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Influence of the reduction of digestible lysine and crude protein and the supplementation of non-essential amino acids, and feed additives on the performance and intestinal health of weaned piglets

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Doctor Scientiae

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AMANDA MEDEIROS CORREIA

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Gabriel Cipriano Rocha

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ABSTRACT

CORREIA, Amanda Medeiros, D.Sc., Universidade Federal de Viçosa, June, 2025. **Influence of the reduction of digestible lysine and crude protein and the supplementation of non-essential amino acids, and feed additives on the performance and intestinal health of weaned piglets**. Adviser: Gabriel Cipriano Rocha.

Three experiments were conducted with weaned piglets. In Experiment I, the objective was to evaluate the effects of temporary dietary lysine restriction on performance and economic viability in nursery piglets. A total of 144 piglets were distributed in a randomized block design with two treatments and 12 replicates. The treatments were: control-lys, lysine levels recommended from 21 to 32 and 32 to 42 days of age, and low-lys, 90% of the lysine levels in the control-lys diet. From 42 to 62 days, all animals received the control diet. From 21 to 32 days, piglets fed the low-lys diet had poorer average daily gain (ADG), feed conversion (FC), and body weight at 32 days (P=0.05). From 32 to 42 days, piglets fed the low-lys diet showed lower average daily feed intake, ADG, and body weight at 42 days (P=0.05). From 21 to 62 days, piglets on the low-lys diet showed lower ADG and final body weight (P=0.05) compared to those on the control-lys diet. On days 27 and 29, piglets on the low-lys diet exhibited higher (P=0.05) diarrhea incidence. The lowest feed cost and highest economic efficiency index were recorded for the low-lys treatment. However, piglets on the control-lys diet achieved 3.9% higher profitability. In conclusion, temporary lysine restriction in piglet diets worsens performance and reduces economic viability. In Experiment II, the effects of crude protein (CP) and non-essential amino acids (NEAA) supplementation on performance, blood profile, intestinal morphology, relative mRNA abundance of inflammatory and antioxidant markers, and tight junction proteins were investigated in piglets during the first two weeks post-weaning. Ninety piglets aged 21 days (7.55 ± 0.72 kg) were assigned in a randomized block design to one of three dietary treatments: (1) high-CP, diet with 24% CP; (2) low-CP, diet with 18% CP; and (3) low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg Arg (L-arginine; purity > 99%) and 10 g/kg Glu+Gln (minimum 10% L-glutamine and minimum 10% L-glutamate). The piglets were fed corn and soybean meal-based diets in a 14-day trial. Feed conversion improved (P<0.05) in piglets fed the high-CP diet compared to low-CP or low-CP + NEAA. Serum urea nitrogen was higher (P<0.05) in piglets on the high-CP diet compared to other treatments. In the duodenum, villus height in piglets fed the low-CP + NEAA diet was greater (P<0.05) than in those fed high-CP or low-CP. Goblet cell proportion was higher (P<0.05) in piglets fed the low-CP +

NEAA or high-CP diet compared to low-CP. In the jejunum, crypt depth was greater (P<0.05) in piglets on the high-CP diet compared to low-CP + NEAA. Jejunal mRNA expression of IFN-? was higher (P<0.05) in piglets on the high-CP diet compared to other treatments. However, superoxide dismutase and occludin expression was higher (P<0.05) in piglets fed the low-CP + NEAA diet compared to the high-CP diet. In the ileum, the number of Peyer's patches was greater (P<0.05) in piglets fed the high-CP diet compared to other treatments. In conclusion, the high-CP diet (24% CP) improves feed conversion in piglets during the first two weeks post-weaning compared to the low-CP diet (18% CP), with or without NEAA supplementation. However, the low-CP diet supplemented with NEAA (Arg, Gln, and Glu) enhances intestinal health by increasing villus height and goblet cell proportion in the duodenum, reducing jejunal crypt depth and Peyer's patch number in the ileum. Additionally, piglets fed the low-CP + NEAA diet showed increased expression of superoxide dismutase and occludin and decreased mRNA expression of IFN-?. In Experiment III, the effects of nucleotide, autolyzed yeast (Saccharomyces cerevisiae), and sodium butyrate supplementation in nursery pig diets were evaluated on growth performance, diarrhea incidence, blood profile, intestinal morphology, mRNA expression of nutrient transporters, inflammatory markers, antioxidant profile, and tight junction proteins in the small intestine. One hundred eighty piglets aged 21 days (5.17 ± 0.57 kg) were assigned in a randomized block design to one of four dietary treatments: (1) CON, control, basal diet; (2) NUC, CON + nucleotides; (3) YSC, CON + autolyzed yeast S. cerevisiae; (4) ASB, CON + sodium butyrate acidifier. Piglets were fed for 24 days, divided into two phases: Phase 1 (21 to 32 days) and Phase 2 (32 to 45 days). During Phase 1, YSC and ASB improved average daily gain (ADG) and feed conversion (FC) compared to CON. Over the total period, ASB improved ADG, and YSC improved FC compared to CON. The NUC diet did not affect growth performance. ASB increased ileal villus height compared to CON. YSC and ASB reduced Peyer's patch number in the ileum compared to CON. YSC increased mRNA expression of nutrient transporters (SMCT2, MCT1, and PepT1), tight junction proteins (OCL and ZO-1), antioxidants (GPX), and IL1-ß in the jejunum compared to CON. ASB increased mRNA expression of nutrient transporters (SGLT1 and MCT1), tight junction proteins (OCL and ZO-1), and antioxidants (GPX and SOD) compared to CON. In conclusion, autolyzed yeast and sodium butyrate improved growth performance by enhancing intestinal barrier integrity, nutrient transporter mRNA expression, and antioxidant enzymes in the jejunum of nursery pigs, whereas nucleotide supplementation did not show such effects.

Keywords: non-essential amino acids; crude protein; intestinal health; pigs; feed additives

RESUMO

CORREIA, Amanda Medeiros, D.Sc., Universidade Federal de Viçosa, junho de 2025. **Influência da redução de lisina digestível e proteína bruta e da suplementação de aminoácidos não essenciais e aditivos alimentares no desempenho e saúde intestinal de leitões desmamados**. Orientador: Gabriel Cipriano Rocha.

Foram realizados três experimentos com leitões desmamados. No experimento I, objetivou-se avaliar os efeitos da restrição temporária de lisina na dieta sobre o desempenho e viabilidade econômica de leitões em fase de creche. Foram utilizados 144 leitões, distribuídos em blocos ao acaso, com dois tratamentos e 12 repetições. Os tratamentos foram controle-lis, nível de lisina recomendado dos 21 aos 32 e 32 aos 42 dias de idade e baixa-lis, 90% do nível de lisina das dietas controle-lis. Dos 42 aos 62 dias, todos os animais receberam dieta controle. De 21 a 32 dias, leitões alimentados com baixa-lis tiveram pior ganho médio diário (GMD), conversão alimentar e peso corporal aos 32 dias (P=0,05). Dos 32 aos 42 dias, leitões alimentados com baixa-lis apresentaram menor consumo médio de ração diário, GMD e peso aos 42 dias (P=0,05). Dos 21 aos 62 dias, leitões alimentados com baixa-lis apresentaram GMD e peso corporal final menores (P=0,05) quando comparados aos alimentados com controle-lis. Aos 27 e 29 dias, leitões alimentados com baixa-lis apresentaram maior (P=0,05) indicência de diarreia. O menor custo de alimentação e o maior índice de eficiência econômica foram registrados para baixa-lis. No entanto, os suínos alimentados com controle-lis apresentaram rentabilidade 3,9% maior. Como conclusão, a restrição temporária de lisina nas dietas de leitões piora o desempenho e diminui a viabilidade econômica. No experimento II, investigou-se o efeito da suplementação de proteína bruta (PB) e aminoácidos não essenciais (ANE) sobre o desempenho, perfil sanguíneo, morfologia intestinal, abundância relativa de mRNA de marcadores inflamatórios e antioxidantes, e proteínas de junção apertada em leitões nas duas primeiras semanas após o desmame. Noventa leitões de 21 dias de idade (7,55 ± 0,72 kg) foram distribuídos em um delineamento em blocos ao acaso em um de três tratamentos dietéticos: (1) alta-PB, dieta com 24% de PB; (2) baixa-PB, dieta com 18% de PB; e (3) baixa-PB + ANE, dieta com 18% de PB suplementada com 5 g/kg de Arg (L-arginina; pureza > 99%) e 10 g/kg de Glu+Gln (mínimo 10% de L-glutamina e mínimo 10% de L-glutamato). Os leitões foram alimentados com dietas à base de milho e farelo de soja em um ensaio de 14 dias. Houve melhora (P<0,05) na conversão alimentar dos leitões alimentados com a dieta alta-PB em comparação com os tratamentos baixa-PB ou baixa-PB + ANE. A concentração sérica de

nitrogênio ureico foi maior (P<0,05) nos leitões alimentados com alta-PB em comparação com outros tratamentos dietéticos. No duodeno, a altura das vilosidades dos animais alimentados com a dieta baixa-PB + ANE foi maior (P<0,05) do que naqueles alimentados com alta-PB e baixa-PB. A proporção de células caliciformes dos leitões alimentados com baixa-PB + ANE ou alta-PB foi maior (P<0,05) em comparação com a baixa-PB. No jejuno, a profundidade das criptas dos leitões com tratamento dietético alta-PB foi maior (P<0,05) em comparação com baixa-PB + ANE. No jejuno, a expressão de mRNA de IFN-? foi maior (P<0,05) nos animais alimentados com dietas alta-PB em comparação com outros tratamentos dietéticos. No entanto, a expressão de superóxido dismutase e occludina foi maior (P<0,05) nos animais alimentados com baixa-PB + ANE do que nos leitões com dietas alta-PB. No íleo, o número de placas de Peyer nos leitões alimentados com alta-PB foi maior (P<0,05) em comparação com outros tratamentos dietéticos. Em conclusão, a dieta alta-PB (24% PB) melhora a conversão alimentar dos leitões nas primeiras duas semanas após o desmame em comparação com a dieta baixa-PB (18% PB), suplementada ou não com ANE. No entanto, a dieta baixa-PB suplementada com ANE (Arg, Gln e Glu) melhora a saúde intestinal dos leitões, promovendo maior altura das vilosidades e proporção de células caliciformes no duodeno, reduzindo a profundidade das criptas jejunais e o número de placas de Peyer no íleo. Além disso, os leitões que receberam a dieta baixa-PB + ANE apresentaram aumento na expressão de superóxido dismutase e ocludina, e menor expressão de mRNA de IFN-?. No experimento III, foram avaliados os efeitos da suplementação de nucleotídeos, levedura autolisada (Saccharomyces cerevisiae) e butirato de sódio em dietas para leitões na fase de creche sobre o desempenho de crescimento, incidência de diarreia, perfil sanguíneo, morfologia intestinal, expressão de mRNA de transportadores de nutrientes, marcadores inflamatórios, perfil antioxidante e proteínas de junção apertada no intestino delgado. Cento e oitenta leitões de 21 dias de idade (5,17 ± 0,57 kg) foram distribuídos em um delineamento em blocos ao acaso em 1 de 4 tratamentos dietéticos: (1) CON: controle, dieta basal, (2) NUC: CON + nucleotídeos, (3) YSC: CON + levedura lisada *S. cerevisiae*, (4) ASB: CON + acidificante butirato de sódio. Os leitões foram alimentados por 24 dias, divididos em duas fases: fase 1 (21 a 32 dias) e fase 2 (32 a 45 dias). Durante a fase 1, YSC e ASB melhoraram o ganho médio diário (GMD) e a conversão alimentar (CA) em comparação com o CON. No período total, ASB melhorou o GMD e YSC melhorou a CA em comparação com o CON. A dieta NUC não afetou o desempenho de crescimento. O ASB aumentou a altura das vilosidades ileais em comparação com o CON. YSC e ASB reduziram o número de placas de Peyer no íleo em comparação com o

CON. O YSC aumentou a expressão de mRNA de transportadores de nutrientes (SMCT2, MCT1 e PepT1), proteínas de junção apertada (OCL e ZO-1), antioxidantes (GPX) e IL1-ß no jejuno em comparação com o CON. O ASB aumentou a expressão de mRNA de transportadores de nutrientes (SGLT1 e MCT1), proteínas de junção apertada (OCL e ZO-1) e antioxidantes (GPX e SOD) em comparação com o CON. Em conclusão, a levedura autolisada e o butirato de sódio promoveram melhorias no desempenho de crescimento ao melhorar a integridade da barreira intestinal, a expressão de mRNA de transportadores de nutrientes e enzimas antioxidantes no jejuno de leitões na fase de creche, enquanto a suplementação de nucleotídeos não apresentou esses efeitos.

Palavras-chave: aminoácidos não essenciais; proteína bruta ; saúde intestinal; leitões; aditivos alimentares

SUMMARY

CHAPTER 1:

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Temporary reduction of digestible lysine in nursery pig diets: performance and economic analysis

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ABSTRACT

To evaluate the effects of temporary dietary lysine restriction on nursery pigs' growth performance and its economic viability compared to control diets, 144 piglets (21-d-old) were assigned to randomized blocks, with two treatments and twelve replicates. The treatments were control-lys: lysine level as recommended from 21-32 and 32-42 days and low-lys: 90% of the

lysine level of the control-lys diets. From 42 to 62 days, all animals received a control diet. From 21 to 32 days, pigs fed low-lys had worse average daily gain (ADG), feed conversion, and 32-d body weight (BW; $P \leq 0.05$). From 32 to 42 days, pigs fed low-lys had lower average daily feed intake, ADG, and 42-d BW ($P \leq 0.05$). From 42 to 62 days, pigs had similar performance ($P > 0.05$). Overall (21 to 62 days), pigs fed the low-lys had lower ($P < 0.05$) ADG and final BW. At 27 and 29 days, pigs fed the low-lys diet had a higher ($P \leq 0.05$) incidence of diarrhea. The lowest feed cost and the highest economic efficiency index were recorded for the low-lys treatment. However, pigs fed the control-lys presented a 3.9% higher profitability. In conclusion, a temporary reduction of lysine in the diets of piglets followed by an unrestricted diet in the subsequent period led to worse growth performance and lower economic viability.

Keywords: amino acids, compensatory growth, nutrition, phase-feeding, pig.

RESUMO

Objetivou-se com este estudo avaliar os efeitos da restrição temporária de lisina na dieta sobre o desempenho e viabilidade econômica de leitões em fase de creche. Foram utilizados 144 leitões, distribuídos em blocos ao acaso, com dois tratamentos e 12 repetições. Os tratamentos foram controle-lis, nível de lisina recomendado dos 21 aos 32 e 32 aos 42 dias de idade e baixa-lis, 90% do nível de lisina das dietas controle-lis. Dos 42 aos 62 dias, todos os animais receberam dieta controle. De 21 a 32 dias, leitões alimentados com baixa-lis tiveram pior ganho médio diário (GMD), conversão alimentar e peso corporal aos 32 dias ($P \leq 0,05$). Dos 32 aos 42 dias, leitões alimentados com baixa-lis apresentaram menor consumo médio de ração diário, GMD e peso aos 42 dias ($P \leq 0,05$). Dos 21 aos 62 dias, leitões alimentados com baixa-lis apresentaram GMD e peso corporal final menores ($P \leq 0,05$) quando comparados aos alimentados com controle-lis. Aos 27 e 29 dias, leitões alimentados com baixa-lis apresentaram maior ($P \leq 0.05$) indicência de diarreia. O menor custo de alimentação e o maior índice de eficiência econômica foram registrados para baixa-lis. No entanto, os suínos alimentados com controle-lis apresentaram rentabilidade 3,9% maior. Como conclusão, a restrição temporária de lisina nas dietas de leitões piora o desempenho e diminui a viabilidade econômica.

Palavras-Chave: aminoácidos, alimentação por fase, crescimento compensatório, nutrição, suíno

1. Introduction

Highly digestible feed ingredients and feed additives are used to help young pigs transition from sow's milk to solid diets (Skinner et al., 2014; Valini et al., 2021). Thus, the cost of swine diets is greatest during the nursery phase of production due to the complexity of ingredients, e.g., whey, blood plasma and industrial amino acids. As compensatory growth has been associated with increased feed efficiency, it is a mechanism that may help to increase profitability (Taylor et al., 2013).

With the increasing availability of industrial amino acids, low-protein diets can be formulated based on the concept of ideal protein (NRC, 2012; Rostagno et al., 2017). Formulating based on ideal protein is an effective way to use fewer protein sources in the diet and thus reduce feed costs. According to the concept of ideal protein, the requirements for amino acids are expressed relative to the requirement for lysine (Edmonds & Baker, 1987).

Therefore, one strategy to minimize costs might be the temporary reduction of lysine in weaned piglets' diets (Gomes et al., 2021), followed by a diet meeting the amino acid requirements for the phase (Skinner et al., 2014). Reducing the dietary amino acid content may reduce growth temporarily, which may lead to compensatory growth during a subsequent realimentation period (Skiba, 2005). During realimentation, pigs that have previously experienced reduced growth may have better performance ratios and reach a similar final body weight (BW) of pigs fed unrestricted diets (Skinner et al., 2014; Totafurno et al., 2020). However, little available research examines compensatory growth during the nursery phase and this, along with an economic analysis, is lacking in the literature.

The temporary reduction of lysine in weaned piglets' diets followed by an unrestricted diet in the subsequent period is hypothesized to lead to similar growth performance as nonrestricted pigs and would be economically feasible at the end of the nursery phase. Thus, the objective of this study was to evaluate the effects of temporary dietary lysine restriction on nursery pigs' growth performance and its economic viability compared to control diets.

2. Material and methods

The experimental protocol followed ethical animal research principles (CONCEA, 2016) and was approved by the Ethical Committee on Animal Use of Universidade Federal de Viçosa (protocol n° 041/2020). The experiment was conducted on 2020, in a commercial–

experimental barn, located in the municipality of Oratórios, in the state of Minas Gerais, Brazil (20° 25' 5"S, 42° 47' 28"W).

A total of 144 pigs (AGPIC 415 × Camborough), castrated males and females, weaned at 21 days old and with an initial BW of 5.58 ± 0.66 kg, were used in a 41-day trial. The pigs were housed in suspended pens (1.70 × 1.20 m). Each pen housed six pigs (0.34 m²/pig) with free access to feed and water. The minimum and maximum temperatures in the nursery room were 20.8 ± 2.49 ºC and 30.3 ± 1.79 ºC, respectively.

The pigs were assigned to a randomized block design according to their initial BW, with twelve replicates. Pigs were fed in three phases from 21 to 32, 32 to 42, and 42 to 62 days of age. In the first two phases, treatments consisted of (1) control-lys: 1.45% and 1.34% of standardized ileal digestible (SID) lysine from day 21 to 32 and 32 to 42, as Rostagno et al. (2017) recommend and (2) low-lys: 1.30% and 1.21% of SID lysine from day 21 to 32 and 32 to 42, equivalent to 90% of the SID lysine level of the control-lys diets. In the last phase, from day 42 to 62, all animals received a common diet recommended for the phase (Table 1).

Throughout the trial, feed was weighed before feeding and feed wastage was collected and weighed daily to determine the average daily feed intake (ADFI). At 21, 32, 42, and 62 days, pigs were individually weighed to calculate BW, average daily gain (ADG), and feed conversion (FC). The incidence of diarrhea was visually assessed at 26, 28, 30, and 32 days and categorized as 0 = absent or 1 = present for each pen.

The economic analyses were performed considering the whole nursery phase (21 to 62 days). To verify the economic feasibility of this strategy, first, the feed cost per kilogram of BW gain (Y_i) was calculated following the methodology proposed by Bellaver et al. (1992):

$$Y_i = (C_i * I_i)/G_i$$

where Y_i is the feed cost per kilogram of BW gain; C_i is the feed cost per kilogram of the i^{th} treatment; I_i = feed intake of the i^{th} treatment; and G_i is the BW gain of the i^{th} treatment.

Thereafter, the economic efficiency index (EEI) for each treatment was calculated following the equation, proposed by Barbosa et al. (1992):

$$EEI = (FCe/CTe) * 100$$

where FCe is the lowest feed cost per kilogram of weight gain observed among the treatments and CTe is the cost of the considered treatment.

Subsequently, the profitability was calculated as follows:

$$\text{Profitability} = ([FBW - IBW] * P) - (C * I)$$

where FBW is the final BW at the end of the nursery phase; IBW is the initial BW at the beginning of the experiment; P is the price per kilogram of pig BW; C is the cost per kilogram of feed; and I is the feed intake per pig.

The feed costs and prices of the ingredients used were those recorded in the municipality of Ponte Nova in the state of Minas Gerais, Brazil, during July 2021. The price of the nursery pig was equivalent to 1.8 times the price of the finished pig/kg, a common local practice for the sale of nursery pigs. All values were converted to USD using the monthly average currency exchange rate according to the Banco Central do Brasil.

The pen was considered the experimental unit for the performance data analysis. The data were analyzed using the GLM procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA). Treatments were compared using an ANOVA F-test and the effects were considered significant at $P \leq 0.05$. Treatments consisted of two levels of digestible lysine (100% vs 90%). Diarrhea score data were analyzed using the FREQ procedure of SAS, in which the pen was considered the experimental unit, the effects were assessed with a chi-squared (X^2) test, and they were considered significant at $P \leq 0.05$.

3. Results and discussion

One of the strategies to minimize costs in pig farms during times of high ingredient costs is the temporary reduction of lysine in the diet of weaned piglets, aiming for compensatory growth in the subsequent period (Taylor et al., 2013; Gomes et al., 2021). However, few studies explore the effects of this reduction as well as the economic viability of modified diets for nursery pigs. Thus, a temporary reduction of lysine in weaned piglets' diets followed by an unrestricted diet in the subsequent period was hypothesized to lead to similar growth performance as nonrestricted pigs and be economically feasible at the end of the nursery phase.

From 21 to 32 days of age, pigs fed low-lys diets had worse ADG, FC, and 32-d BW ($P \leq 0.05$), while ADFI was not affected ($P > 0.05$; Table 2). From 32 to 42 days of age, pigs fed low-lys diets had lower ADFI, ADG, and 42-d BW ($P \leq 0.05$), while FC was not affected ($P > 0.05$).

The lower growth performance of pigs fed low-lys diets was expected when the amino acid level in the diet was below the amount recommended to maximize growth performance in the *Brazilian Tables for Poultry and Swine* (Rostagno et al., 2017). Our results are consistent with other studies that demonstrated that lower lysine diets might impair pigs' performance

(Taylor et al., 2013; Totafurno et al., 2020; Gomes et al., 2021). Moreover, the low-lys diet lowered the pigs' ADFI, similar to Taylor et al. (2013) and Gomes et al.'s (2021) findings. Along with reduced lysine in the low-lys diet, the other essential amino acids were reduced proportionally. Therefore, our results may be explained by the lower level of dietary tryptophan; Liang et al. (2018) have demonstrated that lower levels of tryptophan in the diet may lead to lower feed intake. Moreover, an optimal ratio between digestible lysine and metabolizable energy (ME) must be prioritized when the amino acid or energy contents of the diet change (Ferreira et al., 2019). Researchers have demonstrated that pigs present lower feed intake when fed lower SID lys:ME diets (Sweer et al., 2018; Ferreira et al., 2019), as might have been the case in the low-lys treatment.

At 27 and 29 days of age, pigs fed the low-lys diet had a higher ($P \leq 0.05$) incidence of diarrhea (Table 3). The higher incidence of diarrhea in pigs fed a low-lys diet may partially explain the lower ADG and, consequently, the differences in BW at 32 and 42 days between treatment groups. Our results are consistent with Gomes et al. (2021), who found that weaned piglets fed a low lysine diet experienced more diarrhea than pigs consuming a control diet. , Animals fed low lysine diets had other amino acids proportionally reduced, such as a threonine responsible for mucin synthesis and maintenance of intestinal integrity (Bertolo et al., 1998) and tryptophan related to improvement in intestinal barrier function, decrease in the expression of inflammatory cytokines, in addition to a reduction in the population of intestinal pathogens (Liang et al., 2018).

From 42 to 62 days of age, pigs previously fed the low-lys diet had similar ($P > 0.05$) ADFI, ADG, and FC as those fed the control-lys diet. However, pigs fed the control-lys diet had a greater final BW ($P \leq 0.05$). Thus, although low-lys-fed pigs presented a similar ADG from 42 to 62 days, it was insufficient to reach a similar final BW as pigs fed the control diet.

Notably, pigs from the low-lys treatment might have shown partial compensatory growth from 42 to 62 days. According to Skiba (2005), there are two types of compensatory growth, complete and partial. Complete compensatory growth occurs when compensatory growth is so strong that the pig attains the same BW weight and body composition at the same age as nonrestricted animals. Partial compensatory growth occurs when animals' growth rate increases but the magnitude or duration is insufficient to attain similar performance as nonrestricted animals, which may explain this study's findings.

During the entire nursery phase (21 to 62 days of age), pigs fed the low-lys treatment had lower ADG compared to those fed the control-lys diet ($P \leq 0.05$), while ADFI and FC were

unaffected (P>0.05). Thus, the higher ADG and final BW of the control-lys pigs reinforce the importance of following nutritional manuals' recommendations for maximum growth performance.

During the entire experimental period, the lowest feed cost and the highest EEI were recorded for the low-lys treatment (Table 4). The lower feed cost occurred due to the reduction of lysine and other industrial amino acids in the low-lys initial diets, lowering the total feed costs. Besides diet, the EEI also considers the ADFI and ADG. Thus, the highest EEI indicated that feeding low-lys diets was economically efficient and resulted in low production costs. However, the EEI does not consider the sale value of the pig; therefore, we decided to estimate the profitability.

The pigs fed the control-lys diet presented a 3.9% higher profitability when compared to those fed the low-lys diet. Their improved profitability can be explained by the higher final BW of the control-lys group, associated with the piglets' market price. Thus, in this study, the temporary reduction of lysine in the nursery diets was found to be less practical than the diet in which the phase recommendations were obeyed. However, in other economic scenarios, lysine reduction may be feasible. After feeding a low-lys diet had improved the EEI, the cost to produce one kilogram of BW was lower compared to that of doing so in pigs fed a control diet. Therefore, when pork is devalued, lower dietary lysine levels might provide a higher profit margin.

In this study, a temporary reduction of lysine in the diets of weaned piglets followed by an unrestricted diet in the subsequent period led to partial compensation of growth performance. Thus, contrary to our initial hypothesis, the nursery pigs were not able to attain a similar final BW compared to nonrestricted animals. Moreover, the temporary reduction of lysine in diets was not economically feasible at the end of the nursery phase. However, due to the improved EEI, in other economic scenarios, such alternatives might be considered.

5. Conclusions

A temporary reduction of lysine in the diets of weaned piglets followed by an unrestricted diet in the subsequent period led to worse growth performance and lower economic viability in the examined economic scenarios. Moreover, piglets fed the low lysine diet had a higher incidence of diarrhea.

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Table 1. Ingredients and calculated nutritional composition of diets fed from 21 to 32, 32 to 42 and 42 to 62 days of age

Ingredients g/kg	21-32 d		32-42 d		42-62 d
	Control-lys[1]	Low-lys[2]	Control-lys	Low-lys	Control
Corn	373.3	377.0	399.5	402.5	584.7
Soybean meal	174.0	174.0	225.6	225.6	322.1
Dried whey	150.0	150.0	110.0	110.0	-
Soybean Micronized	100.0	100.0	80.0	80.0	-
Extrude corn	80.0	80.0	80.0	80.0	-
Plasma protein	40.0	40.0	25.0	25.0	-
Sugar	30.0	30.0	30.0	30.0	30.0
Dicalcium phosphate	12.5	12.5	13.1	13.1	15.7
Limestone	8.7	8.7	8.3	8.3	7.4
Soybean oil	6.6	7.9	7.6	8.5	22.5
Fumaric acid	5.0	5.0	5.0	5.0	4.0
Anti-caking[3]	3.0	3.0	2.0	2.0	-
Zinc Oxide	2.5	2.5	2.2	2.2	-
Choline chloride	2.0	2.0	1.5	1.5	0.5
L-lysine	3.7	1.8	2.9	1.2	3.4
DL-methionine	2.2	1.4	1.7	0.9	1.4
L-threonine	2.4	1.3	1.8	0.9	1.4
L-valine	0.9	0.0	0.2	0.0	-
L-tryptophan	0.5	0.2	0.3	0.0	0.1
Salt	0.6	0.6	1.2	1.2	4.7
Copper Sulfate	0.6	0.6	0.6	0.6	0.6
Vit-mineral premix	1.4	1.4	1.4	1.4	1.4
BHT	0.1	0.1	0.1	0.1	0.1
Calculated nutritional composition					
ME, kcal/kg	3,400	3,400	3,375	3,375	3,350
Crude protein, %	21.00	20.64	21.00	20.71	20.10
SID[4] lysine, %	1.451	1.306	1.346	1.211	1.206
SID threonine, %	0.972	0.875	0.902	0.812	0.784
SID met + Cys	0.813	0.731	0.754	0.679	0.687
SID tryptophan	0.276	0.248	0.256	0.231	0.229
SID valine	1.001	0.920	0.929	0.911	0.832
Calcium, %	0.850	0.850	0.825	0.825	0.773
Available P, %	0.505	0.505	0.471	0.471	0.431
Sodium, %	0.280	0.280	0.230	0.230	0.205
Lactose, %	11.25	11.25	8.25	8.25	-

[1]Control-Lys, following the recommendation of Rostagno et al. (2017); [2]Low-Lys, Lysine level of 90% of the recommendation of Rostagno et al. (2017); [3]Tixosil® (Solvay, Brazil) prevent the formation of lumps (caking); [4]SID = standardized ileal digestible.

Table 2. Effects of control lysine and low lysine diets on nursery pig growth performance

Item	Treatments[1]		CV(%)[4]	P-value*
	Control-lys[2]	Low-lys[3]		
21 to 32 d of age				
Initial BW, kg	5.48	5.49	5.82	0.92
ADFI, kg/d	0.24	0.23	9.78	0.26
ADG, kg/d	0.22	0.18	13.55	<0.01
FC	1.11	1.27	10.67	<0.01
32 d BW, kg	7.87	7.50	5.75	0.05
32 to 42 d of age				
ADFI, kg/d	0.55	0.45	12.67	<0.01
ADG, kg/d	0.42	0.35	7.92	<0.01
FC	1.31	1.30	13.78	0.88
42 d BW, kg	12.12	10.97	3.63	<0.01
42 to 62 d of age[3]				
ADFI, kg/d	0.95	0.92	9.16	0.57
ADG, kg/d	0.63	0.64	5.57	0.56
FC	1.50	1.45	8.16	0.29
62 d BW, kg	24.73	23.76	3.25	<0.01
21 to 62 d of age				
ADFI, kg/d	0.66	0.62	7.70	0.08
ADG, kg/d	0.47	0.45	3.89	<0.01
FC	1.41	1.40	6.32	0.82

[1] Experimental treatment diets were fed from 21 to 42 days of age, and a common diet was fed to all pigs from 42 to 62 days of age. [2] Following the recommendation of Rostagno et al. (2017); [3] Lysine level of 90% of the recommendation of Rostagno et al. (2017); [4] CV, coefficient of variation; *P ≤ 0.05 significant.

Table 3. Effects of control lysine and low lysine diets on number of pens (total of 12) with diarrhea

Days of age	Treatments[1]		P-value
	Control-lys[2]	Low-lys[3]	
25	3/12	7/12	0.09
27	1/12	5/12	0.05
29	0/12	4/12	0.02
31	1/12	1/12	1.00

[1] Experimental treatment diets were fed from 21 to 42 days of age, and a common diet was fed to all pigs from 42 to 62 days of age. [2] Following the recommendation of Rostagno et al. (2017); [3] Lysine level of 90% of the recommendation of Rostagno et al. (2017); *P $\leq$ 0.05 significant by X^2 test.

Table 4. Economic analysis of feeding control lysine and low lysine diets for nursery pigs

	Treatments[1]	
Item	Control-Lys[2]	Low-Lys[3]
Feed cost (US$/Kg)	0.55	0.54
EEI (%)[3]	96.9	100
Profitability (US$)	33.5	32.2

[1] Experimental treatment diets were fed from 21 to 42 days of age, and a common diet was fed to all pigs from 42 to 62 days of age. [2] Following the recommendation of Rostagno et al. (2017); [3] Lysine level of 90% of the recommendation of Rostagno et al. (2017). [3]EEI - economic efficiency index.

CHAPTER 2:

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Effects of crude protein and non-essential amino acids on growth performance, blood profile, and intestinal health of weaned piglets

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ABSTRACT

This study investigated the effect of crude protein (CP) and non-essential amino acids (NEAA) supplementation on the growth performance, blood profile, intestinal morphology, mRNA relative abundance of inflammatory and antioxidant markers, and tight junction proteins in piglets over the first two weeks after weaning. Ninety 21-day-old piglets (7.55 ± 0.72 kg) were assigned in a randomized block design to one of three dietary treatments: (1) high-CP, diet with 24% CP, (2) low-CP, diet with 18% CP, and (3) low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg Arg (L-arginine; purity

> 99%), and 10 g/kg Glu+Gln (minimum 10% L-glutamine and minimum 10% L-glutamate). Piglets were fed with corn-soybean meal basal diets in a 14-day trial. There was an improvement ($P < 0.05$) on feed conversion ratio of piglets fed high-CP diet compared to treatments with low-CP or low-CP + NEAA. Serum urea nitrogen was higher ($P < 0.05$) in piglets fed high-CP compared to other dietary treatments. In the duodenum, the villus height of animals fed low-CP + NEAA diets was greater ($P < 0.05$) than those fed with high-CP and low-CP. The goblet cells proportion of piglets fed low-CP + NEAA or high-CP was higher ($P < 0.05$) compared to low-CP. In the jejunum, the crypt depth of the piglets with high-CP dietary treatment was greater ($P < 0.05$) in comparison with low-CP + NEAA. In the jejunum, IFN-γ mRNA expression was higher ($P < 0.05$) in animals fed high-CP diets compared to other dietary treatments. However, superoxide dismutase and occludin mRNA expression were higher ($P < 0.05$) in animals fed low-CP+NEAA than in piglets on high-CP diets. In the ileum, the number of Peyer's patches in piglets fed high-CP was higher ($P < 0.05$) compared to other dietary treatments. In conclusion, high-CP diet (24% CP) improves the feed conversion of piglets in the first two weeks after weaning compared to the low-CP (18% CP) supplemented or not with NEAA However, the low-CP diet supplemented with NEAA (Arg, Gln, and Glu) improves intestinal health in piglets by promoting greater villus height and proportion of goblet cells in the duodenum, reducing jejunal crypt depth and Peyer's number patches in the ileum. In addition, piglets that received the low-CP + NEAA diet showed an increase in superoxide dismutase and occludin, and a lower expression of IFN-γ mRNA.

1. Introduction

The gastrointestinal tract of the piglet undergoes several changes during the post-weaning period until it is able to digest plant-based feed ingredients. Therefore, it is crucial for the gastrointestinal tract to regulate the changes caused by the introduction of a solid diet, such as gastric-intestinal pH regulation, enzymatic secretion, and intestinal motility, to the aim of improving digestion and nutrient absorption processes.

Soybean meal (SBM) is the most widely used plant protein source for weaned piglets' diets. The amino acid (AA) profile, balance, and digestibility in SBM are better than any other plant protein source used in swine diets. However, growth performance, intestinal morphology, and immunological status of weaned piglets may be negatively affected due to the presence of antinutritional compounds in this ingredient (Deng et al.,

2022; Rocha et al., 2022). The reduction of dietary crude protein (CP) coupled with supplementation of industrial AA classified as nutritionally essential (EAA) and non-essential (NEAA) are alternatives to reduce the impacts reported in the post-weaning phase (Zhao et al., 2014; Yi et al., 2018; Gomes et al., 2021).

The EAA is one that cannot be synthesized by pigs from materials ordinarily available in cells at a rate matching the demands for maintenance, growth, development and health, which must be provided in the diet to meet the requirements (NRC, 2012). In contrast, NEAA are those AA that can be synthesized in adequate amounts by the animal organism to meet the requirements for maintenance, growth, development, and health and, therefore, need not be provided in the diet (Wu, 2010). During stress such as health challenges, the synthesis of adequate amounts of NEAA can be limited by the availability of appropriate amounts of metabolic nitrogen (N) (Rochell et al., 2015). However, NEAA have a physiological function and thus the animal may have, in some specific conditions, dietary requirements for NEAA to support their maximal growth and health (Rocha et al., 2022). Because of an incomplete understanding of AA biochemistry, nutrition, and physiology, the concept of "nutritional non-essentiality" has led to a disregard for the importance of NEAA in the practice of nutrition (Hou et al., 2015), causing to reduced growth performance (Gomes et al., 2021).

Among the NEAA, arginine (Arg) (Liao, 2021), glutamine (Gln) (He et al., 2016a), and glutamate (Glu) (Yin et al., 2015), can improve the intestinal health of weaned piglets by reducing inflammation and improving the integrity of the intestinal epithelial mucosa. Wu et al. (2014) suggested that the effects of Arg are mediated by nitric oxide production and regulation of gene expression related to cell proliferation and differentiation in the intestinal mucosa. Glutamine is the main source of energy for enterocytes, and it is important to maintain the structural and functional integrity of the intestinal mucosa (Ji et al., 2019). Similarly, Glu is related to increasing the rate of cell proliferation and differentiation, and reducing the oxidative stress of intestinal cells by increasing glutathione synthesis (Yin et al., 2015).

On the other hand, studies have also shown that higher levels of CP in diets for piglets can be beneficial due to the greater contribution of NEAA, peptides, and total N (Nyachoti et al., 2006; Yue and Qiao, 2008; Batson et al., 2021). According to these authors, CP levels as high as 24% would not compromise piglet's growth performance, although it could reduce gut health. Moreover, according to Rocha et al. (2022), there is a minimum CP level after which the growth performance of pigs can be compromised,

for weaned piglets, the proposed minimum CP level was 18.4%. Apparently, below this minimum level, other nutrients such as NEAA, bioactive compounds, and others become limiting for maximal growth performance.

Based on this knowledge, the hypothesis of this study is that supplementation with NEAA in low-CP diets can improve the performance, intestinal health, and immune response of weaned piglets. Thus, the study investigated the effect of CP and NEAA supplementation on the growth performance, blood profile, intestinal morphology, mRNA relative abundance of inflammatory and antioxidant markers, and tight junction proteins in piglets over the first two weeks after weaning.

2. Material and Methods

2.1. Animals and housing

Ninety piglets [PIC 337 (Large White × Landrace × Duroc × Pietrain) × Camborough (Large White × Landrace)] castrated male and female, weaned at 21 d-old and with 7.55 ± 0.72 kg body weight (BW) were used over the first 2 weeks after weaning. Piglets were housed in suspended pens (0.54 m^2/piglet) at an experimental facility in Universidade Federal de Viçosa, MG, Brazil. Each pen houses three piglets with free access to feed and water. For increased microbial pressure, piglets were raised in rooms that were not disinfected or cleaned after the previous occupation by piglets from the same herd (Le Floc'h et al., 2006; Valini et al., 2021). This procedure was adopted to simulate the commercial condition of a production unit. The minimum and maximum temperatures inside the nursery room were 27.4 ± 0.7ºC and 30.9 ± 0.8ºC, respectively.

2.2. Diets and experimental design

Diets were formulated according to the nutritional recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017) (Table 1), and provided in mash form. At 21 d, piglets were assigned in a randomized block design based on BW to one of three dietary treatments: (1) high-CP, diet with 24% CP, (2) low-CP, diet with 18% CP, and (3) low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg Arg (L-arginine; purity > 99%), and 10 g/kg Glu+Gln (minimum 10% L-glutamine and minimum 10% L-glutamate). There were ten pen replicates for each of the three dietary treatments.

2.3. Performance and diarrhea incidence

Throughout the trial, feed was weighed before feeding and feed wastage was collected and weighed daily to determine average daily feed intake (ADFI). At 21 and 35 d, piglets were individually weighed to estimate BW, average daily weight gain (ADG), and feed conversion ratio (FC). In addition, diarrhea incidence was visually assessed by the same technician at 7:00 h when piglets were 25, 27, 29, 31, and 33 days of age and was classified as 0 = absence or 1 = presence for each pen (Gomes et al., 2021).

2.4. Sample collection

At 35 days of age, blood was collected from one piglet with BW closest to the average weight of the piglets within its respective pen. Blood was collected by orbital sinus puncture with a hypodermic needle (40 × 1.6 mm) into 10 mL tubes without anticoagulants for the determination of serum urea N (SUN; Ureal Cobas C311, Linklab, software PNCQ) and immunoglobulin G concentrations (IgG Atellica CH IgG_2, CH Analyzer, Siemens Healthineers). In addition, blood samples were collected in 10 ml tubes containing sodium heparin to assess the plasma amino acid profile by liquid chromatography-tandem mass spectrometry.

The same blood donor piglet was electrically stunned followed by exsanguination to collect samples. Fragments measuring 2 cm were sampled from the duodenum (10 cm from the pylorus), jejunum (mid-section), and ileum (5 cm to ileocecal junction) for histological evaluation (Yang et al., 2014). The histological sections were then washed in a physiological solution and fixed in 4.0% paraformaldehyde solution for 24 h at room temperature. Another 2 cm of jejunum was collected and immediately frozen in liquid N and stored at −80°C for RNA extraction and gene expression analysis.

2.5. Intestinal morphology, Peyer's patches, and goblet cells

After 24 h of fixation, the tissues of the duodenum, jejunum, and ileum were transferred to a 70% (v/v) ethanol solution. Next, they were cross-sectionally cut and dried in ethyl crescent gradients, diaphanized in HistoChoice®, and embedded in liquid Paraplast® at 65°C. Five transverse cuts with 5 μm thickness each were placed per slide

and were stained with hematoxylin and eosin. The cuts were semi-serial using 1 in 10 cuts. For morphological readings of villus height and crypt depth in the duodenum, jejunum, and ileum, an EVOS M5000 Imaging System (Invitrogen, Thermo Fisher Scientific) optical microscope with a 10-objective lens was used. Afterward, the images were analyzed by the image analyzer ImageJ 1.50i; java1.6.0_20 (National Institutes of Health). The heights of 20 villus and their 20 crypts were selected and measured. Villus to crypt ratios using the length data were then calculated. All measurements were made by a single individual. In the ileum segment, the total count of the Peyer's patches was performed, with a magnification of 4×.

For evaluation of goblet cells in the duodenum, jejunum, and ileum, 10 fields per slide were photographed at a magnification of 20×. Subsequently, the Image J program was used and perpendicular lines were inserted with markings in uniformly sized quadrants under each image. Then, the total count of intersections in the image and of the cells that touched the intersections was performed. The calculation was following the methodology proposed by Mandarim-de-Lacerda (1995):

$$\textit{Goblet cells (\%)} = \frac{\textit{total number of goblet cells } x \, 100}{\textit{total number total number of intersections}}$$

2.6. Relative mRNA abundance

Total RNA extraction was performed using a commercial kit (SV Total RNA isolation kit – Promega, Z3100) following the manufacturer instructions. The RNA concentration was estimated by NanoDrop™ Lite (Thermo Fisher Scientific), and RNA integrity was evaluated through 1% agarose gel electrophoresis. Complementary DNA synthesis was performed according to the GoScript™ Reverse Transcription System protocol (Promega Corporation). GenBank numbers to access the primers of the genes are shown in Table 2. Primers were used for reverse transcription quantitative PCR with GoTaq® qPCR Master Mix (Promega) in QuantStudio® 3 (Applied Biosystems, Thermo Fisher Scientific). Geometric mean of Ct value of *β-actin* was used to normalize target genes expression for the jejunum samples. Gene of interest relative expression was calculated by $2^{(-\Delta\Delta Ct)}$ (Livak and Schmittgen, 2001) for glutathione peroxidase (*GPX*), superoxide dismutase (*SOD*), catalase (*CAT*), occludin (*OCL*), zonula occludens-1 (*ZO-*

1), interferon gamma (*IFN-γ*), tumor necrosis factor alpha (*TNF-α*), interleukin 1 beta (*IL1-β*) and interleukin 10 (*IL-10*).

2.7. Statistical analysis

The pen was considered the experimental unit for growth performance and diarrhea incidence analysis. One piglet from each pen was considered the experimental unit for intestinal morphology, gene expression, and serum results. The statistical model included the fixed effect of treatment, and block and residual error as random factors. The normality of experimental errors was evaluated using Shapiro-Wilk. The data were analyzed using the GLMMMIX procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA) via one-way analysis of variance (ANOVA). When an effect was detected in the ANOVA ($P < 0.05$), means were compared by Tukey's post hoc test. Data of diarrhea were analyzed using the FREQ procedure of SAS, and the effects were determined by the chi-squared test at $P < 0.05$.

3. Results

3.1. Growth performance and fecal consistency score

There was no effect ($P > 0.05$) of dietary treatments on ADFI, ADG, and final BW (Table 3). However, there was an improvement ($P < 0.05$) on FC of piglets fed high-CP diet compared to treatments with low-CP or low-CP + NEAA. Treatments did not alter ($P > 0.05$) the diarrhea incidence (Table 4).

3.2. Blood profile

The SUN was higher ($P < 0.05$) in piglets fed high-CP treatment than those with the low-CP and low-CP + NEAA diets (Table 5). There was no effect ($P > 0.05$) of treatments on IgG concentrations. Plasma Gln+Lys concentration was higher ($P < 0.05$) in piglets on high-CP treatment than in low-CP, while low-CP + NEAA had intermediate results. Plasma Met concentration was higher ($P < 0.05$) in piglets fed low-CP than those piglets that received high-CP, while low-CP + NEAA had intermediate results. Plasma Arg, Orn, and Glu concentrations were higher ($P < 0.05$) in piglets fed high-CP and low-

CP + NEAA dietary treatment compared to low-CP. Plasma Val concentration was higher in piglets receiving low-CP + NEAA dietary treatment than those with high-CP, while low-CP had intermediate results. Plasm Leu + Ile, Tyr, and Phe concentrations were higher ($P < 0.05$) in piglets on high-CP dietary treatment compared to others. Plasma Ala concentration was higher ($P < 0.05$) in piglets from low-CP + NEAA treatment compared to others. Plasma Thr, Try, Gly, and Cit concentrations were not influenced ($P > 0.05$) by dietary treatments.

3.3. Intestinal morphology, Peyer's patches, and goblet cells

In the duodenum, the villus height of animals fed low-CP + NEAA diets was greater ($P < 0.05$) than those fed with high-CP and low-CP (Table 6). Moreover, the goblet cells proportion of piglets fed high-CP or low-CP + NEAA was higher ($P < 0.05$) compared to low-CP. However, there were no effects ($P > 0.05$) of treatments on the crypt depth and villus:crypt ratio. In the jejunum, the crypt depth of the piglets with high-CP dietary treatment was greater ($P < 0.05$) in comparison with low-CP + NEAA, while low-CP had intermediate results. However, dietary treatments had no effect ($P > 0.05$) on villus height, villus:crypt ratio, and proportion of goblet cells. In the ileum, dietary treatments had no effects ($P > 0.05$) on villus height, crypt depth, villus:crypt ratio, and proportion of goblet cells. However, the number of Peyer's patches in piglets fed high-CP was higher ($P < 0.05$) compared to other dietary treatments.

3.4. Relative mRNA abundance

In the jejunum, *IFN-γ* mRNA expression was higher ($P < 0.05$) in animals fed high-CP diets compared to other dietary treatments (Figure 1). However, *SOD* and *OCL* mRNA expression were higher ($P < 0.05$) in animals fed low-CP+NEAA than in piglets on high-CP diets. There was no effect ($P > 0.05$) of dietary treatments on mRNA expression of *GPX*, *CAT*, *TNF-α*, *ZO-1*, *IL1-β*, and *IL-10*.

4. Discussion

The reduction of dietary CP balanced with EAA has been used as part of a strategy to improve intestinal health in pigs and, consequently, improve growth performance

(Zhao et al., 2014). However, under stress such as the post-weaning period, there is a greater demand for NEAA because tissue production does not meet the systemic needs (Hou et al., 2015). Thus, it has been suggested that the generation of NEAA from EAA may become a limiting factor for normal growth performance of weaned pigs (Wu, 2010; Fukatsu et al., 2011; Rocha et al., 2022). In this way, studies have shown that high-CP levels or supplementation of NEAA in diets for newly weaned piglets may be beneficial due to the higher intake of NEAA (Nyachoti et al., 2006; Yue and Qiao, 2008; Batson et al., 2021).

In the present study, three experimental diets were fed to piglets in the first two weeks after weaning. The first diet contained 24% CP, supplemented with Lys, Met, and Thr. The second diet contained 18% CP, supplemented with Lys, Met, Thr, Trp, Val, Ile, Leu, and His. The third diet was similar to the second and supplemented with NEAA Arg, Gln, and Glu. All diets were formulated keeping the EAA at or above the recommended ratio to Lys (Rostagno et al., 2017). The hypothesis of the study was that low CP diets supplemented with NEAA would improve the growth performance, gut health, and immune response of weaned piglets.

High-CP levels may be associated with a higher incidence of diarrhea (Heo et al., 2009) and worse growth performance in weaned piglets (Wu, 2014). However, in the present study, the high-CP diets had no negative effects on the incidence of diarrhea, ADG, ADFI, and BW at 35 days of age. Besides, high-CP diets improved the FC of piglets. Others also demonstrated improved growth performance associated with higher levels of dietary CP (Kim et al., 2011; Tian et al., 2016; Limbach et al., 2021). According to Silva et al. (2020), reducing dietary CP levels decreases the supply of dietary N and NEAA, as well as reduces the expression of digestive enzyme genes for carbohydrates and proteases in pigs (He et al., 2016b). Therefore, it is assumed that in the present study, inadequate endogenous NEAA synthesis limited the growth of piglets fed low-CP diets. Moreover, the supplementation of NEAA in the low-CP+NEAA treatment may have not been sufficient to recover growth performance, probably because the animals required a higher level of NEAA or other non-supplemented NEAA. According to Gloaguen et al. (2014), the rate of NEAA synthesis can be limited by the availability of dietary or metabolic N, originating from the deamination of EAA, which will further limit the growth performance of animals.

The SUN is indicative of the efficiency of N utilization by the animals. In the present study, piglets fed low-CP and low-CP+NEAA diets had lower SUN

concentrations compared to high-CP diets. According to Heo et al. (2009), AA absorbed beyond what is necessary for biosynthesis cannot be stored and undergo catabolism, which has urea as its final product. The present result indicated that there was an excess of AA in the high-CP diet. Thus, animals fed low-CP diets may have been more efficient in N utilization, corroborating the results reported by other authors (Nyachoti et al., 2006; Yue and Qiao, 2008; Gomes et al., 2021).

Plasma concentration of AA can be influenced either by the uptake of AA from the diet and by the tissue absorption of circulating AA (Ren et al., 2015). The lower plasma concentrations of Arg and Glu in piglets fed low-CP diet may be related to the lower level in the diet and lower availability of N for the synthesis of these AA as compared to low-CP+NEAA and high-CP. Piglets fed the high-CP diet had lower concentrations of Met and Val in the plasma, which can be explained by the lower dietary supplementation of these AA in industrial form. Supplemented industrial AA are readily available for absorption and are promptly absorbed in the proximal small intestine, while CP-bound AA need to be broken down by luminal and brush border enzymes before absorption (Morales et al., 2017; Yang and Liao, 2019). Plasma concentrations of Leu + Ile, Phe, and Tyr were higher in piglets fed the high-CP diet, which is explained by the fact that this diet contained a higher concentration of those AA as a result of the higher CP content. Moreover, low-CP+NEAA treatment increased plasma concentrations of Orn and Ala compared to low-CP, showing that dietary NEAA supplementation may reduce the intestinal catabolism of other AA and elevate their entry into the portal vein, as reported by Yi et al. (2018).

Gut health has significant implications for swine health status and nutrient utilization, due to its various functions including digestion and absorption of nutrients, secretion of mucins and immunoglobulins, and selective barrier protection against harmful antigens and pathogens (Yang and Liao, 2019). Thus, the evaluation of intestinal morphometry, goblet cells, and Peyer's patches associated with gene expression of tight junction proteins, pro- and anti-inflammatory cytokines, and antioxidant enzymes can be used as tools for assessing intestinal health (Valini et al., 2021).

In the present study, animals fed the low-CP + NEAA diet had higher villus height in the duodenum, indicating greater absorptive capacity for the available nutrients (Zhang et al., 2013). This result may be related to the supplementation of Gln and Glu. Glutamine is a major metabolic fuel for rapidly dividing cells, such as enterocytes, and together with Glu is related to increasing the rate of cell proliferation and differentiation (Yin et al.,

2015; Ji et al., 2019). Piglets fed low-CP + NEAA also had shorter crypt depth in the jejunum, indicating decreased metabolic cost of epithelium turnover associated with inflammation response (Yang and Liao, 2019). Besides shorter crypt depth, those piglets had reduced Peyer's patches in the ileum, suggesting less intestinal challenge compared to the high-CP diet. Peyer's patches are aggregated lymphoid follicles, with a protective function against pathogens (Mair et al., 2014). The high-CP content, as a result of the high SBM level, may have increased the proliferation of pathogenic bacteria in the ileum (although not evaluated in the present study), stimulating the immune system and increasing the number of Peyer's patches. These results are supported by a study conducted by Deng et al. (2022), who reported that the higher the SBM content in the diet, the higher the content of indigestible carbohydrates (stachyose and raffinose) and antigenic proteins (glycinin and β-conglycinin) considered antinutritional factors. In addition, the high-CP content can increase the proliferation of pathogenic bacteria and their potential toxins for the gastrointestinal tract, such as ammonia and polyamines (Duarte and Kim, 2022).

Goblet cells are responsible for the production of mucus that acts as a physical barrier against the invasion of pathogens, while tight junction proteins form a selective physical barrier to prevent endotoxins absorption (Moeser et al., 2017). In the present study, it was demonstrated higher proportion of goblet cells and higher expression of OCLN in animals fed low-CP + NEAA diets and thus improved intestinal integrity. Additionally, it was evaluated the expression of antioxidant enzymes and cytokines in the jejunum of weaned piglets, because the antioxidant capacity and the immune response are fundamental for the promotion of intestinal health. According to Yin et al. (2014), weaning causes an increase in reactive oxygen species that can cause oxidative stress at the intestinal level and in other tissues. In this way, it has been shown that supplementation of Arg, Gln, and Glu in diets can improve the intestinal antioxidant response in pigs (Jiao et al., 2015; Yi et al., 2018). Corroborating this report, animals fed a low-CP + NEAA diet showed increased SOD expression in the jejunum, which suggested greater antioxidant capacity associated with NEAA supplementation.

The IFN-γ is a pro-inflammatory cytokine considered an immunological marker produced in response to inflammation (Andrade et al., 2015). Animals fed the high-CP diet had higher expression of IFN-γ, which may be related to higher SBM levels compared to the low-CP treatments (261 × 73 g/kg). High levels of indigestible proteins in the diet might result in inflammatory response, especially increasing pro-inflammatory cytokines

levels, which might decrease gut integrity (Long et al., 2021). Actually, in the present study, the higher IFN-γ in pigs fed high-CP diets was associated with reduced OCL expression.

Altogether, the results indicated that the low-CP + NEAA diet improves N utilization efficiency, and intestinal architecture and modulates the response expression of genes related to the immune system and antioxidant capacity in piglets in the first two weeks after weaning. Therefore, supplementation with Arg, Gln, and Glu in diets for weaned piglets is a promising nutritional approach to support a formulation with low dietary CP levels.

5. Conclusion

The high-CP diet (24% CP) improves the feed conversion of piglets in the first two weeks after weaning compared to the low-CP (18% CP) supplemented or not with NEAA However, the low-CP diet supplemented with NEAA (5 g/kg of Arg and 10 g/kg of Gln + Glu) improves intestinal health in piglets by promoting greater villus height and proportion of goblet cells in the duodenum, reducing jejunal crypt depth and Peyer's number patches in the ileum. In addition, piglets that received the low-CP + NEAA diet showed an increase in *SOD* and *OCL* mRNA expression and a lower expression of *IFN-γ* mRNA.

6. Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

7. Ethics statement

The experimental protocol follows the ethical principles in animal research (CONCEA, 2016) and was approved by the Ethical Committee on Animal Use of Universidade Federal de Viçosa (UFV) protocol nº 066/2021.

8. Author contributions

AMC, AS, and GCR: conceptualization and designed the study. AMC: conducted the project. AMC, JLG, and GCR: methodology, statistical analysis and formal analysis. AMC, AS, and GCR: writing—original draft preparation. AMC, JLG, and GCR: writing—review and editing. All authors contributed to the article and approved the submitted version.

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11. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

12. Publisher's note

13. References

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TABLE 1 Ingredients and calculated nutritional composition of diets fed to weaned piglets (g/kg, as-fed basis)[1]

Ingredients, g/kg	**High-CP**	**Low-CP**	**Low-CP + NEAA**
Corn, 7.8% CP	318.5	495.5	495.4
Soybean meal, 46.0% CP	261.5	73.5	73.5
Dried whey, 12.5% CP	150.0	150.0	150.0
Soybean micronized, 36.0% CP	100.0	100.0	100.0
Extrude corn, 7.6% CP	55.0	55.0	55.0
Plasma protein, 78.0% CP	40.0	40.0	40.0
Sugar	30.0	30.0	30.0
Dicalcium phosphate	11.7	13.3	13.3
Limestone	8.5	9.1	9.1
Soybean oil	11.0	3.0	3.0
Anti-caking[2]	3.0	3.0	3.0
Zinc oxide	2.5	2.5	2.5
Choline chloride	2.0	2.0	2.0
L-lys, 78.0%	1.3	7.0	7.0
DL-met, 99.0%	1.4	3.1	3.1
L-thr, 98.5%	1.1	3.8	3.8
L-trp, 99.0%	-	1.0	1.0
L-val, 96.5%	-	2.6	2.6
L-ile, 98.0%	-	1.8	1.8
L-leu, 99.5%	-	0.6	0.6
L-his, 98.0%	-	0.7	0.7
L-arg, 98.0%	-	-	5.0
Gln + Glu, 98.0%	-	-	10.0
Salt	0.4	0.4	0.4
Copper sulfate	0.6	0.6	0.6
Vitamin-mineral premix	1.4	1.4	1.4
Calculated and analyzed[3] composition			
Metabolizable energy, kcal/kg	3,400	3,400	3,453
Crude protein, %	24.0 (23.4)	18.0	19.6 (19.5)
SID[4] lys, %	1.45 (1.52)	1.45	1.45 (1.51)
SID met, %	0.43 (0.48)	0.52	0.52 (0.52)
SID met + cys, %	0.81 (0.84)	0.81	0.81 (0.83)
SID thr, %	0.97 (1.11)	0.97	0.97 (1.05)
SID trp, %	0.27 (0.29)	0.27	0.27 (0.28)
SID val, %	1.06 (1.29)	1.00	1.00 (1.19)
SID ile, %	0.94 (0.96)	0.79	0.79 (0.83)
SID leu, %	1.84 (1.93)	1.45	1.45 (1.55)
SID his, %	0.59 (0.57)	0.47	0.47 (0.50)
SID arg, %	1.44 (1.30)	0.90	1.37 (1.22)
Total calcium, %	0.85	0.85	0.85

Available P, %	0.50	0.50	0.50
Sodium, %	0.28	0.28	0.28
Lactose, %	11.2	11.2	11.2

[1]Dietary treatment: High-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg arg (L-arginine, purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate).
[2]Tixosil® (Solvay, Brazil) prevent the formation of lumps (caking).
[3]Total amino acid analyzed included in the parenthesis.
[4]Standardized ileal digestible.

TABLE 2 List of primers used in reverse transcription quantitative-PCR gene expression analysis in weaned piglets

Genes[1]	GenBank number	Sequence[2]
GPX	NM_214201.1	F: 5′GCCCAACTTCATGCTCTTC3′ R: 5′CAGGATCTCCCCATTCTTGGC3′
SOD	NM_001190422.1	F: 5′ATCAAGAGAGGCACGTTGGA3′ R: 5′TCTGCCCAAGTCATCTGGTT3′
CAT	NM_214301.2	F: 5′GCTTTAGTGCTCCCGAACAG3′ R: 5′AGATGACCCGCAATGTTCTC3′
OCL	NM_001163647.1	F: 5′TCCTGGGTGTGATGGTGTTC3′ R: 5′CGTAGAGTCCAGTCACCGCA3′
ZO-1	XM_003353439.2	F: 5´AAGCCCTAAGTTCAATCACAATCT3′ R: 5´ATCAAACTCAGGAGGCGGC3′
IFN- γ	NM_213948	F: 5′TGGTAGCTCTGGGAAACTGAATG3′ R: 5′GGCTTTGCGCTGGATCTG3′
TNF-α	NM_214022.1	F: 5′CATCGCCGTCTCCTACCA3′ R: 5′CCCAGATTCAGCAAAGTCCA3′
IL1- β	NM_214055.1	F: 5′TCTGCCCTGTACCCCAACTG3′ R: 5′CCCAGGAAGACGGGCTTT3′
IL-10	NM_214041.1	F: 5′GAAGGACCAGATGGGCGACTT3′ R: 5′CACCTCCTCCACGGCCCTTG3′
β-actin	U07786.1	F: 5′CTCTTCCATCGTGTCCTTCTAC3′ R: 5′CCTCAGACTTGTCGATCTTCTG3′

[1]*GPX*, glutathione peroxidase; *SOD*, superoxide dismutase; *CAT*, catalase; *OCL*, occludin; *ZO-1*, zonula occludens-1; *IFN-γ*, interferon gamma; *TNF-α*, tumor necrosis factor alpha; *IL1- β*, interleukin 1 beta; *IL-10*, interleukin 10.

[2]F and R indicate Forward and Reverse primers, respectively.

TABLE 3 Effects of crude protein and non-essential amino acids on growth performance of piglets (at 35 d-old)[1]

Item[2]	**Dietary treatment**[3]			**SEM**[4]	***P*-value**
	High-CP	**Low-CP**	**Low-CP + NEAA**		
Initial BW, kg	7.56	7.56	7.55	-	-
ADFI, g/d	399	395	424	22.28	0.78
ADG, g/d	345	298	333	19.32	0.31
FC, g:g	1.16[b]	1.33[a]	1.27[a]	0.02	<0.01
Final BW, kg	12.37	11.75	12.22	0.31	0.47

[a,b]Means with different superscript letters are different by tukey's post hoc test at 5% probability.

[1]Data are means of 10 pens replicates per dietary treatment and 3 piglets per pen as an experimental unit.

[2]Average daily feed intake (ADFI, g/d), average daily weight gain (ADG, g/d), feed conversion ratio (FC).

[3]Dietary treatment: High-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg arg (L-arginine, purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate).

[4]Pooled standard error of the mean.

TABLE 4 Effects of crude protein and non-essential amino acids on diarrhea incidence of piglets[1]

Days of age	**Dietary treatment[2]**			***P*-value**
	High-CP	**Low-CP**	**Low-CP + NEAA**	
25	0	0	1	0.37
27	2	1	2	0.85
29	1	0	1	0.78
31	2	0	0	0.26
33	2	0	0	0.09

[1]Data are means of 10 pen replicates per dietary treatment and 3 piglets per pen as an experimental unit.

[2]Dietary treatment: High-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg arg (L-arginine, purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate).

TABLE 5 Effects of crude protein and non-essential amino acids on blood profile of piglets (at 35 d-old)[1].

Item[2]	**Dietary treatment**[3]			**SEM**[4]	***P*-value**
	High-CP	**Low-CP**	**Low-CP + NEAA**		
SUN, mg/dL	21.0[a]	5.7[b]	7.0[b]	0.97	<0.01
IgG, mg/dL	203.3	161.6	170.0	23.09	0.37
Amino acids, µmol/L					
Glutamine + lysine	51.2[a]	37.1[b]	44.9[ab]	4.17	0.03
Methionine	39.6[b]	87.4[a]	61.0[ab]	11.61	0.01
Arginine	93.0[a]	40.0[b]	81.1[a]	3.53	<0.01
Threonine	57.2	88.3	79.8	14.24	0.28
Tryptophan	25.2	21.7	24.1	1.74	0.34
Valine	129.3[b]	144.1[ab]	181.6[a]	12.20	0.01
Leucine + isoleucine	146.1[a]	92.2[b]	97.5[b]	5.81	<0.01
Glycine	448.2	410.5	405.3	34.66	0.63
Tyrosine	74.4[a]	30.6[b]	25.2[b]	3.63	<0.01
Ornithine	72.2[a]	39.3[b]	58.1[a]	5.13	<0.01
Phenylalanine	40.0[a]	23.9[b]	16.5[b]	2.58	<0.01
Citrulline	46.4	34.2	36.9	4.32	0.12
Glutamate	146.1[a]	84.5[b]	129.8[a]	8.50	<0.01
Alanine	199.3[b]	173.7[b]	254.5[a]	11.65	<0.01

[a,b]Means with different superscript letters are different by tukey's post hoc test at 5% probability.

[1]Data are means of 10 piglets per dietary treatment.

[2]SUN: serum urea nitrogen, IgG: immunoglobulin G.

[3]Dietary treatment: High-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg arg (L-arginine, purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate).

[4]Pooled standard error of the mean.

TABLE 6 Effects of crude protein and non-essential amino acids on intestinal morphology of piglets (at 35 d-old)[1].

Item	Dietary treatment[2]			SEM[3]	*P*-value
	High-CP	Low-CP	Low-CP + NEAA		
Duodenum					
Villus height, μm	381[b]	383[b]	427[a]	11.49	0.01
Crypt depth, μm	208	213	229	8.83	0.40
Villus:crypt ratio	1.8	1.8	1.9	0.07	0.58
Goblet cells, %	53.2[a]	45.3[b]	53.4[a]	2.24	0.01
Jejunum					
Villus height, μm	365	324	299	22.00	0.16
Crypt depth, μm	161[a]	143[ab]	141[b]	5.05	0.01
Villus:crypt ratio	2.3	2.2	2.1	0.12	0.67
Goblet cells, %	45.3	44.8	43.6	2.88	0.45
Ileum					
Villus height, μm	249	239	244	15.97	0.86
Crypt depth, μm	135	131	132	6.07	0.95
Villus:crypt ratio	1.9	1.9	1.9	0.07	0.90
Goblet cells, %	40.9	44.7	43.0	1.99	0.57
Peyer's patches, n	48[a]	38[b]	41[b]	2.20	0.02

[a,b]Means with different superscript letters are different by tukey's post hoc test at 5% probability.

[1]Data are means of 10 piglets per dietary treatment.

[2]Dietary treatment: High-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet with 18% CP supplemented with 5 g/kg arg (L-arginine, purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate).

[3]Pooled standard error of the mean.

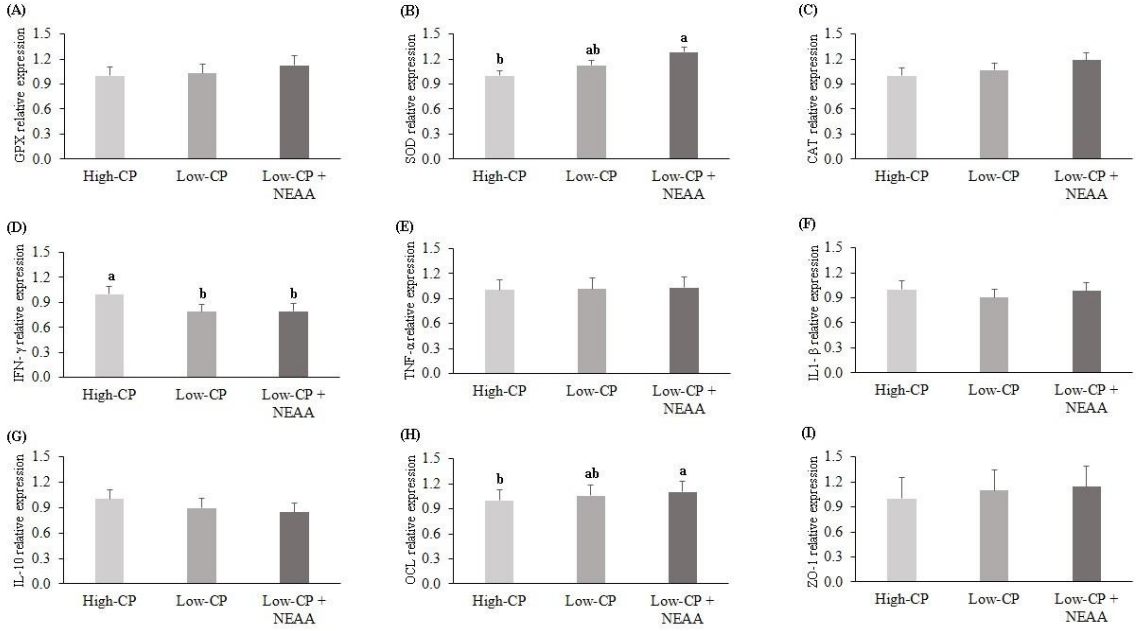

FIGURE 1

Effects of crude protein and non-essential amino acids on mRNA relative abundance of inflammatory and antioxidants markers, and tight junction proteins of jejunum of piglets (at 35 d-old). Dietary treatments: high-CP, diet with 24% CP; low-CP, diet with 18% CP; low-CP + NEAA, diet supplemented with 5 g/kg arg (L-arginine; purity > 99%), and 10 g/kg glu+gln (minimum 10% L-glutamine with minimum 10% L-glutamate). Results are relative to the high-CP treatment. Data are means of 10 piglets per dietary treatment. [a,b]Means with different superscript letters are different by tukey's post hoc test at 5% probability. GPX, glutathione peroxidase **(A)**; SOD, superoxide dismutase **(B)**; CAT, catalase **(C)**; IFN-γ, interferon gamma **(D)**; TNF-α, tumor necrosis factor **(E)**; IL1- β, interleukin 1 beta **(F)**; IL-10, interleukin 10 **(G)**; OCL, occludin **(H)**; ZO-1, zonula occludens-1 **(I)**.

CHAPTER 3:

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Autolyzed yeast and sodium butyrate supplemented alone to diets promoted improvements in performance, intestinal health and nutrient transporter in weaned piglets

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ABSTRACT

This study investigated the effects of supplemental nucleotides, autolyzed yeast (*Saccharomyces cerevisiae*), and sodium butyrate in diets for nursery pigs on growth performance, diarrhea incidence, blood profile, intestinal morphology, mRNA expression of nutrient transporters, inflammatory markers, antioxidant profile, and tight junction proteins in the small intestine. One hundred eighty 21-d-old pigs (5.17 ± 0.57 kg) were assigned in a randomized block design to 1 of 4 dietary treatments: (1) CON: control, basal diet, (2) NUC: CON + nucleotides, (3) YSC: CON + lysed yeast *S. cerevisiae*, (4) ASB: CON + acidifier sodium butyrate. Pigs were fed for 24 d, phase 1 (21 to 32 d) and 2 (32 to 45 d). During phase 1, YSC and ASB improved average daily gain (ADG) and

feed conversion (FC) compared with CON. At the overall period, ASB improved ADG and YSC improved FC compared with CON. The NUC diet did not affect growth performance. The ASB increased ileal villus height compared to CON. The YSC and ASB reduced the number of Peyer's patches in the ileum compared with CON. The YSC increased mRNA expression of nutrient transporters (SMCT2, MCT1, and PepT1), tight junction proteins (OCL and ZO-1), antioxidants (GPX), and IL1-β in the jejunum compared with CON. The ASB increased mRNA expression of nutrient transporters (SGLT1 and MCT1), tight junction proteins (OCL and ZO-1), and antioxidants (GPX and SOD) compared with CON. In conclusion, autolyzed yeast and sodium butyrate promoted growth performance by improving the integrity of the intestinal barrier, the mRNA expression of nutrient transporters, and antioxidant enzymes in the jejunum of nursery pigs whereas supplementation of nucleotides did not show such effects.

Keywords: autolyzed yeast, gut health, performance, piglets, nucleotides, sodium butyrate

1. Introduction

Weaning is considered the "critical window" in the piglet's life because it is associated with several stress factors, such as loss of contact with the mother and original litter, solid diet, environmental and structural changes, and the establishment of a new hierarchy[1,2]. Abrupt separation from the sow and dietary transition are critical events causing intestinal challenges and compromising immune functions affecting growth of pigs[3,4]. Dietary interventions with various functional additives have been introduced and implemented to cope with challenges to the intestine and immune functions upon weaning. Nucleotides[5,6], yeast[7,8] and organic acids[9,10] have appeared on the market as feeding strategies to improve intestinal health and, consequently, promote better growth performance of weaned piglets. Globally, their use as in-feed additives in pig diets has become more frequent, especially during the weaning period[11,12]. However, factors such as the form of supplementation, the type of additive, and the site of action make each application unique and complex in animal responses and should be better understood in studies involving pigs fed different feed additives[13,14,15].

Nucleotides are monomers that serve as building blocks of nucleic acids (DNA and RNA) and are synthesized by animals through the *de novo* pathway or the salvage

pathway. These monomers function as physiological mediators, coenzyme components, and contributors to cell growth and division and are crucial to the rapid proliferation of intestinal mucosa cells and immune cells[16,17]. Thus, the supplementation of nucleotides to the diet can bring benefits in periods of rapid growth and development (e.g. digestive organs, immune systems, cell renewal) helping in circumstances associated with intestinal injuries and stress, such as post-weaning period[5,6].

The lysed yeast (*S. cerevisiae*) contains in the cell wall a complex polymer and is composed of β-glucans, α-mannans, mannoproteins, and a minor component of chitin[18], in addition, the cytoplasm contains B complex vitamins, highly digestible proteins and free amino acids[15]. However, monogastric animals do not have enzymes to digest polysaccharides such as those present in the yeast cell wall, therefore, the breakdown of yeast cell wall by lysing would improve the bioavailability of yeast components for animal nutrition[7]. The major polysaccharide constituents of the yeast cell wall, β-glucans and α-mannans, have been recognized to be capable of modulation of the mucosa immune system, promoting beneficial effects on pig health (e.g. inhibition of pathogen adhesion to gastrointestinal epithelial tissue and stimulation of immunocompetent cells in Peyer's patches)[19,20]. Thus, yeast products, mainly based on *S. cerevisiae*, are widely used as feed additives.

Organic acids (e.g. sodium butyrate) act by reducing the pH of digesta in the gastrointestinal tract, stimulating the activity of digestive enzymes, inhibiting the proliferation of pathogenic bacteria, and improving the digestibility of nutrients[9,21]. Sodium butyrate is a short-chain fatty acid that acts by improving villus height, decreasing crypt, and improving the expression of tight junction protein in the gut[14]. As a result, it beneficially affects the reabsorption of fluids and electrolytes which is associated with reduced diarrhea[22], moreover modulating intestinal permeability will reduce translocation of intraluminal toxins and antigens from the lumen into subepithelial tissues and systemic blood circulation[23]. Together with improving gut health, the ability of sodium butyrate to alleviate oxidative stress and improve immunological profile will result in improved pig weight gain[22,14,24]. However, free organic acids dissociate and lose most of their antibacterial capacity before reaching the distal part of the digestive system[25]. Therefore, sodium butyrate in encapsulated form allows progressive reach to the distal parts of the gastrointestinal tract without being fully dissociated, because the product is gradually released compared to an uncoated product[10].

In view of the above, the hypothesis of the study was that the dietary supplementation of purified nucleotide, lysed yeast (*S. cerevisiae*), or encapsulated sodium butyrate would improve the growth performance of weaned piglets by beneficially stimulating the immune response and supporting intestinal physiological and health functions. Therefore, the objective of this study was to evaluate the effects of purified nucleotide, lysed yeast, and sodium butyrate in diets for nursery pigs on growth performance, diarrhea incidence, blood profile, intestinal morphology, mRNA expression of nutrients transporter, inflammatory markers, antioxidant profile, and tight junction proteins.

2. Results

2.1. Growth performance

During phase 1 (21 to 32 d), pigs fed YSC or ASB diets showed ($P < 0.05$) improvements in ADG, FC, and BW compared with pigs fed CON diet; however, ADFI was not affected by dietary treatments (Table 1). In phase 2 (32 to 45 d), pigs fed ASB diet had higher ($P < 0.05$) BW compared to pigs fed CON diet. In addition, there was a trend to increase the BW ($P = 0.095$) in pigs fed YSC diet compared to those fed CON diet. Pigs fed NUC diet tended to present lower ADG ($P = 0.080$). However, FC was not affected by dietary treatments. During the overall period, ADG was higher ($P < 0.05$) in pigs fed ASB diet than those with CON diet. In addition, FC was better ($P < 0.05$), and a trend was observed to improve ADG ($P = 0.095$) in pigs fed YSC diet compared with pigs on CON diet. Moreover, ADFI was not affected by dietary treatments.

2.2. Diarrhea incidence

There was a trend to reduce the diarrhea incidence ($P = 0.098$) in pigs fed NUC diet and improved fecal score ($P = 0.090$) in pigs fed YSC diet than pigs fed CON diet in the phase 1 (Table 2). In phase 2, there was a trend to reduce the diarrhea incidence ($P = 0.089$) and improved fecal score ($P = 0.091$) in pigs fed YSC diet than in those fed CON diet.

2.3. Blood profile

There was no effect of dietary treatments on IgG, creatinine, and urea concentrations (Table 3).

2.4. Intestinal morphology

Dietary treatments had no effects on villus height, crypt depth, villus:crypt ratio, and proportion of goblet cells in the duodenum and jejunum (Table 4). In the ileum, the dietary treatments had no effects on crypt depth and proportion of goblet cells. However, villus height was increased ($P < 0.05$) and villus:crypt ratio trended to increase ($P = 0.066$) in pigs fed ASB diet than pigs fed CON diet. In addition, the number of Peyer's patches in pigs fed YSC or ASB diets was lower ($P < 0.05$) compared to pigs that received CON diet. There was a trend towards an increase ($P = 0.071$) villus height and reduce ($P = 0.078$) the number of Peyer's patches in pigs fed NUC diet than those fed the CON diet.

2.5. mRNA relative abundance of nutrient transporters

In the jejunum, there was no effect of dietary treatments on the mRNA expression of GLUT2, EAAC1, and y^+LAT1 (Fig. 1). However, mRNA expression of SMCT2 and PepT1 was higher ($P < 0.05$) in pigs fed YSC diet compared to pigs fed CON diet. The mRNA expression of MCT1 in pigs fed ASB and YSC diets was higher ($P < 0.05$) than in those fed CON diet. In addition, SGLT1 mRNA expression in pigs fed ASB diet was higher ($P < 0.05$), and YSC diet ($P = 0.055$) trended upwards compared to pigs fed CON diet.

2.6. mRNA relative abundance of inflammatory and antioxidants markers, and tight junction proteins

There was no effect of dietary treatments on the mRNA expression of CAT, IFN-γ, and IL-10 (Fig. 2). However, mRNA expression of GPX, OCL, and ZO-1 was higher ($P < 0.05$) in pigs fed YSC or ASB diets compared to pigs fed CON diet. The mRNA expression of SOD in pigs fed ASB diet, and IL1-β in pigs fed YSC diet was higher ($P < 0.05$) than in those fed CON diet. In addition, TNF-α mRNA expression in pigs fed YSC

diet (P = 0.082) and ASB diet (P = 0.061) trended upwards compared to pigs fed CON diet. There was a downward trend (P = 0.058) in IL1-β mRNA expression of pigs fed NUC diet than pigs that received CON diet.

3. Discussion

The hypothesis of the current experiment was that the dietary supplementation of purified nucleotide, *S. cerevisiae* yeast, or encapsulated acidifier sodium butyrate would improve the growth performance of weaned piglets and beneficially affect gut parameters. The present results showed that feeding nursery pigs with YSC or ASB diets increased growth performance and improved gut health, whereas no improvements were observed feeding the NUC diet.

Although nucleotides can be synthesized from other precursors in pigs' diets, in the post-weaning a stressful and limited nutrient intake period, nucleotides could be considered an essential nutrient[6]. However, in the present study, performance was not influenced by NUC diet, while others have reported positive effects of nucleotide supplementation on piglet growth performance[26,27] and feed intake[5]. The NUC diets correspond to 100 mg nucleotides/kg diet in phase 1 and 75 mg nucleotides/kg in phase 2 in a 24-d trial and were fed purified. On the other side, Superchi et al.[26] used a nucleotide yeast-derived source that also contains viable cells, cell wall components, inositol, and functional amino acids. Weaver and Kim[27] demonstrated improved growth performance with up to 1,000 mg nucleotides/kg during a 28-d trial. Working with different levels of nucleotides (0, 50, 150, 250, and 500 mg/kg) in a 20-d trial with one pig per pen, Jang and Kim[5] only found significant improvements in the feed intake, and when feeding the lower supplementation levels (50 and 150 mg/kg). Thus, the discrepancies in growth performance results between studies are justified due to the different sources, the different dosages used, and administration time.

Regarding the YSC diet, the improved growth performance results are attributed to the improvements in intestinal health promoted by the supplementation of *S. cerevisiae* yeast. This additive contains high amounts of highly digestible protein, essential amino acids, nucleotides, mannanoligosaccharides, and β-glucans[15]. These components present in yeast may have anti-inflammatory properties to reduce intestinal inflammation and, consequently, minimize diarrheal disorders (as observed in the current study) and nutrient malabsorption[1]. According to Kogan and Kocher[19], β-glucans are capable of blocking

fimbriae of pathogenic bacteria and preventing their adhesion to the epithelium of the intestinal mucosa, acting to prevent or eliminate infection. Yeast-based additives support the immune system of piglets by modulating the intestinal microbiota[28], which may contribute to improve growth performance[29].

An increase in nutrient digestibility as reported by Barbosa et al.[30] and Boontiam et al.[8] is another mechanism by which yeast supplementation in nursery pigs' diets can improve the growth performance. Nutrient transporters are proteins expressed in the apical membrane of intestinal cells that absorb nutrients and, therefore, it is possible to improve digestibility by increasing the expression of these transporters[31]. This is supported by the results of our study, which showed increased mRNA expression of nutrient transporters (SGLT1, SMCT2, MCT1, and PepT1) in the jejunum of pigs. The SGLT1 is responsible for glucose absorption, while PepT1 acts on peptide absorption. The increase in the expression of these transporters suggested that there was greater availability of glucose and amino acids at the cellular level, in agreement with the findings by Clarke et al.[31]. Similarly, the increased expression of SMCT2 and MCT1 indicated a greater availability of monocarboxylates (e.g. lactate, short-chain fatty acids, and ketone bodies)[32], which represent substrates for maintaining the energetic state in cells like the enterocytes.

Regarding the ASB diet, the improvement in growth performance is supported by the increase in SGLT1 and MCT1 mRNA expression in the jejunum of nursery pigs. The MCT1 is expressed in both the small and large intestines, and its function is to transport butyrate into the cell[33]. However, in the small intestine, microbial butyrate formation is low or absent[34], but the addition of butyrate to diets exerts trophic effects[35] and stimulates the secretion of digestive enzymes[21], and this can result in more efficient digestion and absorption of dietary nutrients, leading to improved performance. Furthermore, butyrate is a source of energy for enterocytes and additionally has an antiapoptotic effect[33]. Therefore, greater MCT1 and SGLT1 expression may enhance the absorption of nutrients and energy to cope with post-weaning stress[36]. In the present study, sodium butyrate was added in encapsulated form (composed of a vegetable fat-based coating material) to be enzymatically broken down by lipase secreted in the duodenum, as mentioned by Maito et al.[10]. According to Tugnoli et al.[25], the encapsulation process provides protection that allows a gradual release of the acidifier along the length of the gastrointestinal tract, reducing the dissociation in the stomach and maintaining their efficacy in jejunum and ileum.

The weaning transition promotes physiological changes in the structural and functional aspects of the intestine, causing villous atrophy and increased crypt depth, which in turn reduces the small intestine capacity to absorb nutrients[3]. In the present study, villus height and villus:crypt ratio in the ileum were higher in pigs fed the ASB diet, indicating improvement in intestinal morphology. Moreover, a greater abundance of jejunal mucosal barrier function-related genes (OCL and ZO-1) was observed in pigs fed the ASB diet. These results indicated that ASB diet was efficient in the structural maintenance of the small intestine, as suggested by others[35]. The gradual release of sodium butyrate throughout the intestinal tract seems to be critical in achieving the expected target[9], allowing the additive to affect different portions of the intestine such as jejunum and ileum. According to Guilloteau et al.[33], supplementation with sodium butyrate stimulates the proliferation of epithelial cells, resulting in a greater absorption surface and preservation of villi length, reflecting the improvements observed in growth performance of pigs.

A greater abundance of jejunal OCL and ZO-1 mRNA expression was also observed in pigs fed the YSC. According to Rose et al.[37], OCL and ZO-1 are classified as intestinal junction proteins with a role in regulating epithelial permeability. Decreased intestinal permeability can be correlated with reduced oxidative stress, because this results in attenuation of damage to the intestinal mucosal barrier[14]. Oxidative stress is a physiological stage in which antioxidant defense is inadequate to detoxify the reactive oxygen species, this oxidative process damages essential biomolecules, leading to reduced growth performance[29]. The GPX and SOD as the key enzymes of the antioxidant system play a crucial role in eliminating free radicals, reducing oxidative damage, and maintaining cell structure is well known[38]. In our study, in addition to the increase in the expression of tight junction proteins, pigs fed the ASB and YSC diet had greater expression of GPX and SOD mRNA. These results suggested that nursery pigs fed with additives had greater antioxidant capacity than those fed the CON diet, demonstrating that there was an improvement in the intestinal redox state.

The impacts of weaning stress are not only limited to intestinal barrier function and oxidative stress, but an increase in the activation of the immune system in weaned piglets is also observed[3]. An unregulated enhanced immune response may trigger a negative effect on other metabolic processes and as a result impair growth performance[39]. Peyer's patches are the major organized lymphoid structures involved in the induction of mucosal immune responses in the intestine[40]. In the present study, a reduction in Peyer's

patch counts was observed in the ileum of pigs fed YSC or ASB diet. This suggested that there was less induction of the mucosal immune system in pigs that received YSC and ASB diets and, consequently, less stimulus to the immune system because Peyer's patches can be considered as immunological sensors of the intestine[41]. These results suggested that both yeast and sodium butyrate could help enhance the small intestine epithelial barrier, antioxidant capacity, and immune system. Thus, the enhancement of overall gut health helps explain the improvement in the growth performance of pigs fed YSC and ASB diets.

Regarding the production of pro-inflammatory cytokines, it was found that pigs fed the YSC diet showed an increase in IL1-β mRNA expression and a tendency to increase TNF-α, while piglets fed the ASB diet showed a tendency to increase TNF-α mRNA expression. On the other hand, pigs fed the NUC diet had a tendency to reduce IL1-β mRNA expression. Nucleotides, yeast, and acidifier-based additives can improve pig immune responses in the post-weaning period[16]. These additives can activate immune cells, including macrophages, which produce IL-1β as part of the immune response[42, 43]. The results of the present study indicated a sustained condition of immune response, because in some cases, a controlled and transient increase in IL-1β may be part of a healthy immune response to support the ability of pigs to fight infections or maintain intestinal health[18]. According to Grimble[39], it is considered beneficial the presence of cytokines (e.g. IL-1β and TNF-α) in adequate concentrations during an inflammatory response to infection. It is important to avoid overstimulation of the immune system, as greater expression of pro-inflammatory cytokines can trigger pathological responses in inflammatory conditions[44]. However, in a previous study, it was observed that TNF-α increases the expression of specific anti-apoptotic proteins, as well as triggers the expression of the survival gene BCL2A1 (not evaluated in the current study) in the intestine of weaned piglets[45]. Also, the β-glucans present in yeast are recognized by specific receptors (pattern recognition receptors) on immune cells, such as macrophages and neutrophils. In particular, they are recognized by the dectin-1 receptor[46]. Once β-glucans bind to these receptors, they can trigger an immune response. Upon recognition of β-glucans, immune cells can produce pro-inflammatory cytokines, such as TNF-α, and IL-1β[47]. Collectively, although these cytokines play a central role in initiating and magnifying the inflammatory response, they did not negatively affect the biological response of nursery pigs fed YSC and ASB diets.

4. Conclusions

Based on the evaluated criteria, dietary supplementation of autolyzed yeast *S. cerevisiae* or sodium butyrate promotes better growth performance by improving the integrity of the intestinal barrier, the mRNA expression of nutrient transporters and antioxidant enzymes in the jejunum of nursery pigs, but without major differences in intestinal morphology in those fed with *S. cerevisiae* yeast. Furthermore, none of the dietary treatments promoted changes in the observed blood metabolites, but a diet containing *S. cerevisiae* yeast or sodium butyrate provided a sustained immune response in the jejunum of nursery pigs. On the other hand, dietary nucleotide supplementation did not improve growth performance and gut health.

5. Material and Methods

The experimental protocol follows the ethical principles in animal research (CONCEA, 2016) and was approved by the Ethical Committee on Animal Use of Universidade Federal de Viçosa, under protocol nº 0110/2022. All methods were carried out in accordance with relevant guidelines and regulations. All methods were reported in accordance with ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines).

5.1. Animals, experimental design, housing, and diets

The experiment was conducted on a commercial farm in the municipality of Santo Antônio do Grama, MG, Brazil. A total of 180 piglets [PIC 337 (Large White × Landrace × Duroc × Pietrain) × DB 90 (Large White × Landrace)] castrated males and females, weaned at 21 d-old and with 5.17 ± 0.57 kg BW were used in a 24-d trial. Piglets were assigned to a randomized block design based on their initial BW (light 4.7 ± 0.25 kg and heavy 5.6 ± 0.34 kg) into 4 dietary treatments and 9 pens replicates (5 pigs/pen).

The experiment was conducted only in one series, without an adaptation period. The pigs were housed in suspended pens (1.75 × 1.00 m, 0.35 m²/pig), with a plastic floor, semi-automatic feeders and nipple drinkers, with free access to diet and water. The minimum and maximum temperatures in the nursery room were 22.9 ± 1.50ºC and 31.8 ± 2.54ºC, respectively.

Pigs were fed in a two-phase feeding regimen (phase 1: 21 to 32 d, and phase 2: 32 to 45 d). All diets were corn and soybean meal-based with industrial amino acids and formulated according to the nutritional recommendations of the Brazilian Tables for Poultry and Swine[48] (Table 5), and provided in mash form. During phases 1 and 2, the dietary treatments consisted, respectively, of: (1) CON: control, basal diet, (2) NUC: CON + 1 g/kg and 0.5 g/kg of nucleotides, (3) YSC: CON + 20 g/kg and 10 g/kg of lysed yeast *S. cerevisiae*, (4) ASB: CON + 1.5 g/kg and 1 g/kg of acidifier sodium butyrate. Feed additives were added in replacement with inert in the CON based on the manufacturer's recommendations.

5.2. Composition of feed additives

The source of nucleotides (Nucleobase 1.5, Aleris Animal Nutrition, Jundiaí, SP, Brazil) obtained from yeast extract purified with autolyzed yeast, contained 15% free nucleotides and 85% vehicle, corresponding to 100 mg free nucleotides/kg diet (phase 1) and 75 mg free nucleotides/kg (phase 2). The yeast source (Sinergis, Aleris Animal Nutrition, Brazil) contained 100% autolyzed yeast *S. cerevisiae*. The acidifier source (Nuttritiva B90CNa, Additiva Nutrition, Monte Alegre do Sul, SP, Brazil) contained 90% sodium butyrate in the form of a protected and encapsulated salt with palm oil, microgranulated, corresponding to 1.35 g sodium butyrate/kg diet (phase 1) and 0.90 g sodium butyrate/kg diet (phase 2).

5.3. Growth performance and diarrhea incidence

Throughout the trial, the offered diet and leftovers were weighed to calculate average daily feed intake (ADFI). Pigs were individually weighed on d 21, 32, and 45 to determine BW, average daily weight gain (ADG), and feed conversion (FC). The fecal consistency of each pig was visually assessed during phase 1 (24 to 32 d) and phase 2 (32 to 45 d), using the method described by Liu et al.[49]. Fresh feces were ranked on a 4-point scale as follows: 0 = solid, 1 = semi-solid, 2 = semi-liquid, and 3 = liquid. The diarrhea incidence was defined as the consistency of feces at scale 2 or 3 for 2 continuous days. The diarrhea incidence for the pigs was calculated as follows: diarrhea incidence (%) = [(the number of pigs with diarrhea in each pen × number of days of diarrhea) ÷ (total

number of pigs in each pen × number of days)] × 100%. Observations were made in the morning, every day throughout the experimental period by a trained evaluator.

5.4. Sample collection

At 32 d-old, blood was collected from 1 piglet with BW closest to the average weight of the pigs within its respective pen. The animals were not fasted. Blood was collected (09h00) by orbital sinus puncture with a hypodermic needle (40 × 1.6 mm) into 10 mL tubes without anticoagulants. Samples were immediately sent at room temperature to the Viçosa Clinical Laboratory (Viçosa, MG - Brazil) for determination of serum urea nitrogen (SUN; Ureal Cobas C311, Linklab, software PNCQ), creatinine (Creatinine, WS-Kovalent, kinetic method, ASB-380, Mindray), and immunoglobulin G concentrations (IgG Atellica CH IgG_2, CH Analyzer, Siemens Healthineers).

The same blood donor piglet was electrically stunned (240 volts for 3 s) followed by exsanguination to collect samples on d 32. Fragments measuring 2 cm were sampled (8 pigs/treatment) from the duodenum (10 cm from the pylorus junction), jejunum (mid-section), and ileum (5 cm to ileocecal junction) for histological evaluation[50]. The histological sections were then washed in a physiological solution (0.9% sodium chloride) and fixed in 4% paraformaldehyde solution (100 mL 40% paraformaldehyde, 900 mL distilled water, 2.28 g monobasic sodium phosphate, and 21.74 g dibasic sodium phosphate) for 24 h at room temperature[6]. Another 2 cm of jejunum was collected and immediately frozen in liquid nitrogen, stored at −80°C for RNA extraction and gene expression analysis.

5.5. Intestinal morphology, Peyer's patches, and goblet cells

After 24 h of fixation, the fragments of the duodenum, jejunum, and ileum were transferred to an ethanol solution 70% (v/v). Then, the samples were cut into cross-sections and dried in increasing gradients of ethyl, diaphanized in HistoChoice®, and embedded in liquid Paraplast® at 65°C. Five cross-sections (5 μm thickness each) were placed per slide and stained with hematoxylin and eosin. The sections were semi-serial using 1 in 10 cuts[51]. For morphological readings of villus height and crypt depth in the duodenum, jejunum, and ileum, an EVOS™ M5000 Cell Imaging System optical microscope (Invitrogen, Thermo Fisher Scientific) with a 10-objective lens was used. The

images were then analyzed using ImageJ 1.50i (Java1.6.0_20; National Institutes of Health, USA). Heights of 20 villus and their 20 crypts were selected and measured. Villus to crypt ratios using the length data were then calculated. All measurements were made by a single trained individual. In the ileum fragment, the total count of the Peyer's patches was performed at 4× magnification[52].

To assess the goblet cells in the duodenum, jejunum, and ileum, 10 fields per slide were photographed at 20× magnification. Subsequently, the ImageJ program was used, and perpendicular lines were inserted with markings in uniformly sized quadrants under each image. Then, the total number of intersections in the image and the cells that touched the intersections were counted. The calculation was made according to the methodology proposed by Mandarim de Lacerda[53]:

$$Goblet\ cells\ (\%) = \frac{total\ number\ of\ goblet\ cells\ x\ 100}{total\ number\ total\ number\ of\ intersections}$$

5.6. Relative mRNA abundance

Total RNA was extracted using a commercial kit (SV Total RNA isolation kit – Promega, Z3100), following the manufacturer's instructions. The RNA concentration was estimated using NanoDrop™ Lite (Thermo Fisher Scientific), and RNA integrity was assessed using 1% agarose gel electrophoresis. Complementary DNA was synthesized according to the GoScript™ Reverse Transcription System protocol (Promega Corporation). GenBank numbers used to access the gene primers are shown in Table 6. Primers were used for reverse transcription quantitative PCR with GoTaq® qPCR Master Mix (Promega) in QuantStudio® 3 (Applied Biosystems, Thermo Fisher Scientific). Geometric mean of Ct value of β-actina was used to normalize the expression of the target genes for the jejunum samples. The relative expression of the gene of interest was calculated by ΔCt[54]for glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), occludin (OCL), zonula occludens-1 (ZO-1), interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL1-β), interleukin 10 (IL-10), glucose transporter type 2 (GLUT2), Na^+/glucose co-transporter 1 (SGLT1), sodium-coupled monocarboxylate transporter (SMCT2), monocarboxylate transporter 1 (MCT1), peptide transporter 1 (PepT1), excitatory amino acid carrier 1 (EAAC1), Na^+-independent cationic and Na^+-dependent neutral amino acid transporter 1 (y^+LAT1).

5.7. Statistical procedures

The pen was considered the experimental unit for growth performance and diarrhea incidence analysis. One pig from each pen was considered the experimental unit for intestinal morphology, gene expression, and blood profile. The statistical model included the fixed effect of dietary treatment, and block and residual error as random factors. The normality of experimental errors was evaluated using Shapiro-Wilk. The data were analyzed using the mixed procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA) via one-way analysis of variance (ANOVA). When an effect was detected in the ANOVA ($P < 0.05$), differences between means were determined by the preplanned contrasts, in which each of the treatments (NUC, YSC, and ASB) were compared versus the control treatment (CON). The statistical significance and tendency were declared at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

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Author contributions

GCR and AMC: conceptualization, data curation, and project management. GCR, AMC, and FFA: methodology. GCR: software. GCR, AMC, JLG and SWK: statistical analysis, formal analysis, and writing—original draft preparation. GCR and SWK: validation. AMC and FFA: investigation. AMC, JLG, SWK and GCR: writing—review and editing. GCR: supervision. All authors contributed to the article and approved the submitted version.

Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Table 1. Growth performance of piglets fed diets supplemented or not with feed additives (n = 9 pens replicates per dietary treatment and 5 piglets per pen as an experimental unit). [a]Average daily feed intake (ADFI, g/d), average daily weight gain (ADG, g/d), feed conversion ratio (FC, g:g). [b]Dietary treatment: phase 1 (21 to 32 d) and phase 2(32 to 45 d), respectively: (1) CON: control, basal diet, (2) NUC: CON + 1 g/kg and 0.5 g/kg of nucleotides, (3) YSC: CON + 20 g/kg and 10 g/kg of lysed yeast *S. cerevisiae*, (4) ASB: CON + 1.5 g/kg and 1 g/kg of acidifier sodium butyrate. [c]Pooled standard error of the mean.

Item[a]	Dietary treatment[b]				SEM[c]	*P*-value		
	CON	NUC	YSC	ASB		CON × NUC	CON × YSC	CON × ASB
Body weight, kg								
Initial	5.22	5.22	5.22	5.22	-	-	-	-
32 d of age	6.83	6.89	7.18	7.18	0.11	0.693	0.034	0.031
45 d of age	12.64	12.36	13.12	13.32	0.19	0.321	0.095	0.019
Phase 1, 21 to 32 d								
ADFI, g/d	296	286	281	302	7.02	0.319	0.133	0.546
ADG, g/d	146	152	178	178	10.08	0.687	0.035	0.030
FC, g/g	2.05	1.97	1.61	1.74	0.09	0.549	<0.010	0.034
Phase 2, 32 to 45 d								
ADFI, g/d	627	600	638	668	17.35	0.263	0.678	0.105
ADG, g/d	486	454	494	510	12.57	0.080	0.674	0.187
FC, g/g	1.29	1.32	1.29	1.31	0.02	0.336	0.940	0.602
Overall period								
ADFI, g/d	449	431	448	473	10.88	0.237	0.902	0.141
ADG, g/d	309	297	329	337	8.20	0.326	0.095	0.019
FC, g/g	1.46	1.45	1.36	1.40	0.02	0.848	<0.010	0.103

Table 2. Diarrhea incidence and fecal score of piglets fed diets supplemented or not with feed additives (n = 9 pens replicate per dietary treatment and 5 piglets per pen as an experimental unit). [a]Dietary treatment: phase 1 (24 to 32 d) and phase 2 (32 to 45 d), respectively: (1) CON: control, basal diet, (2) NUC: CON + 1 g/kg and 0.5 g/kg of nucleotides, (3) YSC: CON + 20 g/kg and 10 g/kg of lysed yeast *S. cerevisiae*, (4) ASB: CON + 1.5 g/kg and 1 g/kg of acidifier sodium butyrate. [b]Pooled standard error of the mean.

Item	Dietary treatment[a]				SEM[b]	*P*-value		
	CON	NUC	YSC	ASB		CON × NUC	CON × YSC	CON × ASB
Phase 1								
Diarrhea incidence, %	20.01	12.10	14.33	15.56	3.28	0.098	0.230	0.345
Fecal score	0.80	0.64	0.61	0.69	0.07	0.161	0.090	0.341
Phase 2								
Diarrhea incidence, %	15.50	16.00	6.70	7.16	3.55	0.927	0.089	0.106
Fecal score	0.53	0.60	0.32	0.37	0.11	0.516	0.091	0.193

Table 3. Blood profile of piglets fed diets supplemented or not with feed additives on d 32 (n = 9 pigs replicates per dietary treatment). [a]Dietary treatment: CON, control, basal diet; NUC, CON + 1 g/kg of nucleotides; YSC, CON + 20 g/kg of lysed yeast *S. cerevisia*; ASB: CON + 1.5 g/kg of acidifier sodium butyrate. [b]Pooled standard error of the mean.

Item	Dietary treatment[a]				SEM[b]	*P*-value		
	CON	NUC	YSC	ASB		CON × NUC	CON × YSC	CON × ASB
Urea, mg/dL	16.55	16.89	15.55	13.11	1.58	0.882	0.658	0.133
Creatinine, mg/dL	0.64	0.70	0.68	0.66	0.06	0.416	0.330	0.458
IgG, mg/dL	272.74	306.67	266.07	273.69	23.42	0.157	0.777	0.968

Table 4. Intestinal morphology of piglets fed diets supplemented or not with feed additives on d 32 (n = 9 pigs replicates per dietary treatment). [a]Dietary treatment: CON, control, basal diet; NUC, CON + 1 g/kg of nucleotides; YSC, CON + 20 g/kg of lysed yeast *S. cerevisiae*; ASB: CON + 1.5 g/kg of acidifier sodium butyrate. [b]Pooled standard error of the mean.

Item	Dietary treatment[a]				SEM[b]	*P*-value		
	CON	NUC	YSC	ASB		CON × NUC	CON × YSC	CON × ASB
Duodenum								
Villus height, μm	307	297	298	300	19.09	0.662	0.689	0.753
Crypt depth, μm	178	176	182	177	9.23	0.840	0.761	0.926
Villus:crypt ratio	1.72	1.70	1.63	1.71	0.08	0.812	0.387	0.857
Goblet cells, %	32.50	34.25	33.51	36.79	3.00	0.656	0.790	0.227
Jejunum								
Villus height, μm	264	255	261	268	24.09	0.771	0.922	0.880
Crypt depth, μm	150	139	143	157	11.12	0.474	0.640	0.590
Villus:crypt ratio	1.80	1.83	1.82	1.71	0.10	0.804	0.851	0.486
Goblet cells, %	23.15	24.62	25.16	22.79	2.76	0.683	0.562	0.912
Ileum								
Villus height, μm	210	241	234	255	12.99	0.071	0.150	<0.010
Crypt depth, μm	132	139	138	138	9.17	0.534	0.573	0.576
Villus:crypt ratio	1.64	1.66	1.69	1.81	0.07	0.896	0.603	0.066
Goblet cells, %	50.43	53.07	46.65	45.94	4.59	0.661	0.514	0.405
Peyer’s patches, n	48	40	39	38	3.33	0.078	0.042	0.017

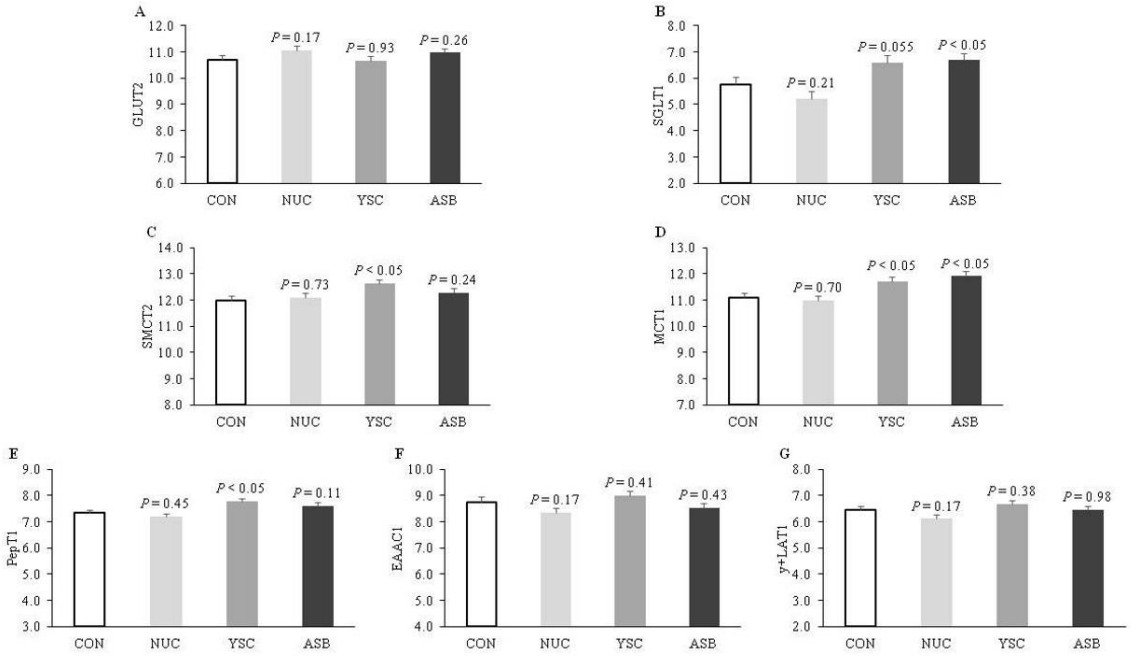

Figure 1. mRNA relative abundance of nutrient transporters in the jejunum of piglets fed diets supplemented or not with feed additives on d 32. Dietary treatment: CON, control, basal diet; NUC, CON + 1 g/kg of nucleotides; YSC, CON + 20 g/kg of lysed yeast *S. cerevisiae*; ASB: CON + 1.5 g/kg of acidifier sodium butyrate. Data are means of 9 pigs replicates per dietary treatment. *P*-value, preplanned contrasts of each of the treatments (NUC, YSC, and ASB) versus the control treatment (CON). **(A)** GLUT2, glucose transporter type 2; **(B)** SGLT1, Na^+/glucose co-transporter 1; **(C)** SMCT2, sodium-coupled monocarboxylate transporter; **(D)** MCT1, monocarboxylate transporter 1; **(E)** PepT1, peptide transporter 1; **(F)** EAAC1, excitatory amino acid carrier 1; **(G)** y^+LAT1, Na^+-independent cationic and Na^+-dependent neutral amino acid transporter 1.

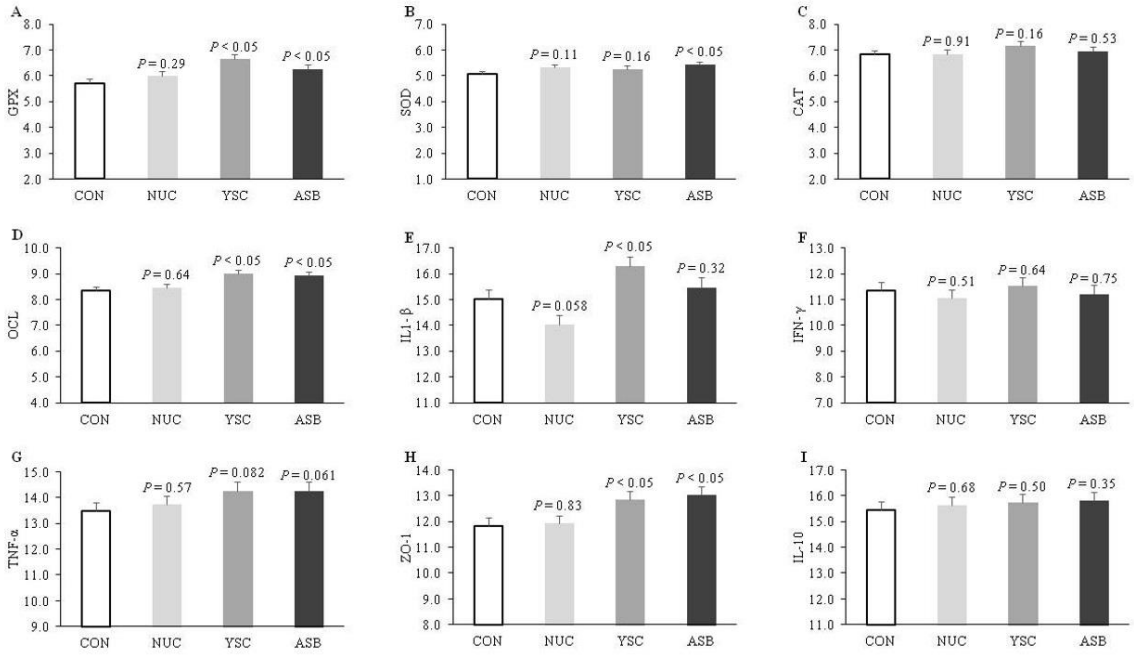

Figure 2. mRNA relative abundance of inflammatory and antioxidants markers, and tight junction proteins in the jejunum of pigls fed diets supplemented or not with feed additives on d 32. Dietary treatment: CON, control, basal diet; NUC, CON + 1 g/kg of nucleotides; YSC, CON + 20 g/kg of lysed yeast *S. cerevisiae*; ASB: CON + 1.5 g/kg of acidifier sodium butyrate. Data are means of 9 pigs replicates per dietary treatment. *P*-value, preplanned contrasts of each of the treatments (NUC, YSC, and ASB) versus the control treatment (CON). **(A)** GPX, glutathione peroxidase; **(B)** SOD, superoxide dismutase; **(C)** CAT, catalase; **(D)** OCL, occludin; **(E)** ZO-1, zonula occludens-1; **(F)** IFN-γ, interferon gamma; **(G)** TNF-α, tumor necrosis factor alpha; **(H)** IL1- β, interleukin 1 beta; **(I)** IL-10, interleukin 10.

Table 5. Ingredients and chemical composition of control diets fed to nursery piglets (g/kg, as-fed basis). [a]Feed additives were included as a replacement for the inert in the diet. Phase 1 (21 to 32 d) and phase 2(32 to 45 d), respectively: (1) CON: control, basal diet, (2) NUC: CON + 1 g/kg and 0.5 g/kg of nucleotides (Nucleobase 1.5, Aleris Animal Nutrition, Jundiaí, SP, Brazil), (3) YSC: CON + 20 g/kg and 10 g/kg of lysed yeast *S. cerevisiae* (Sinergis, Aleris Animal Nutrition, Brazil), (4) ASB: CON + 1.5 g/kg and 1 g/kg of acidifier sodium butyrate (Nuttritiva B90CNa, Additiva Nutrition, Monte Alegre do Sul, SP, Brazil). [b]Composition per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 2,250 IU; vitamin E, 65 IU; vitamin K, 3 mg; thiamine, 2.25 mg; riboflavin, 6 mg; pyridoxine, 2.25 mg; vitamin B_{12}, 27 mcg; folic acid, 400 mcg; biotin, 150 mcg; pantothenic acid, 22.5 mg; niacin, 45 mg; copper sulfate, 10 mg; iodine, 1.5 mg; iron sulfate, 100 mg; manganese sulfate, 40 mg; sodium selenite, 0,3 mg; zinc oxide, 100 mg. [c]Natuphos®, Basf enzyme. [d]Standardized ileal digestible.

Item[a]	Phase 1	Phase 2
	21 to 32 d	32 to 45 d
Ground corn, 7.8% CP	388.1	470.9
Soybean meal, 46.0% CP	219.7	242.2
Dried whey, 12.5% CP	150.0	100.0
Soybean micronized, 36.0% CP	100.0	70.0
Extrude corn, 7.6% CP	20.0	20.0
Plasma protein, 78.0% CP	20.0	10.0
Sugar	30.0	30.0
Inert (kaolin)	20.0	10.0
Dicalcium phosphate	9.35	10.6
Limestone calcitic	7.69	7.34
Soybean oil	15.93	11.1
Zinc oxide	2.50	2.20
Choline chloride	1.95	1.50
L-lys, 78.0%	4.69	4,45
DL-met, 99.0%	2.32	1.96
L-thr, 98.5%	2.30	2.14
L-trp, 99.0%	0.32	0.28
L-val, 96.5%	1.03	0.76

Salt	1.90	2.44
Copper sulfate	0.60	0.60
Vitamin-mineral premix[b]	1.40	1.40
Phytase[c]	0.05	0.05
BHT	0.10	0.10
Calculated composition		
ME, kcal/kg	3,400	3,375
Crude protein, g/kg	213.00	213.00
SID[d] lys, g/kg	14.50	13.46
SID met, g/kg	4.90	4.53
SID met + cys, g/kg	8.13	7.54
SID thr, g/kg	9.72	9.02
SID trp, g/kg	2.76	2.56
SID val, g/kg	10.01	9.29
SID ile, g/kg	8.01	7.70
Total Ca, g/kg	8.50	8.25
Available P, g/kg	5.19	5.00
Total Na, g/kg	2.80	2.30
Lactose, g/kg	112.50	75.00

Table 6. List of primers used in reverse transcription quantitative-PCR gene expression analysis in weaned piglets. [a]GPX, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; OCL, occludin; ZO-1, zonula occludens-1; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; IL1- β, interleukin 1 beta; IL-10, interleukin 10; GLUT2, glucose transporter type 2; SGLT1, Na+/glucose co-transporter 1; SMCT2, sodium-coupled monocarboxylate transporter; MCT1, monocarboxylate transporter 1; PepT1, peptide transporter 1; EAAC1, excitatory amino acid carrier 1; y+LAT1, Na+-independent cationic and Na+-dependent neutral amino acid transporter 1. 2F and R indicate Forward and Reverse primers, respectively.

Genes[a]	GenBank number	Sequence[2]
GPX	NM_214201.1	F: 5′GCCCAACTTCATGCTCTTC3′ R: 5′CAGGATCTCCCCATTCTTGGC3′
SOD	NM_001190422.1	F: 5′ATCAAGAGAGGCACGTTGGA3′ R: 5′TCTGCCCAAGTCATCTGGTT3′
CAT	NM_214301.2	F: 5′GCTTTAGTGCTCCCGAACAG3′ R: 5′AGATGACCCGCAATGTTCTC3′
OCL	NM_001163647.1	F: 5′TCCTGGGTGTGATGGTGTTC3′ R: 5′CGTAGAGTCCAGTCACCGCA3′
ZO-1	XM_003353439.2	F: 5´AAGCCCTAAGTTCAATCACAATCT3′ R: 5´ATCAAACTCAGGAGGCGGC3′
IFN- γ	NM_213948	F: 5′TGGTAGCTCTGGGAAACTGAATG3′ R: 5′GGCTTTGCGCTGGATCTG3′
TNF-α	NM_214022.1	F: 5′CATCGCCGTCTCCTACCA3′ R: 5′CCCAGATTCAGCAAAGTCCA3′
IL1- β	NM_214055.1	F: 5′TCTGCCCTGTACCCCAACTG3′ R: 5′CCCAGGAAGACGGGCTTT3′
IL-10	NM_214041.1	F: 5′GAAGGACCAGATGGGCGACTT3′ R: 5′CACCTCCTCCACGGCCCTTG3′
GLUT2	FJ209732.1	F: 5′TTGCCTTGGATGAGTTATGTGA3’ R: 5′GCGTGGTCCTTGACTGAAAA3’
SGLT1	MW_280290.1	F: 5′GGTTGGAGCTTCTCTGTTTG3’ R: 5′CCAGAACAACCACCCAAATC3’
SMCT2	XM_003122908.1	F: 5′AGGTCTACCGCTTTGGAGCAT3’ R:5′GAGCTCTGATGTGAAGATGATGACA3’
MCT1	AM_286425.1	F: 5′GGTGGAGGTCCTATCAGCAG3’ R: 5′AAGCAGCCGCCAATAATCAT3’
PepT1	AY_180903.1	F: 5′CATGGATGCTGTGGTGTATC3’ R: 5′CATGGAAGCCAGGAACATC3’
EAAC1	NM_001164649.1	F: 5′CAGTGGATGCCATGTTAGAC3’ R: 5′CGTCTCTGGCTCACTAGAA 3’
y^+LAT1	EU_047705.1	F: 5′ TGCGTGGAGGACATCTT3’

		R: 5′CTCCTTCCAGCGCAAATAG3’
β-actin	U07786.1	F: 5′CTCTTCCATCGTGTCCTTCTAC3′ R: 5′CCTCAGACTTGTCGATCTTCTG3′