. Since neither LAB populations increased nor coliform counts decreased individually, the ratio remained similar to that observed during Period I.

These findings suggest that, for *P. guilliermondii* to exert beneficial effects during the final stages of fattening, modulation should occur indirectly as a carryover effect from supplementation during gestation, lactation, or the piglet phase, when microbiome functionality and immunity are improved during critical developmental stages (Bass *et al.*, 2022; Thayer *et al.*, 2022; Choi, 2023). The impact of the yeast during the finishing stages appears to be marginal when health status and microbiota are already optimized.

CONCLUSIONS

This experiment demonstrated that supplementation with the postbiotic derived from Pichia guilliermondii in finishing pigs exhibits only marginal effectiveness when administered during the finishing stage of fattening pigs. The results showed no impact on the analyzed variables, with the exception of increased total cholesterol during the Finishing I stage in pigs supplemented with the postbiotic, providing an indirect evaluation of the additive-host interaction in physiologically mature animals.

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