FULL ARTICLE

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Can *Saccharomyces cerevisiae* supplementation improve piglets' performance and intestinal health after weaning?*

A suplementação com Saccharomyces cerevisiae pode melhorar o desempenho e saúde intestinal de leitões após o desmame?

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ABSTRACT

This study aimed to assess the impact of two commercial Saccharomyces cerevisiae strains (CHY1 and CHY2) on the intestinal health and performance of weaned piglets challenged with enterotoxigenic *Escherichia coli* during the nursery phase. One hundred ninety-two piglets with an average weight of 6.70 ± 0.92 kilograms were allocated in a randomized block design to one of four treatments: a negative control (C) without *E. coli* challenge and no yeast supplementation; a positive control (CH) with *E. coli* challenge and no yeast supplementation; and two treatment groups receiving an *E. coli* challenge with a CHY1 and CHY2 yeast strain supplementation. The challenge involved inoculating piglets with two dosages of *E. coli* F4 (106 CFU/ml and 109 CFU/ml) and a saline solution for the C group. Samples of intestinal tissue, blood, and cecal content were collected on the trial's 11th, 28th, and 42nd days. All variables were subjected to analysis of variance, and upon detecting significant differences via the F-test ($p < 0.05$), Tukey's test was applied to compare treatment means. For the analysis of diarrhea occurrence, the Kruskal-Wallis test was applied. When variables were rejected at a 5% probability level, a Dunn's test was conducted as a post-hoc analysis for paired multiple comparisons ($p < 0.05$), with statistical significance set at this level. Weaned piglets supplemented with CHY1 exhibited superior performance metrics, including higher average daily gain (15.3% increase), body weight (3.4% increase), feed-to-gain ratio (9.5% increase), and average daily feed intake (12.3% increase) at 28 days compared to the CH group across two different nutritional phases. No discernible effects were observed on measuring blood parameters, intestinal morphology, or cecal short-chain fatty acids. Both yeast-treated groups displayed improved performance during the most challenging periods. However, the CHY1 yeast strain contributed to enhanced piglet performance in the initial 28 days without inducing changes in intestinal morphology.

Keywords: *Escherichia coli*. Gut microbiota. Nursery phase. Probiotics. Swine.

RESUMO

O presente estudo teve como objetivo avaliar duas cepas comerciais de *Saccharomyces cerevisiae* (designadas como CHY1 e CHY2) sob a saúde intestinal e desempenho de leitões desmamados desafiados com *Escherichia coli* enterotoxigênica durante a fase de creche. Um total de 192 leitões com peso médio 6,70 ± 0.92 quilogramas foram distribuídos em um delineamento em blocos casualizados com quatro tratamentos: um controle negativo (C) sem desafio de *E. coli* e sem suplementação da levedura; um controle positivo (CH) com desafio de *E. coli* e sem a suplementação da levedura; e dois grupos com a suplementação das dietas com as cepas comerciais das leveduras intituladas CHY1 e CHY2, juntamente ao desafio de *E. coli.* O desafio envolveu a inoculação de duas doses de *E. coli* F4 (106 UFC/ml e 109 UFC/ml) nos leitões e uma inoculação de solução salina para o grupo C. Amostras de tecido intestinal, sangue e conteúdo cecal foram

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coletadas nos 11º, 28º e 42º dias do experimento. Todas as variáveis foram submetidas a análise de variância e quando detectada diferença significativa pelo teste de F (p<0,05), o teste de Tukey foi aplicado para comparar as médias. Para a avalição da ocorrência de diarreia, o teste de Kruskal-Wallis foi aplicado e quando as variáveis foram rejeitadas ao nível de 5% de probabilidade, o teste de Dunn foi conduzido como uma análise post-hoc para comparações múltiplas (p<0,05) com significância estatística nesse nível. Leitões desmamados suplementados com CHY1 apresentaram métricas de desempenho superiores, incluindo maior ganho de peso diário (aumento de 15.3%), peso vivo (aumento de 3.4%), consumo de ração diário (aumento de 9.5%) e melhor eficiência alimentar (aumento de 12.3%) até os 28 dias de experimento em comparação com o grupo CH. Não foram observados efeitos dos tratamentos sobre os parâmetros sanguíneos mensurados, morfologia intestinal ou ácidos graxos de cadeia curta presentes no conteúdo cecal. Ambos os grupos tratados com leveduras apresentaram melhor desempenho durante os períodos mais desafiadores. No entanto, a cepa de levedura CHY1, especificamente, contribuiu para um melhor desempenho dos leitões nos primeiros 28 dias, sem induzir alterações na morfologia intestinal.

Palavras-chave: *Escherichia coli.* Microbiota intestinal. Creche. Probióticos. Suínos.

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Introduction

Immediately following the weaning process, piglets undergo a series of social, physiological, and dietary changes that significantly impact the composition of their gut microbiota (Gao et al., 2019; St-Pierre et al., 2023). This period post-weaning is a time of dynamic adjustments, as piglets deftly reshape their gut microbiota in response to dietary adjustments (Karasova et al., 2021). However, during this weaning phase, the stage is set for a complex interplay: the genesis of intestinal issues and ailments (St-Pierre et al., 2023). Post-weaning diarrhea, a multi-faceted affliction affecting piglets, emerges from variables such as altered dietary patterns, genetic predispositions of piglets, and the potential presence of specific pathogens, notably enterotoxigenic *Escherichia coli* (Cremonesi et al., 2022; Karasova et al., 2021). Moreover, the stressors accompanying this weaning period often coalesce, leading to reduced growth, decreased overall performance, and an amplified vulnerability to post-weaning diarrhea among these young pigs (Gao et al., 2019; Markowiak & Śliżewska, 2018).

Historically, antimicrobials have been prescribed by veterinarians, and incorporated into nursery-phase diets to offset these adverse effects and promote gut health (Cremonesi et al., 2022). However, global pressures enforcing the reduction and elimination of antibiotic use in livestock have resulted in a growing demand to identify viable and effective alternatives to mitigate post-weaning diarrhea and delayed growth performance (Maron et al., 2013).

One promising alternative studied in the literature includes the use of probiotics, defined as live microorganisms that, when ingested in adequate quantities, impart advantageous effects on the host's health (Food and Agricultural Organization of the United Nations, 2002; Hill et al., 2014), more specifically, yeast strains, into the nursery phase diet to mitigate diarrhea and improve productivity (Boontiam et al., 2022). Yeast strains have shown immense potential for providing positive nutritional benefits in swine (Chaucheyras-Durand & Durand, 2010), and recent work by Zhaxi et al. (2020) indicates that certain yeast strains, such as *Saccharomyces cerevisiae* can influence the intestinal microbiota by outcompeting bacteria for adhesion sites. Thus, limiting bacterial proliferation, enhancing IgA secretion in the intestinal mucosa, and improving the integrity of the intestinal wall (Zhaxi et al., 2020). These physiological benefits are associated with enhanced productive performance and a reduction in the occurrence of diarrhea (Bontempo et al., 2006; Kiros et al., 2018).

To date, there are 34 yeast products available for use as an additive in swine feed in the Brazilian market (Brasil, 2020). However, limited research directly evaluates the efficacy of such commercially available yeast probiotics and compares each product within the same experimental

environment (Jiang et al., 2015). Therefore, the primary objective of this study was to assess the impact of two distinct commercial strains of *Saccharomyces cerevisiae* (designated as CHY1 and CHY2) on the intestinal health and overall performance of weaned piglets subjected to a challenge with enterotoxigenic *Escherichia coli* (ETEC) during the nursery phase.

Material and Methods

The study was conducted at the Swine Research Laboratory (LPS) located at the University of São Paulo (USP), Pirassununga, São Paulo, Brazil (21°59'46"S and 47°25'36"W). The research complied with the Guidelines of the Institutional Ethics Committee on Animal Use set from FMVZ/USP (Protocol: CEUA 3743220518).

Animals, facilities, and experimental design

A total of 192 24 days-old, large white-landrace barrows and gilts (n= 96 barrows, n=96 gilts; Choice Genetics' terminal cross lineage; 6.70 ± 0.92 kg) were purchased from a commercial herd located within the state and used for the study. Weaned piglets were transported to the university and housed in 48 pens, into groups of four piglets at the USP nursery facility. The pens had a stocking density of 0.35m^2 per pig, and the flooring comprised solid and slatted floors. Piglets were provided ad-libitum feed and water via semi-automatic feeders and nipple drinkers. The room temperature control was achieved by manually manipulating curtains and heating lamps.

The experimental design followed a randomized block structure based on initial weight and sex, comprising four treatments, each with 12 replications.

Treatment groups:

1. C – control group without an ETEC challenge and no inclusion of a yeast strain in the feed;
2. The CH control group was subjected to an ETEC challenge but without adding a yeast strain probiotic in the feed;
3. CHY1 – yeast strain probiotic 1 (2.0 kg per ton of probiotic during pre-starter 1, reduced to 1 kg per ton on the pre-starter 2 and starter diets) and challenged with ETEC;
4. CHY2 – yeast strain probiotic 2 (2.0 kg per ton of probiotic during pre-starter 1, reduced to 1.0 kg per ton on the pre-starter 2 and starter diets, also with an ETEC challenge.

Each treatment group consisted of 48 weaned piglets, and the experimental unit was defined as one pen containing four piglets. The overall experimental period spanned 42 days and was divided into three distinct feeding phases: pre-starter 1 (1 to 7 days), pre-starter 2 (8 to 28 days), and starter (29 to 42 days). Diets were formulated to meet or exceed the nutritional requirement for each age group, following the recommendations outlined in the National Research Council (2012).

The probiotics utilized consisted of live yeast strains of *Saccharomyces cerevisiae* sourced from two manufacturers. The concentration of live yeasts in the feed was 1.0 x 1010 CFU/g, which remained consistent for both CHY1 and CHY2 groups. The probiotics were introduced into the feed by replacing inert material (kaolin), with a proportion of 2 kg per ton during the pre-starter 1 phase and subsequently reduced to 1 kg per ton for the pre-starter 2 and starter diets (Table 1).

Experimental procedures

The weaned piglets allocated to the CH, CHY1, and CHY2 treatment groups were orally inoculated with a 1 ml solution containing 106 CFU/mf of *Escherichia coli* F4 on the 8th and 9th days of the experiment. In contrast, piglets in the C group were administered 1 ml saline solution orally (amounting to a total of 2 ml saline solution per piglet). A subsequent inoculation was carried out on the 17th day, during which the CH, CHY1, and CHY2 groups were exposed to a bacterial inoculation (2 ml, 109 CFU/ml of *E. coli* F4). In contrast, the C group received an equivalent volume of saline solution. The bacterial solution used was prepared from a field strain of *Escherichia coli* F4 (LT+, Sta+, and STb+), following the method described by Silveira (2014).

Performance analysis

Throughout the study, piglet weight was recorded at the onset of the trial and on days 7, 11, 14, 21, 28, 35, and 42. Parameters such as average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F: G) were calculated based on the feed offered and leftovers present on the pen floor (both assessed daily).

Incidence of diarrhea

During the experimental period, fecal scores were assessed for consistency by pen using Pedersen & Toft's 4-point scoring method (2011); score 1: firm and shaped, 2: soft and shaped, 3: soft and unshaped, 4: liquid. To determine the fecal score percentage by week, the fecal score was calculated by dividing each score classification by the total observations in a given period, multiplied by 100. Variables

Table 1 - Centesimal composition and nutritional diet values were calculated for each diet offered during the experiment.

Ingredients[1] (kg)	Pre-starter 1[2]	Pre-starter 2[3]	Starter[4]
Corn	24.300	39.500	61.500
Soybean Meal (45%)	15.000	20.000	27.000
Micronized Soybeans	9.000	1.750	7.000
Soy Protein Concentrate	6.000	6.250	-
Starch	23.449	15.821	0.757
Whey Permeate (80% Lactose)	12.097	6.250	-
Milk lactose (90% Lactose)	6.000	6.250	-
DL- methionine	0.227	0.187	0.101
L- tryptophan	0.052	0.040	0.008
L-threonine	0.185	0.158	0.091
L-lysine	0.500	0.434	0.312
L-valine	0.114	0.071	-
Phytase	0.010	0.010	0.010
Premix Vit/Min[5]	1.000	1.000	1.000
Salt	0.200	0.250	0.400
Limestone	-	-	0.579
Dicalcium phosphate	0.542	0.704	0.668
Calcium sulfate	0.550	0.750	-
Zinc oxide (80%)	0.385	0.288	-
Doxycycline (50%)	0.040	-	-
Antioxidant	0.025	0.025	0.025
Flavoring	0.050	0.050	0.050
Kaolin	0.274	0.112	0.399
Values			
Metabolizable energy (Kcal/kg)	3694	3607	3259
Crude Protein	21.48	19.08	20.44
Total Lysine (%)	1.51	1.44	1.32
Digestible Lysine (%)	1.45	1.39	1.17
Ca (%)	0.73	0.63	0.60
P (%)	0.52	0.47	0.45
Lactose (%)	15.45	9.98	-

[1]Values on a fed-basis. [2]pre-starter 1, 1 to 7 days. [3]pre-starter 2, 8 to 28 days. [4]starter, 29 to 42 days. [5]Composition per kg of product: Cu (12.00 mg); Fe (80.00 mg); I (1.00 mg); Mn (40.00 mg); Se (0.36 mg); Zn (110.00 mg); vit. A (6875.00 U.I.); vit. D3 (1505.00 U.I.); vit. E (40.00 mg); vit. K3 (3.07 mg); vit. B1 (1.00 mg); vit. B2 (3.13 mg); vit. B6 (2.00 mg); vit. B12 (0.02 mg); niacin (30.00 mg); folic acid (0.30 mg); pantothenic acid (15.00 mg); biotin (0.10 mg); choline (200.97 mg).

considered included the percentage of each score, the average fecal score for each treatment, and the overall fecal score.

Intestinal health parameters

At the 11th, 28th, and 42nd days of trial, serial slaughters were performed to assess the time required for the yeast to initiate alterations in the piglets' intestinal microbiota and morphology and to ascertain if specific times rendered piglets more responsive to yeast supplementation. Nine weaned piglets per treatment were selected for slaughter, each with an average weight closest to the pen average (36 piglets per slaughter event, 108 piglets total). Piglets were rendered unconscious via head-heart electrocution. Once insensibility was determined, exsanguination via cardiac perforation was performed as a secondary step. All slaughter procedures were conducted using trained staff at the USP's slaughterhouse in Pirassununga.

Intestinal morphometry

Post-slaughter samples of jejunum (2.0 cm) were collected to evaluate the integrity of the intestinal mucosa. The first 10 cm of intestines after the pylorus were considered the duodenum, and jejunum samples were collected approximately 55 cm from the end of the duodenum. Samples were prepared using the method Zhaxi et al. outlined (Zhaxi et al., 2020). A microscope equipped with a camera and the ImageJ software was utilized to measure parameters such as villi height, crypt depth, and the villus: crypt ratio.

Cecal content microbiological and short chain fatty acids composition

In addition to jejunal samples, cecal content samples were obtained to analyze the populations of intestinal bacteria, including *Escherichia coli*, *Enterobacterium*, *Bifidobacterium*, and *Lactobacilli*, as well as the concentrations of short-chain

fatty acids (SCFA), according to Ferreira et al. (2016) methods. The samples were diluted in phosphate-buffered saline (PBS), with *E. coli*, *Enterobacterium*, and *Bifidobacterium* samples being fractionated from 10-1 to 10-3 and Lactobacillus samples from 10-1 to 10-5. Selective culture media were employed for the cultivation of each bacterium group. Prior to statistical analysis, all colony counts (CFU/g) underwent logarithmic transformation (log10).

Hemogram

Lastly, blood was also collected at the time of cardiac perforation to assess red blood cell count, leukocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Additionally, platelet count and morphological analysis of leukocytes were conducted using the May-Grunwald-Giemsa blood smear technique, following Schalm's method (Schalm, 2011).

Statistical analysis

The normality of data distribution was assessed using the Shapiro-Wilk test. When the data did not exhibit a normal distribution, the PROC RANK (SAS Institute Inc., 2009) was employed to perform data transformations. The variables that were found to be non-normal and subsequently transformed include initial weight and feed-to-gain ratio from 0 to 7 days; the intestinal morphometry variables Crypt depth, and Villus: Crypt ratio; blood parameters variables such as Erythroblasts/ 100 Leukocytes, Rod Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets, Plasmatic protein, Neutrophil/lymphocyte ratio; microbiological composition variables like *E. coli, Enterobacterium, Lactobacillus,* and *Bifidobacterium;* and the short chain fatty acids compositions variables, including isobutyrate, isovalerate, and valerate.

All variables were subjected to analysis of variance using the MIXED procedure in SAS statistical software (2009). Tukey's test was applied to compare treatment means after detecting significant differences via the F-test ($p < 0.05$). For the analysis of diarrhea occurrence, the NPAR1 WAY procedure was used. In instances where the Kruskal-Wallis test rejected variables at a 5% probability level, a Dunn's test was carried out as a post-hoc analysis for paired multiple comparisons ($p < 0.05$), with statistical significance set at this level. The statistical model employed for analysis was as follows (Equation 1):

$$Y_{ij} = \mu + T_i + \delta_j + \varepsilon_{ij} \quad (1)$$

In which: Y_{ij} is an observation in unit j in treatment I, μ is the overall mean, T_i is the dietary treatment effect, δ_j is the block effect j, d ε_{ij} is the error associated with observation in block j in treatment i.

Results and Discussion

In the present study, supplementation of the CHY1 yeast to the piglets during the pre-starter 1 period yielded notable improvements in performance parameters. Specifically, weaned piglets fed with CHY1 exhibited weight gains 3.4% higher (p=0.031), an ADG increase of 15.3% (p=0.033), and a 9.5% (p=0.009) enhancement in F:G compared to piglets in the CH group (Table 2). Similarly, piglets receiving CHY2 treatment displayed improved F:G when contrasted with those in the CH group (8.0% higher; p=0.009). The probiotic treatments also positively influenced piglets' performance during the subsequent pre-starter 2 period. Piglets in the CHY1 group demonstrated greater live weights (7.7% increase; p=0.0009), an 11.3% greater ADG (p=0.002), and a 12.3% greater ADFI (p=0.012) when compared to the CH group piglets. Despite these encouraging results during challenging periods, no overall performance enhancement was observed across the entire experimental duration. Detailed performance data can be found in Table 2.

The findings of this study suggest that the probiotic impact of *Saccharomyces cerevisiae* becomes more apparent when weaned piglets undergo heightened challenges. This is evident from the performance enhancement observed immediately following weaning (pre-starter 1 period) and after the *E. coli* challenge (pre-starter 2 period). Similar positive probiotic effects under stressful conditions have been reported by other researchers (Che et al., 2017; Upadhaya et al., 2019). For instance, Che et al. (2017) noted that weaned piglets fed live yeasts exhibited comparable ADG to groups fed antibiotics, especially following a sanitary challenge.

Beyond the initial phases, all groups demonstrated similar performance by day 28, when piglets had acclimated to their surroundings and were distanced from the *E. coli* challenge (Table 2). This phenomenon might indicate compensatory growth in the control groups, which is consistent with findings from Surek et al. (2019). Zhaxi et al. (2020) associated the performance improvements linked to probiotics, including strains tested in this study, with modulation of the intestinal microbiota, stimulation of the intestinal immune system, and subsequent reduction of local inflammation and diarrhea.

During the first week of the trial, the CHY2 group showed a higher percentage of fecal score 2 (41.6%; p=0.042)

Table 2 - Performance of piglets challenged with enterotoxigenic *E.coli* and supplemented with yeast strains probiotics on diets during the nursery phase.

Performance variable	Treatments[1]				SEM[2]	P value
	C	CH	CHY1	CHY2		
Initial Weight (kg)	6.71	6.70	6.70	6.70	0.2678	0.625
Weight 7 days (kg)	8.70^{ab}	8.54^{b}	8.83^{a}	8.75^{ab}	0.3650	0.031
ADG, 0-7 days (kg)	0.284^{ab}	0.264^{b}	0.304^{a}	0.293^{ab}	0.0163	0.033
ADFI, 0-7 days (kg)	0.383	0.385	0.400	0.391	0.0208	0.605
F:G 0-7 days	0.762^{ab}	0.687^{b}	0.752^{a}	0.742^{a}	0.0165	0.009
Weight 28 days (kg)	18.61^{ab}	17.88^{b}	19.26^{a}	18.66^{ab}	0.6591	0.001
ADG, 8-28 days (kg)	0.470^{ab}	0.443^{b}	0.493^{a}	0.472^{ab}	0.0160	0.002
ADFI, 8-28 days (kg)	0.715^{ab}	0.673^{b}	0.756^{a}	0.719^{ab}	0.0319	0.012
F:G 8-28 days	0.661	0.659	0.657	0.663	0.0124	0.975
Weight 42 days (kg)	26.01	25.72	26.48	26.28	0.7565	0.322
ADG, 29-42 days (kg)	0.528	0.534	0.504	0.520	0.0203	0.680
ADFI, 29-42 days (kg)	0.937	0.971	0.958	0.960	0.0407	0.900
F:G 29-42 days	0.573	0.554	0.527	0.546	0.0197	0.382
ADG, 0-42 days (kg)	0.458	0.443	0.466	0.458	0.0135	0.255
ADFI, 0-42 days (kg)	0.734	0.725	0.764	0.744	0.0296	0.354
F:G 0-42 days	0.629	0.614	0.612	0.619	0.0110	0.573

[1]C, control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHY1, yeast strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge. [2]SEM, Standard error of the mean. ADG is the average daily gain; ADFI is the average daily feed intake; F:G is the feed-to-gain ratio. Means followed by equal letters do not differ, by Tukey test, at 5% probability.

than the control group. Additionally, CHY2 and C groups displayed differences during weeks two to four (W2-4), with CHY2 showing a lower score of 1 (13.6%; p=0.040) and a higher score of 2 percentage (46.4%; p=0.035), resulting in an elevated average fecal score (9.88%; p=0.044) compared to the C group. However, the CHY2 group presented a higher percentage of score 3 (85.3%; p=0,007) when compared to the CHY1 group. Throughout the week one to six (W1-6), the C group demonstrated a higher percentage of score 1 compared to CHY2 (13.4%) and CH (10.0%) groups (p=0.020), as well as a more significant percentage of score 2 (25.0%; p=0.003) than CH piglets. Remarkably, the CHY2 and CH groups had the same average total fecal score (1.489), which was 6.0% higher (p=0.049) than the C group's mean score (Table 3).

These results suggest that yeast strain 1 exhibited greater efficacy in countering the *E. coli* challenge than yeast strain 2, which induced diarrhea in treated piglets, as evidenced by the increased percentage of score 3 during W2-4 (Table 3). The impact of yeast use on diarrhea prevention or promotion varies across studies (Che et al., 2017; Yang et al., 2016). Such discrepancies might stem from differences in manufacturing processes that influence probiotic interaction with the immune system (Jiang et al., 2015).

Although differences in fecal scores were evident, evaluation of intestinal morphology (Table 4) did not reveal significant disparities between treatments. While there is inconsistent information on the effects of yeast on intestinal morphology in the technical-scientific literature, maintaining intestinal wall integrity is considered a key benefit of probiotic use (Bontempo et al., 2006; Che et al., 2017; Zhaxi et al., 2020). Similar findings to those of this trial were reported by Che et al. (2017), who did not identify differences in villi height and crypt depth associated with probiotics use. On the contrary, other authors have reported increased villi height and a higher villus/ crypt ratio (Bontempo et al., 2006; Zhaxi et al., 2020) in yeast-fed piglets, suggesting the potential of yeasts to preserve intestinal morphology. These measures are indicators of intestinal health in swine (Duttlinger et al., 2021), with longer villi, shallower crypts, and higher villus/crypt ratios being associated with improved nutrient digestion and absorption (Bontempo et al., 2006).

Blood parameters were no different across treatments (Table 5). These results are similar to work conducted by Keimer et al. (2018) that found that even when weaned piglets were fed commercial yeast cultures and exhibited improved performance, no changes in blood cell count were observed. These outcomes might suggest that yeast's anti-inflammatory effect is localized to the digestive system rather than being systemic, thus limiting its detectability in peripheral blood samples.

Regarding the modulation of intestinal microbiota (Table 6), the current study did not observe any significant alterations induced by the probiotic's supplementation. This could be attributed to the possibility that the challenge

Table 3 - Fecal score[1] prevalence of piglets in the nursery phase challenged with *E. coli* F4 and supplemented with yeasts probiotics on diets.

Fecal scores variable[2]	Treatments[3]				SEM[4]	P value
	C	CH	CHY1	CHY2		
Score 1 W1 (%)	83.02	76.56	76.96	75.45	12.40	0.245
Score 2 W1 (%)	13.55^{b}	19.94^{ab}	19.91^{ab}	23.21^{a}	44.99	0.042
Score 3 W1 (%)	2.72	3.26	3.13	1.24	105.71	0.402
Score 4 W1 (%)	0.71	0.23	0.00	0.10	287.46	0.243
Fecal Score W1	1.211	1.272	1.262	1.260	9.33	0.599
Score 1 W2-4 (%)	86.30^{a}	80.21^{ab}	81.45^{ab}	74.56^{b}	11.21	0.040
Score 2 W2-4 (%)	12.50^{b}	17.79^{ab}	18.19^{ab}	23.34^{a}	46.32	0.035
Score 3 W2-4 (%)	1.21^{ab}	1.82^{ab}	0.31^{b}	2.11^{a}	126.68	0.007
Score 4 W2-4 (%)	0.00	0.18	0.05	0.00	379.30	0.098
Fecal Score W2-4	1.149^{b}	1.220^{ab}	1.190^{ab}	1.275^{a}	8.29	0.044
Score 1 W5-6 (%)	29.05	22.19	25.89	23.68	33.52	0.266
Score 2 W5-6 (%)	56.68	59.19	58.09	62.92	11.07	0.127
Score 3 W5-6 (%)	13.10	14.77	12.94	10.63	41.52	0.268
Score 4 W5-6 (%)	1.17	3.85	3.08	2.77	124.34	0.179
Fecal Score W5-6	1.864	2.003	1.932	1.925	8.65	0.145
Score 1 W1-6 (%)	66.67^{a}	60.26^{b}	62.18^{ab}	57.75^{b}	11.70	0.020
Score 2 W1-6 (%)	27.40^{b}	31.95^{ab}	31.78^{ab}	36.51^{a}	19.46	0.003
Score 3 W1-6 (%)	5.42	6.38	4.99	4.80	37.73	0.413
Score 4 W1-6 (%)	0.51	1.41	1.05	0.94	116.06	0.230
Fecal Score W1-6	1.398^{b}	1.489^{a}	1.449^{ab}	1.489^{a}	6.64	0.049

[1]Fecal consistency categories (Pedersen & Toft, 2011): score 1, firm and shaped (standard); score 2, soft and shaped (standard); score 3 (diarrhea), soft and score 4, liquid (diarrhea); [2]W1, first week of trial; W2-4, second to fourth week of trial; W5-6, fifth to sixth week of trial, and W1-6, the whole period of the trial; [3] C, control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHY1, yeast strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge; [4] SEM, Standard error of the mean; Fecal Score, weighted average of fecal scores. Means followed by equal letters do not differ, by Dunn´s test, at 5% probability.

Table 4 - Histological analysis of jejunum samples of piglets challenged with *E. coli* F4 and supplemented with yeast, on nursery phase, at each slaughter day on treatments.

Histological variable	Treatments[1]				SEM[2]	P value
	C	CH	CHY1	CHY2		
Slaughter at 11th day						
Villus Height (µm)	270.43	285.82	310.01	279.07	22.74	0.620
Crypt Depth (µm)	225.34	201.07	186.96	232.27	34.04	0.925
Villus: Crypt Ratio	1.25	1.43	1.92	1.30	0.262	0.361
Slaughter at 28th day						
Villus Height (µm)	302.05	318.97	281.11	253.38	36.11	0.536
Crypt Depth (µm)	273.16	318.8	292.62	254.17	31.05	0.429
Villus: Crypt Ratio	1.08	1.08	1.00	1.06	0.182	0.985
Slaughter at 42nd day						
Villus Height (µm)	257.89	313.22	327.7	249.39	60.34	0.399
Crypt Depth (µm)	248.22	272.39	326.7	306.96	44.78	0.378
Villus: Crypt Ratio	1.07	1.20	1.01	0.98	0.29	0.734

[1]C, control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHY1, yeast strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge. [2]SEM, Standard error of the mean.

imposed on the piglets was insufficient to induce changes in bacterial counts in cecal content samples, as yeasts struggle to establish themselves in healthy microbiotas (Pecquet et al., 1991). The capacity of probiotics to modulate intestinal microbiota appears to be inconsistent. For instance, Mathew et al. (1998) found no impact of probiotic use on intestinal microbiota composition despite observing improved piglet performance with the supplementation. In contrast, Che et al. (2017) reported lower total bacterial counts and reduced *E. coli* shedding in feces due to probiotic supplementation. The intestinal microbiota plays a vital role in partially digesting nutrients

Table 5 - Blood count of piglets challenged with *E. coli* F4 and supplemented with yeast, on nursery phase, at each slaughter day on treatments.

Blood variable	Treatments[1]				SEM[2]	P value
	C	CH	CHY1	CHY2		
Slaughter at 11th day						
Red Cells (x10⁶/µL)	6.28	6.04	6.00	6.05	0.223	0.781
Hemoglobin (g/dL)	11.39	10.98	10.79	10.90	0.405	0.731
Hematocrit (%)	35.69	34.39	34.41	34.62	1.276	0.869
MCV (fL)	57.08	57.09	57.39	57.30	0.767	0.978
MCH (pg)	18.11	18.10	17.91	17.99	0.307	0.923
MCHC (%)	31.80	31.83	31.31	31.47	0.282	0.321
Erythroblasts/ 100 Leukocytes	1.75	2.00	3.44	5.50	1.697	0.377
Corrected Total Leukocytes (µL/µL)	16067	16795	16852	19036	1664.110	0.611
Myelocytes (%)	0.000	0.000	0.000	0.000	-	-
Metamyelocytes (%)	0.000	0.000	0.000	0.000	-	-
Rod Neutrophils (%)	0.500	0.875	1.222	1.200	0.524	0.739
Segmented Neutrophils (%)	45.625	42.875	45.111	42.200	4.923	0.943
Lymphocytes (%)	50.375	53.375	50.333	52.400	4.929	0.951
Monocytes (%)	2.250	1.750	2.111	2.800	0.514	0.707
Eosinophils (%)	0.375	0.625	0.444	0.700	0.312	0.811
Basophils (%)	0.875	0.500	0.778	0.700	0.315	0.833
Platelets (x10³/µL)	353.250	217.500	346.440	391.300	65.936	0.261
Plasmatic protein (g/dL)	5.175	4.975	4.978	5.260	0.136	0.216
Neutrophil/lymphocyte ratio	1.040	0.976	1.087	1.028	0.266	0.852
Slaughter at 28th day						
Red Cells (x10⁶/µL)	6.19	6.92	6.93	6.62	0.426	0.676
Hemoglobin (g/dL)	10.25	10.94	11.37	11.28	0.685	0.760
Hematocrit (%)	33.56	35.98	37.42	36.84	2.254	0.764
MCV (fL)	48.49	52.23	54.10	55.74	3.192	0.189
MCH (pg)	14.76	15.82	16.36	17.00	0.984	0.262
MCHC (%)	27.10	30.36	30.35	30.54	1.711	0.988
Erythroblasts/ 100 Leukocytes	1.22	2.78	2.11	2.11	1.197	0.551
Corrected Total Leukocytes (µL/µL)	13673.00	15957.00	14089.00	16401.00	1422.570	0.586
Rod Neutrophils (%)	0.44	0.22	0.11	0.11	0.162	0.337
Segmented Neutrophils (%)	36.22	41.11	45.00	43.33	3.939	0.440
Lymphocytes (%)	49.88	56.41	53.15	53.38	4.540	0.708
Monocytes (%)	1.33	1.33	1.33	2.22	0.361	0.239
Eosinophils (%)	0.77	0.44	0.66	1.11	0.304	0.394
Basophils (%)	0.11	0.00	0.22	0.33	0.124	0.289
Platelets (x10³/µL)	531.89	433.67	633.44	566.00	94.974	0.502
Plasmatic protein (g/dL)	5.00	5.38	5.54	5.40	0.325	0.561
Neutrophil/lymphocyte ratio	0.78	0.84	0.94	0.84	0.134	0.780
Slaughter at 42nd day						
Red Cells (x10⁶/µL)	7.45	7.62	7.52	7.31	0.182	0.621
Hemoglobin (g/dL)	12.49	12.33	12.59	12.06	0.325	0.636
Hematocrit (%)	40.79	40.87	41.23	39.74	1.119	0.774
MCV (fL)	54.69	53.83	54.88	54.39	0.940	0.880
MCH (pg)	16.68	16.18	16.67	16.42	0.284	0.540
MCHC (%)	30.58	30.11	30.50	30.32	0.198	0.327
Erythroblasts/ 100 Leukocytes	2.45[b]	5.62[ab]	3.04[ab]	6.79[a]	1.174	0.050
Corrected Total Leukocytes (µL/µL)	20388.00	21406.00	22429.00	23343.00	2903.210	0.938
Rod Neutrophils (%)	0.30	0.52	0.51	0.68	0.310	0.780
Segmented Neutrophils (%)	53.00	49.00	55.67	55.89	4.783	0.707
Lymphocytes (%)	40.21	44.97	37.96	37.93	4.828	0.678
Monocytes (%)	5.42	3.43	5.62	4.69	1.146	0.357
Eosinophils (%)	0.66	1.63	0.43	0.78	0.401	0.244
Basophils (%)	0.22	0.38	0.00	0.11	0.160	0.418
Platelets (x10³/µL)	427.22	440.00	582.11	526.22	79.977	0.443
Plasmatic protein (g/dL)	5.94	6.02	6.26	6.03	0.131	0.187
Neutrophil/lymphocyte ratio	1.72	1.31	1.74	1.91	0.422	0.634

[1]C, control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHY1, yeast strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge. [2]SEM, standard error of the mean. Means followed by equal letters do not differ, by Tukey test, at 5% probability.

Table 6 - Microbiological composition of the cecum content of piglets challenged with *E. coli* F4 and supplemented with yeast, on nursery phase, at each slaughter day on treatments.

Microbiological composition	Treatments[1]				SEM[2]	P value
	C	CH	CHY1	CHY2		
Slaughter at 11th day						
E. coli	3.15	2.98	2.63	2.68	0.302	0.743
Enterobacterium	2.97	3.54	2.91	2.86	0.499	0.488
Lactobacillus	7.68	7.98	7.60	7.46	0.213	0.257
Bifidobacterium	4.95	5.23	4.88	4.68	0.236	0.399
Slaughter at 28th day						
E. coli	4.26	5.13	4.41	4.60	0.352	0.216
Enterobacterium	4.04	4.54	4.05	3.99	0.382	0.490
Lactobacillus	6.94	7.55	7.35	7.12	0.198	0.184
Bifidobacterium	5.34ab	5.86a	5.28b	5.24b	0.172	0.018
Slaughter at 42nd day						
E. coli	4.71	4.04	3.33	4.33	0.741	0.485
Enterobacterium	3.43	3.70	-	5.06	0.042	-
Lactobacillus	8.06	7.68	7.46	7.87	0.278	0.566
Bifidobacterium	4.35	4.50	4.38	4.52	0.217	0.941

[1]C, Control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHCHY1, yarest strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge. [2]SEM, Standard error of the mean. According to Tukey's test, means followed by eq equal letters do not differ at 5% probability.

and producing SCFAs, constituting the main products of bacterial fermentation in swine's large intestine. These SCFAs provide up to 70% of the energy utilized by colonocytes, underscoring the importance of enhancing cecal fermentation for improved energy provision and intestinal health (Wang et al., 2021).

In this study, variations in SCFA production tendencies between groups were noted for yeast-supplementation groups (Table 7), even though analysis of total bacterial counts did not reveal any probiotic-induced changes in bacterial composition. Specifically, during the pre-starter 1 period, there was a tendency to increase (24.7%; p=0.058) acetate levels in the cecal content of piglets supplemented with probiotics, compared to the CH group. Previous research by Fukuda et al. (2011) highlighted that elevated acetate levels can induce anti-inflammatory effects on the colon mucosa, which could explain the increased feed intake in this study.

Furthermore, a tendency was observed to increase (p=0.094) the butyric acid concentration in both yeast-treated groups, particularly during the pre-starter 2 period (31.27% increase, Table 7). As reported by Liu et al. (2014), probiotics can accelerate carbohydrate breakdown, influence SCFA synthesis, and elevate butyric acid levels in the colon. Notably, the increased concentration of butyric acid coincided with the period of *E. coli* inoculation, corroborating the reported pathogen-controlling properties of butyric acid (Guilloteau et al., 2010).

While the reports on probiotic yeast's effects are inconsistent, this study suggests several factors might explain the absence of differences between groups. It is suggested that animals facing challenging environments are more responsive to probiotic effects (Upadhaya et al., 2019). After the pre-starter 2 period (from 2 days onwards), various factors could have contributed to a less challenging environment for the piglets. These factors include adaptation to the nursery facilities, time elapsed since the last sanitary challenge (10 days), and lower pen density. Another possibility is the lower *E. coli* inoculation dose utilized in this trial compared to that used by the Che et al. (2017) for example, which might account for some of the observed variations. Nevertheless, even if other analyses did not reveal significant probiotic effects, both yeast-treated groups performed better during the most challenging periods (pre-starter 1 and pre-starter 2), indicating the successfulness of the *E. coli* challenge.

Age at weaning could also affect piglets' physiology, making them less susceptible to environmental challenges and attenuating probiotics' positive effects. Piglets weaned later, at 28 days of age, for example, have an easier time adapting to the nursery changes and are less susceptible to diseases. Piglets weaned at 28 days of age, for example, have a more developed antioxidant system, which helps eliminate harmful free radicals and promoters of inflammation produced following weaning (Ming et al., 2021). Physiological changes such as this improve piglets' health status, observed by a linear reduction in injectable antibiotic use with increased weaning ages (Faccin et al., 2020). While the pigs used in this trial were not weaned at 28 days of age, they were weaned older than the age adopted by most swine farms in Brazil (24 vs 21 days) thus

Table 7 - Short Chain Fatty Acids (SCFA) concentration on cecum content of piglets challenged with *E. coli* F4 and supplemented with yeast on the nursery phase at each slaughter day on treatments.

SCFA concentration	Treatments[1]				SEM[2]	P value
	C	CH	CHY1	CHY2		
Slaughter at 11th day						
Acetate	19.70	18.19	21.83	24.16	1.632	0.058
Propionate	7.32	8.44	8.68	8.51	0.692	0.453
Isobutyrate	0.117	0.125	0.088	0.150	0.037	0.722
Butyrate	2.70	2.91	3.35	3.70	0.373	0.187
Isovalerate	0.115	0.123	0.103	0.176	0.046	0.846
Valerate	0.293	0.557	0.433	0.517	0.085	0.062
Slaughter at 28th day						
Acetate	14.33	15.74	18.49	15.25	1.962	0.492
Propionate	6.46	6.84	8.98	7.96	0.744	0.100
Isobutyrate	0.162	0.216	0.281	0.276	0.058	0.633
Butyrate	2.47	2.90	4.15	4.22	0.570	0.094
Isovalerate	0.187	0.268	0.416	0.372	0.088	0.379
Valerate	0.438	0.557	0.796	0.823	0.132	0.314
Slaughter at 42nd day						
Acetate	21.51	23.05	22.90	19.63	1.613	0.403
Propionate	12.14	12.95	12.33	10.34	0.984	0.202
Isobutyrate	0.108	0.079	0.075	0.037	0.108	0.538
Butyrate	5.03	6.56	6.71	5.04	0.846	0.305
Isovalerate	0.139	0.107	0.074	0.069	0.065	0.647
Valerate	1.151	1.634	1.559	1.238	0.252	0.242

[1]C, control without *E. coli* challenge and yeast strain. CH, control with *E. coli* challenge and yeast strain. CHY1, yeast strain probiotic 1 (2.0 kg per ton during pre-starter 1 decreased to 1 kg per ton on the other diets) with *E. coli* challenge. CHY2, yeast strain probiotic 2 (2.0 kg per ton during pre-starter 1, decreased to 1 kg per ton on the other diets) with *E. coli* challenge. [2]SEM, Standard error of the mean. According to Tukey's test, means followed by equal letters do not differ at 5% probability.

making the piglets more prepared to the challenges proposed and thus, less susceptible to the probiotic benefits.

Conclusion

Incorporating Saccharomyces cerevisiae strain 1 improved body weight, ADG, and ADFI in the initial 28 days, coinciding with weaning and the sanitary challenge. Enhanced performance and better fecal scores during weeks 2 to 4 suggest strain 1's potential in mitigating *E. coli* F4 challenge effects. Strain 1 outperformed yeast strain 2, which exhibited increased post-challenge diarrhea (score 3). Thus, using strain 1 during nursery phase stress is advised, acknowledging uncertain underlying mechanisms.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

Informed consent was obtained from all subjects involved in the study.

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