FERNANDA FIALHO ABRANCHES

SUPPLEMENTATION OF BUTYRIC AND MEDIUM-CHAIN FATTY ACIDS MONOGLYCERIDES IN DIETS WITHOUT GROWTH PROMOTERS: EFFECTS ON PERFORMANCE AND INTESTINAL HEALTH STATUS IN NURSERY PIGS

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Animal Science, to obtain the title of Magister Scientiae.

Adviser: Gabriel Cipriano Rocha.

VIÇOSA - MINAS GERAIS
2024

Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa

T

A161s
2024

Abranches, Fernanda Fialho, 1999-

Supplementation of butyric and medium-chain fatty acids monoglycerides in diets without growth promoters: effects on performance and intestinal health status in nursery pigs / Fernanda Fialho Abranches. – Viçosa, MG, 2024.

1 dissertação eletrônica (38 f.): il.

Texto em inglês.

Inclui anexo.

Orientador: Gabriel Cipriano Rocha.

Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2024.

Referências bibliográficas: f. 21-27.

DOI: https://doi.org/10.47328/ufvbbt.2024.597

Modo de acesso: World Wide Web.

1. Suínos - Nutrição. 2. Intestinos - Doenças. 3. Suínos - Registros de desempenho. 4. Ácidos graxos. I. Rocha, Gabriel Cipriano, 1983-. II. Universidade Federal de Viçosa.
Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.4084

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

FERNANDA FIALHO ABRANCHES

SUPPLEMENTATION OF BUTYRIC AND MEDIUM-CHAIN FATTY ACIDS MONOGLYCERIDES IN DIETS WITHOUT GROWTH PROMOTERS: EFFECTS ON PERFORMANCE AND INTESTINAL HEALTH STATUS IN NURSERY PIGS

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Animal Science, to obtain the title of Magister Scientiae.

APROVED: 25 de julho de 2024.

Assent:

Documento assinado digitalmente
FERNANDA FIALHO ABRANCHES
Data: 16/09/2024 09:57:16-0300
Verifique em https://validar.iti.gov.br

Fernanda Fialho Abranches
Autor

Documento assinado digitalmente
GABRIEL CIPRIANO ROCHA
Data: 16/09/2024 21:01:43-0300
Verifique em https://validar.iti.gov.br

Gabriel Cipriano Rocha
Orientador

ACKNOWLEDGMENTS

To God, firstly, for life, health, and for having given me strength to fight and pursue my goals.

To my parents, João and Maria de Fátima, for their unconditional support from the beginning and for being my foundation, never sparing efforts for my happiness and the realization of my dreams.

To my brothers Genimarcio and Vanessa, to my siblings-in-law Amanda and João Lucas, and to my niece Isabela for their affection, support, and contribution to my personal and professional growth. I also extend my gratitude to Camila for her encouragement during this journey.

To my advisor, Prof. Dr. Gabriel Ciprinao Rocha, for all the teachings and opportunities provided to me, and for the guidance and execution of this study. I also thank Prof. Dr. Jansller L. Genova for his availability, attention, and contributions to this study.

To my friends and team members, Amanda, Lucas, Maykelly, and Laís, for all their help and teachings during this journey. To friends Jeferson, Pedro "Careli", Damares, and Hellen for their assistance in executing this project. With you, the journey becomes less arduous.

To my postgraduate friends Caroline “Carol”, Gustavo, Nathana and Bianca, for their friendship, help and teaching over the years.

To the employees of UEPE – Suinocultura, Arlindo, José Alberto (Dedeco), Leandro and Fernando, for their friendship, assistance in executing the experiment, and for making the work environment so welcoming.

To the Federal University of Viçosa and the Department of Animal Science for providing me with the opportunity to pursue my master's degree.

I greatly thank BASF SE for financing this study.

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), to granting the scholarship.

To you all, thank you very much

“If you don't know where you are going, any road will get you there”.

(Lewis Carroll)

ABSTRACT

ABRANCHES, Fernanda Fialho, M.Sc., Universidade Federal de Viçosa, July, 2024. **Supplementation of butyric and medium-chain fatty acids monoglycerides in diets without growth promoters: effects on performance and intestinal health status in nursery pigs**. Adviser: Gabriel Cipriano Rocha.

The use of short-chain and medium-chain fatty acids has demonstrated an important role in intestinal function and physiology during stressful periods such as post-weaning, promoting improvements in intestinal epithelial structure and differentiation, intestinal wall integrity, and immune response to support the performance of piglets fed diets without growth promoters. Therefore, the study aimed to evaluate the effects of supplementing butyric and medium-chain fatty acids in diets without growth promoters on zootechnical performance, diarrhea occurrence, blood profile, intestinal morphology and pH, mRNA expression of nutrient transporters, inflammatory markers, antioxidant enzymes, and junction proteins in weaned piglets. Forty piglets aged 21 days (6.70 ± 0.75 kg) were randomly allocated to one of five dietary treatments: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet; (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet; (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet; (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet. Results were described by comparing the mean of each tested diet vs. CON. Zootechnical performance variables were not affected ($P > 0.05$) by dietary treatments. The LA diet reduced ($P < 0.05$) the occurrence of diarrhea while the PA diet tended to reduce ($0.05 > P < 0.1$). The pH of intestinal contents was reduced ($P < 0.05$) in piglets receiving PA, HPA, and HLA diets. LA diet increased ($P < 0.05$) villus height in the duodenum, while others tended to increase ($0.05 > P < 0.1$). In the jejunum, supplementation of fatty acids in both forms and doses increased ($P < 0.05$) the proportion of goblet cells. In the ileum, PA diet reduced ($P < 0.05$) crypt depth and increased ($P < 0.05$) villus:crypt ratio, and PA, HPA, and HLA diets increased ($P < 0.05$) goblet cell proportion. Also in the ileum, HPA and LA diets showed a trend towards reduction ($0.05 > P < 0.1$) in crypt depth and Peyer's patch number. In the jejunum, LA and HLA diets increased ($P < 0.05$) expression of Occludin and HPA expression of Interleukin 10, respectively. The results suggest that supplementation with a blend of fatty acids in both forms improves intestinal health, modulates pH, intestinal morphology, and local immune response, as an alternative to conventional performance enhancers.

Keywords: Butyric Acid; Capric Acid; Caprylic Acid; Short-chain Fatty Acids; Medium-chain Fatty Acids; Organic Acids; Performance; Piglets; Intestinal Health; Monoglycerides.

RESUMO

ABRANCHES, Fernanda Fialho, M.Sc., Universidade Federal de Viçosa, julho, 2024. **Suplementação de monoglicerídeos de ácido butírico e de ácidos graxos de cadeia média em dietas isentas de promotores de crescimento: efeitos no desempenho e no estado de saúde intestinal em leitões de creche**. Orientador: Gabriel Cipriano Rocha.

O uso de ácidos graxos de cadeia curta e média tem demonstrado importante papel na função e fisiologia intestinal durante períodos de estresse como o pós-desmame, atuando na promoção de melhorias nas estruturas e diferenciação do epitélio intestinal, integridade da parede intestinal e resposta imune para apoiar o desempenho dos leitões alimentados com dietas livres de melhoradores de desempenho. Portanto, o estudo objetivou avaliar os efeitos da suplementação dos ácidos graxos butírico e de cadeia média em dietas sem melhoradores de desempenho sobre o desempenho zootécnico, a ocorrência de diarreia, o perfil sanguíneo, a morfologia e pH do conteúdo intestinal, a expressão de RNAm de transportador de nutrientes, os marcadores inflamatórios, as enzimas antioxidantes e as proteínas de junção em leitões desmamados. Quarenta leitões de 21 dias de idade (6,70 ± 0,75 kg) foram distribuídos aleatoriamente para um dos cinco tratamentos dietéticos: (1) CON: controle, dieta basal; (2) powder acid (PA): CON + 0,75 g de um blend de ácidos graxos em pó/kg dieta, (3) high powder acid (HPA): CON + 3,00 g de um blend de ácidos graxos em pó/kg dieta, (4) liquid acid (LA): CON + 0,50 g de um blend de ácidos graxos na forma liquida/kg dieta, (5) high liquid acid (HLA): CON + 2,00 g de um blend de ácidos graxos na forma liquida/kg dieta. Os resultados foram descritos comparando a média de cada uma das dietas testadas vs CON. As variáveis de desempenho não foram afetadas ($P > 0,05$) pelos tratamentos dietéticos. A dieta LA reduziu (P < 0,05) a ocorrência de diarreia enquanto a dieta PA tendeu a reduzir (0,05 > P < 0,1). O pH do conteúdo intestinal apresentou redução ($P < 0,05$) em leitões que receberam as dietas PA, HPA e HLA. A dieta LA aumentou ($P < 0,05$) a altura das vilosidades no duodeno enquanto as demais tenderam a aumentar ($0,05 > P < 0,1$). No jejuno, a suplementação de ácidos graxos em ambas as formas e doses aumentaram ($P <0,05$) a proporção de células caliciformes. No íleo, a dieta PA reduziu ($P < 0,05$) a profundidade de cripta e aumentou ($P < 0,05$) a relação vilo:cripta, e as dietas PA, HPA e HLA aumentaram ($P < 0,05$) a proporção de células caliciformes. Ainda no íleo, as dietas HPA e LA apresentaram tendência à redução ($0,05 > P < 0,1$) da profundidade das criptas e do número de placas de Peyer. No jejuno, as dietas LA e HLA aumentaram ($P < 0,05$) a expressão de Ocludina e HPA a expressão da Interleucina 10, respectivamente. Os resultados sugerem que a suplementação de um blend composto por ácidos graxos em ambas as formas de fornecimento melhora a saúde intestinal, modula o pH, a morfologia intestinal e a resposta imunitária local, como uma alternativa aos melhoradores de desempenho convencionais.

Palavras-chave: Ácido Butírico; Ácido Cáprico; Ácido Caprílico; Ácidos Graxos De Cadeia Curta; Ácidos Graxos De Cadeia Média; Ácidos Orgânicos; Desempenho; Leitões; Saúde Intestinal; Monoglicerídeos.

LIST OF TABLES

SUMÁRIO

1. INTRODUCTION

Weaning is considered a critical period in the piglet's life because it is related to several stressors, such as loss of contact with the mother and litter of origin, environmental and dietary changes, and the establishment of a new social hierarchy (Genova et al., 2020; Correia et al., 2024). These changes, together with the immaturity of the gastrointestinal tract and the immune system, result in physiological disorders, impairment of intestinal epithelial structures, and the expression of pro- and anti-inflammatory cytokines in the post-weaning period (Pluske et al., 2018; Correia et al., 2023).

In recent decades, due to the search for improving the zootechnical performance of weaned piglets, a range of molecules (e.g. antibiotics, copper sulfate, and zinc oxide) have been used as performance enhancers, resulting in the selection of resistant bacterial strains and the accumulation of copper (Cu) and zinc (Zn) in the environment (Correa et al., 2021; Ding et al., 2021; Valini et al., 2021). Due to the risks associated with the inappropriate use of antibiotics, Cu and Zn, some restrictions and prohibitions have been implemented regarding the use of these molecules as performance-enhancing additives to reduce the incidence of resistant pathogens (Papatsiros et al., 2012; Vahjen et al., 2015) and mitigate environmental risks arising from Cu and Zn toxicity (Jensen et al., 2016).

In this context, the European Union banned the use of antibiotics in 2006 and Zn oxide as a performance enhancer in animal diets in 2022 (Gaggìa et al., 2010; Satessa, 2020), and there is pressure to reduce Cu levels in diets (Wu et al., 2020). These bans stimulated the search for alternative additives (e.g., short and medium-chain fatty acids) that mitigate the negative effects of the post-weaning period and meet new market demands. In addition to these environmental characteristics, it is important to consider the form and dose of supplementation, environmental challenges, and how this influences discrepant results found in the literature when researching new additive alternatives (Huang et al., 2015; Ruggeri et al., 2018).

Organic acids can be classified into two main functional categories which are short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) (Tugnoli et al., 2020b). SCFA (e.g. butyric acid monoglyceride) and MCFA (e.g. caprylic and capric acid monoglyceride) are supplemented in piglet diets due to their antibacterial potential against Salmonella spp. and E. coli (Ferronato and Prandini, 2020). These effects favor the reabsorption of fluids and electrolytes, associated with a reduction in the occurrence of diarrhea (Fang et al., 2013).

In addition to this effect, SCFA, specifically butyric acid monoglyceride, increases villus height and reduces crypt depth (Huang et al., 2015), regulates the profile of pro-inflammatory cytokines (e.g. tumor necrosis factor alpha and interleukin 1-beta) (Vinolo et al., 2011), and increases the expression of tight junction proteins (e.g. occludin and zonula occludens) in the small intestine (Jiang et al., 2015). These effects favor the reabsorption of fluids and electrolytes, associated with a reduction in the occurrence of diarrhea (Fang et al., 2013).

The MCFA are a readily available energy source for young animals producing energy via mitochondrial β-oxidation in the liver (Odle, 1997). The MCFAs (e.g. capric and caprylic acid) are transported to the liver via the portal system, producing ketones that are subsequently directed to peripheral tissues, serving as an energy substrate (Zentek et al., 2011; Ferronato and Prandini, 2020). Another function associated with capric and caprylic acid monoglycerides is the growth of the *Lactobacillus* bacteria population, which through fermentation helps to reduce the pH of the intestinal content and, consequently, increase the absorption of nutrients by the intestine (Kuang et al., 2015; Nguyen et al., 2018b).

Unlike previous studies that used diets containing antibiotics (Dibner and Richards, 2005; Ma et al., 2021), Cu sulfate or Zn oxide (Kuang et al., 2015; Correia et al., 2024) as growth promoters, the present study evaluated fatty acid sources as the only performance-enhancing additive in diets, which diverges from the conventional approach. However, the current study is supported by several studies on the effects of butyric acid monoglyceride (Marchiori et al., 2024), capric acid and caprylic acid (Yang et al., 2019) on intestinal health and animal performance, which allows these acids to be an alternative to the conventional additives.

Combined supplementation of SCFA and MCFA monoglyceride, in powder or liquid form, in diets for weaned piglets, induces the search for feeding strategies to promote healthier and more resilient growth, reducing dependence on performance enhancers traditionally used in diets, being in line with current concerns about antimicrobial resistance and environmental sustainability in swine production (Upadhaya et al., 2020).

In view of the above, the hypothesis of the study was that dietary supplementation composed of short and medium-chain fatty acid monoglyceride provided in powder or liquid form would improve the zootechnical 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 combined supplementation of short- and medium-chain fatty acid monoglyceride in diets without performance enhancers for nursery pigs on performance, diarrhea occurrence, blood profile, morphology, and pH of intestinal content, expression of nutrient transporter mRNA, inflammatory markers, antioxidant enzymes, and tight junction proteins in piglets during the first two weeks post-weaning.

2. MATERIAL AND METHODS

The experimental protocol follows the ethical principles in animal research of National Animal Experimentation Control Council and was approved by the Ethics Committee on the Use of Production Animals of the Federal University of Viçosa, under protocol nº 069/2023.

2.1 Animals, experimental design, housing, and diets

The experiment was conducted at the research facility of Universidade Federal de Viçosa, Viçosa, MG, Brazil. A total of 40 Camborough piglets (Agroceres PIC), castrated males and females, weaned at 21 d-old and weighing 6.70 ± 0.75 kg were allotted randomly into five dietary treatments, eight replications, and one animal per experimental unit, represented by the pen (1 piglet/pen).

The experiment was conducted only in one series, without an adaptation period. The piglets were housed in suspended pens (1.60 m × 1.00 m, 1.60 m²/piglet), with plastic flooring, semi-automatic feeders and nipple drinkers, with free access to feed and water. The nursery room ventilation was provided with the aid of tilt-and-turn glass windows. The heating of the experimental pens was controlled using one heat lamp in each pen and side heaters. The minimum and maximum temperatures in the nursery room were 26.6 ± 2.06ºC and 31.1 ± 1.73ºC, respectively. The minimum and maximum relative humidity in the nursery room were 77.1 ± 9.92 and 55.1 ± 9.73, respectively.

Piglets were fed a single-phase feeding regimen (21 to 35 days of age). 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 (Rostagno et al., 2017) (Table 1), and provided in mash form. During the experimental phase, dietary treatments consisted of: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet. Organic acids (powder or liquid) were added in place of the inert in the CON diet.

For PA and LA treatment, the doses of butyric acid monoglycerides and capric plus caprylic acid monoglycerides were between 0.13 to 0.15 g/kg and 0.04 to 0.06 g/kg, respectively. For HPA and HLA treatment, the doses of butyric acid monoglycerides and capric plus caprylic acid monoglycerides were between 0.50 to 0.61 g and 0.15 to 0.24 g, respectively.

2.2 Feed additive composition

The source of organic acid powder tested (Balangut™ LS P, BASF, Cidade Monções, SP, Brazil) contained 17% to 21% butyric acid monoglycerides (C4) and 5% to 8% caprylic (C8) plus capric (C10) acid monoglycerides, with the remainder being a vehicle.

The source of liquid organic acid tested (Balangut™ LS L, BASF, Cidade Monções, SP, Brazil) contained 25% to 30% butyric acid monoglycerides (C4) and 2.5% to 5.5% caprylic (C8) plus capric (C10) acid monoglycerides, with the remainder being a vehicle.

2.3 Growth performance, and diarrhea occurrence

Throughout the trial, the offered diet and leftovers were weighed to calculate average daily feed intake (ADFI). Piglets were weighed individually on days 21 and 35 to determine body weight, average daily weight gain (ADG) and feed conversion ratio (FC). The fecal consistency of each pig was visually assessed from d 21 to 35, using the method described by Liu et al. (2010). Fresh feces were scored on a 4-point scale as follows: 0 = solid, 1 = semi-solid, 2 = semi-liquid, and 3 = liquid. The diarrhea occurrence was defined as piglets without diarrhea having a fecal score of 0 and 1, and piglets with diarrhea having a fecal score of 2 and 3.

Observations were made in the morning (9h00), every day throughout the experimental period by a trained evaluator.

2.4 Sample collection

At 34 days of age, blood was collected from each experimental unit. The animals were not fasting. Blood was collected (08h00) by orbital sinus puncture with a hypodermic needle (40 mm × 1.6 mm) into 10 mL tubes without anticoagulants. Samples were immediately sent at room temperature to the Viçosa Clinical Laboratory (Viçosa, MG, Brazil), where they were centrifuged for 12 min at 7,000 rpm for subsequent determination of urea (Ureal Cobas C311, Linklab, PNCQ software), creatinine (WS Kovalent, kinetic method, ASB-380, Mindray) and immunoglobulin G (IgG) concentrations (Atellica® CH IgG_2 assay, CH Analyzer, Siemens Healthineers).

After weighing, the piglets were electrically stunned (240 volts for 3 s) followed by exsanguination to collect samples on d 35. The viscera were exposed by a central incision. The intestinal contents were collected by sectioning the intermediate portions of each segment of the proximal intestine (duodenum and jejunum) and immediately used to assess pH. Fragments measuring (2 cm length) were sampled (08 piglets/treatment) from the duodenum (10 cm from the pylorus junction), jejunum (mid-section), and ileum (5 cm from the ileocecal junction) for histological evaluation (Yang et al., 2014).

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) (Valini et al., 2021) for 24 h at room temperature. Another 2 cm of jejunum was collected and immediately frozen in liquid nitrogen, stored at −80°C for RNA extraction and gene expression analysis.

2.5 pH of intestinal contents

The pH was obtained by coupling the pH electrode inside a pot (50 mL) containing digestive contents from the proximal intestine segment. The pH meter used (Tec-3MP, Tecnal) was calibrated in a calibration solution of known pH (4.0 and 7.0), following the manufacturer's recommendations. The electrode was washed with distilled water and the pH meter recalibrated between measurements.

2.6 Intestinal morphology, Peyer's patches, and goblet cells

All the sampling and preparation procedures were done according to Correia et al. (2024). 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. 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 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 (Correia et al., 2023).

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. 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 (1995):

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

2.7 Relative mRNA abundance

All the sampling and preparation procedures were done according to Correia et al. (2024). 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 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 the expression of the target genes for the jejunum samples. The relative expression of the gene of interest was calculated by ΔCt (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), interleukin 10 (IL-10), sodium-coupled monocarboxylate transporter (SMCT2), and monocarboxylate transporter 1 (MCT1).

2.8 Statistical procedures

The pen was considered the experimental unit for growth performance, diarrhea occurrence, intestinal morphology, gene expression, and blood profile. The statistical model included the fixed effect of dietary treatment 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 were determined by the preplanned contrasts. Averages of each of the treatments (PA, HPA, LA, and HLA) 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 (Correia et al., 2024).

The diarrhea occurrence values were transformed into piglets without diarrhea (score 0) and with diarrhea (score 1). These data were then fitted to a generalized linear model with binomial distribution and logit link function, using PROC GENMOD. The results of diarrhea occurrence were presented as observed proportions (relative frequency in %). The dietary treatment effect was verified using type III analysis. Significant differences and tendency were defined as $P < 0.05$ and $0.05 \leq P < 0.10$, respectively, and diarrhea occurrence estimates were compared using orthogonal contrasts.

3. RESULTS

There was no effect of dietary treatments on the growth performance (Table 3), as well as on IgG, creatinine, and urea concentrations (Table 4). The dietary treatments LA reduced ($P < 0.05$) diarrhea occurrence, whereas the PA diet tended ($P = 0.099$) to reduce (Table 3). The dietary treatments PA, HPA, and HLA reduced ($P < 0.05$) pH of the intestinal contents, whereas the LA diet tended ($P = 0.054$) to reduce (Table 5).

In the duodenum, villus height was increased ($P < 0.05$) in piglets fed LA diet and tended to increase in piglets fed PA ($P = 0.066$), HPA ($P = 0.092$), and HLA ($P = 0.057$) diets (Table 6). However, there were no effects on crypt depth, villus:crypt ratio, and proportion of goblet cells in the duodenum. In the jejunum, the dietary treatments had no effects on villus height, crypt depth, and villus:crypt ratio. The proportion of goblet cells increased ($P < 0.05$) in piglets fed PA, HPA, LA, and HLA diets. In the ileum, dietary treatments had no effect on villus height. There was a reduction ($P < 0.05$) in crypt depth and an increase ($P < 0.05$) in the villus:crypt ratio in piglets fed PA diet. In addition, the proportion of goblet cells increased ($P < 0.05$) in piglets fed PA, HPA, and HLA diets, and a trend was observed ($P = 0.061$) in piglets fed LA diet. There was a trend towards reduced ($0.05 \leq P < 0.10$) crypt depth and the number of Peyer's patches in piglets fed HPA and LA diets.

In the jejunum, OCL mRNA expression was higher ($P < 0.05$) in piglets fed LA and HLA diets, and the HPA diet trended ($P = 0.053$) to increase (Table 7). The mRNA expression IL-10 in piglets fed HPA diet was higher ($P < 0.05$). There was no effect of dietary treatments on the mRNA expression of GPX, SOD, CAT, ZO-1, IFN-γ, TNF-α, IL1-β, SMCT2, and MCT1.

4. DISCUSSION

The post-weaning transition period is challenging for piglets due to the immaturity of the gastrointestinal tract. Consequently, there is an increase in intestinal disorders, causing a reduced ability to digest and absorb nutrients, in addition to a higher occurrence of diarrhea and reduced cell development and differentiation (Huang et al., 2015; Pluske et al., 2018; Upadhaya et al., 2020). Our study demonstrated that supplementation with a monoglyceride blend of short- and medium-chain fatty acids improves intestinal health, villus height, crypt depth, and tight junctions, thus aiding piglet development. However, there was no effect on performance variables.

Previous studies (Yang et al., 2019; Zhang et al., 2020; Correia et al., 2024) tested different levels and sources of SCFA and MCFA, together or separately, and found divergent results between studies regarding piglet performance variables. These discrepant results can be attributed to environmental issues, form of supplementation, and source of the acids tested (Biagi et al., 2007; Weber and Kerr, 2008; Gomes et al., 2023). In addition, due to the sanitation of the experimental facilities and the weaning weight of the piglets, the CON diet provided in this study may have been effective in sustaining zootechnical performance throughout the experimental period, as reported by Valini et al. (2021).

Regarding the occurrence of diarrhea, our study demonstrated that animals fed the LA and PA diets presented a reduction and a tendency to reduce the occurrence of diarrhea, respectively. According to Zentek et al. (2013), the combination of SCFA and MCFA can modify the intestinal microbiota, helping to reduce post-weaning diarrhea. The reduction in the occurrence of diarrhea due to the addition of butyric acid monoglycerides to the diet can be attributed to the improvement in intestinal integrity (Fang, et al., 2014).

Capric and caprylic acids, and to a lesser extent butyric acid, have antimicrobial action in reducing diarrhea. Together, they can reduce the cytoplasmic pH of microorganisms by diffusing into the bacterial cell in an undissociated form. These acids will dissociate within the bacterial cell, resulting in reduced intracellular pH, suppression of cytoplasmic enzymes and the nutrient transport system, leading to cell death (Bassan et al, 2008; Hanczakowska et al., 2016).

The bacterial population present in the intestinal microbiota is influenced by the pH of the digesta because the more acidic the pH, the lower the growth capacity of pathogenic bacteria in the intestine (Upadhaya et al., 2014; Marchiori et al., 2024). Consequently, reducing pH throughout the gastrointestinal tract contributes to increasing antimicrobial potential and aids in the proliferation of beneficial bacteria (Marchiori et al., 2024). Our study showed that the supply of fatty acid monoglyceride in different forms (PA, HPA, and HLA) reduced the pH of the intestinal content, as well as the LA diet tended to reduce it. This result can be explained by the ability of MCFA (especially capric and caprylic acid monoglycerides) to promote the selection of lactic bacteria in the proximal portion of the intestine, which contributed to the reduction of pH (Zentek et al., 2013).

In addition to capric and caprylic acid monoglycerides, butyric acid monoglycerides, due to their low pKa value (4.82), help reduce the pH of the intestinal content, beneficially influencing the intestinal microbiota and, consequently, the fermentation and production of microbial metabolites throughout the gastrointestinal tract, corroborating the results found (Ficagna et al., 2022; Marchiori et al., 2024). This result suggests an improvement in intestinal health because there is a reduction in the passage rate and an increase in digestive enzymes (e.g. pepsin and trypsin) due to changes in pH, and this promotes better absorption of ingested nutrients (e.g. proteins, minerals, and amino acids) (Ma et al., 2021; Muniyappan et al., 2021).

Intestinal morphology is one of the main indices used to assess the digestive process and intestinal health (Jha et al., 2019; Ma et al., 2021). The height of the villi and the depth of the crypts represent the capacity for nutrient absorption and the rate of cell formation and renewal, respectively (Pluske et al., 1996). The higher the villi, the more epithelial cells and lymphocytes are present, improving the immune response (Diao et al., 2015; Ferrara et al., 2017). The greater depth of the crypts refers to a higher speed of cell renewal of the villi due to the increase in the rate of epithelial desquamation or inflammatory damage to the intestinal mucosa, resulting in an increase in immature cells with lower secretory, digestive and absorptive capacity (Nari et al., 2020; Correia et al., 2024).

The present results indicated that the LA diet promoted an increase in villus height in the duodenum, while the other treatments tended to increase villus height. In the ileum, the PA diet reduced crypt depth and improved the villus:crypt ratio, while the HPA and LA treatments tended to reduce crypt depth, corroborating the results found in previous studies that evaluated SCFA and MCFA supplementation (Li et al., 2018; Nguyen et al., 2018a; Lee et al., 2021). Diao et al. (2015) mentioned that lower pH values of intestinal content positively affect cell growth and division, as well as influence bacterial proliferation in the intestine. This is in agreement with what was observed in the present study regarding the characteristics of intestinal morphology and occurrence of diarrhea.

The goblet cells present in the gastrointestinal tract have the function of producing mucus layer, which acts as the first line of defense of the mucosa, playing a fundamental role in intestinal homeostasis and epithelial integrity (Jung and Saif, 2017; Wu et al., 2018; Tugnoli et al., 2020a). The results of the present study demonstrated an increase in the number of goblet cells in the jejunum and ileum associated with the improvement observed in intestinal morphology. Liu et al. (2014) consider the increase in the number of goblet cells as an indicator of improved intestinal maturation and, consequently, benefits in morphological characteristics as observed in the present study.

Higher jejunal OCL mRNA expression was observed in piglets fed LA and HLA. Higher OCL expression denotes an important role in the regulation of epithelial permeability because it is a claudin protein belonging to tight junctions (Rose et al., 2021; Correia et al., 2024), which constitute one of the main components of the intestinal physical barrier and is crucial for epithelial integrity (Buckley and Turner, 2018; Slifer and Blikslager, 2020). The correct function of the intestinal barrier is directly associated with adequate intestinal health, with a fundamental role in intestinal development, in addition to improving the digestive and absorptive capacity of nutrients (Chen et al., 2013; Diao et al., 2019). In the present study, the improved OCL expression results are consistent with the results of pH, intestinal morphology, and goblet cells.

Peyer's patches are the main lymphoid structures found in the lining of the small intestine, mainly in the ileum, playing a crucial role in the induction and regulation of intestinal mucosal immune responses (Makala et al., 2000; Correia et al., 2023). In the present study, a reduction in the Peyer's patch count was observed in the ileum of piglets fed the HPA or LA diet. This suggested that there was less induction of the mucosal immune system in piglets from these dietary treatments, resulting in less stimulation of the immune system because Peyer's patches can be considered as immune sensors of the intestine (Gribble and Reimann, 2016; Kobayashi et al., 2019).

Furthermore, an increase in IL-10 mRNA expression as an anti-inflammatory marker was observed in piglets fed the HPA diet. According to Groot et al. (2021), the increase in the cytokine IL-10 helps protect against intestinal inflammation, in addition to maintaining the barrier function, and is important in regulating intestinal homeostasis during the animal's defense (Chen et al., 2018). IL-10 produced by effector T cells can be considered a self-limiting mechanism, which regulates immune responses by preventing an excessive reaction of T cells in the intestine and, consequently, helping to maintain the balance between protection against pathogens and prevention of damage to animal tissues (Sun et al., 2018; Ma et al., 2022).

The anti-inflammatory properties of butyric acid monoglyceride suggest a lower need for energy mobilization to activate the immune system, which can be allocated to the development of other systems such as the digestive system (Ficagna et al., 2022). Like butyric acid monoglyceride, capric and caprylic acid monoglycerides have immunomodulatory activities, negatively regulating the expression of pro-inflammatory cytokines (e.g. interferon gamma) and helping to control an increased response (Ferrara et al., 2017; Jackman et al., 2020).

5. CONCLUSION

Based on the criteria evaluated, the supplementation of a blend composed of SCFA and MCFA, in both forms and doses, was efficient in promoting better intestinal and immunological health of weaned pigs and, based on practical and financial criteria, the lowest dose of the blend, in either form, would be the most recommended.

6. REFERENCES

Bassan, J. D. L., Flôres, M. L., Antoniazzi, T., Bianchi, E., Kuttel, J., Trindade, M. M., 2008. Control of the infection caused by Salmonella Enteritidis with organic acids and mannanoligosaccharide in broiler. Ciência Rural, 1961-1965(38). Doi: https://doi.org/10.1590/S0103-84782008000700025.

Biagi, G., Piva, A., Moschini, M., Vezzali, E., Roth, F. X., 2007. Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. Journal of Animal Science, 1184-1191(85). Doi: https://doi.org/10.2527/jas.2006-378.

Buckley, A., Turner, J. R., 2018. Cell biology of tight junction barrier regulation and mucosal disease. Cold Spring Harbor perspectives in biology, a029314 (10). Doi: https://doi.org/10.1101/cshperspect.a029314

Correa, F., Luise, D., Castillo, M., Peris, S., Palomo-Yague, A., Bosi, P., Trevisi, P., 2021. Effect of dietary supplementation with a blend of protected aromatic compounds, including benzoic acid, on growth performance and faecal microbial profile of weaned piglets as an alternative to Zinc Oxide. Livestock Science, 104455 (246). Doi: https://doi.org/10.1016/j.livsci.2021.104455.

Correia, A. M., Genova, J. L., Saraiva, A., Rocha, G. C., 2023. Effects of crude protein and non-essential amino acids on growth performance, blood profile, and intestinal health of weaned piglets. Front. Vet. Sci. 10:1243357. Doi: https://doi.org/10.3389/fvets.2023.1243357.

Correia, A. M., Genova, J. L., Kim, S. W., Abranches, F. F., Rocha, G. C. (2024). Autolyzed yeast and sodium butyrate supplemented alone to diets promoted improvements in performance, intestinal health and nutrient transporter in weaned piglets. Scientific Reports, 11885 (14). Doi: https://doi.org/10.1038/s41598-024-62551-9.

Chen, H., Mao, X., He, J., Yu, B., Huang, Z., Yu, J., Chen, D., 2013. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. British Journal of Nutrition, 1837-1848(110). Doi: https://doi.org/10.1017/S0007114513001293.

Chen, L., Li, S., Zheng, J., Li, W., Jiang, X., Zhao, X., Wu, D., 2018. Effects of dietary Clostridium butyricum supplementation on growth performance, intestinal development, and immune response of weaned piglets challenged with lipopolysaccharide. Journal of Animal Science and Biotechnology, 1-14(9). Doi: https://doi.org/10.1186/s40104-018-0275-8.

Groot, N. de, Fariñas, F., Cabrera-Gómez, C. G., Pallares, F. J., Ramis, G., 2021. Weaning causes a prolonged but transient change in immune gene expression in the intestine of piglets. Journal of Animal Science, skab065(99). Doi: https://doi.org/10.1093/jas/skab065.

Diao, H., Zheng, P., Yu, B., He, J., Mao, X., Yu, J., Chen, D., 2015. Effects of benzoic acid and thymol on growth performance and gut characteristics of weaned piglets. Asian-Australasian Journal of Animal Sciences, 827(28). Doi: http://dx.doi.org/10.5713/ajas.14.0704.

Diao, H., Jiao, A. R., Yu, B., Mao, X. B., Chen, D. W., 2019. Gastric infusion of short-chain fatty acids can improve intestinal barrier function in weaned piglets. Genes & nutrition, 1-16(14). Doi: https://doi.org/10.1186/s12263-019-0626-x.

Dibner, J. J., and Richards, J. D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry science, 634-643 (84). Doi: https://doi.org/10.1093/ps/84.4.634.

Ding, H., Zhang, Q., Xu, H., Yu, X., Chen, L., Wang, Z., Feng, J., 2021. Selection of copper and zinc dosages in pig diets based on the mutual benefit of animal growth and environmental protection. Ecotoxicology and Environmental Safety, 112177 (216). Doi: https://doi.org/10.1016/j.ecoenv.2021.112177.

Fang, C.L., Sun, H., Wu, J., Niu, H. H., Feng, J., 2014. Effects of sodium butyrate on growth performance, haematological and immunological characteristics of weanling piglets. Journal of animal physiology and animal nutrition, 680-685(98). Doi: https://doi.org/10.1111/jpn.12122.

Ferrara, F., Tedin, L., Pieper, R., Meyer, W., Zentek, J., 2017. Influence of medium-chain fatty acids and short-chain organic acids on jejunal morphology and intra-epithelial immune cells in weaned piglets. Journal of animal physiology and animal nutrition, 531-540 (101). Doi: https://doi.org/10.1111/jpn.12490.

Ferronato, G. and Prandini, A., 2020. Dietary supplementation of inorganic, organic, and fatty acids in pig: A review. Animals, 1740(10). Doi: https://doi.org/10.3390/ani10101740.

Ficagna, C. A., Galli, G. M., Zatti, E., Sponchiado, B. M., Cecere, B. G. D. O., Deolindo, G. L., da Silva, A. S., 2022. Butyric acid glycerides in the diet of broilers to replace conventional growth promoters: effects on performance, metabolism, and intestinal health. Archives of Animal Nutrition, 191-204(76). Doi: https://doi.org/10.1080/1745039X.2022.2162796.

Gaggìa, F., Mattarelli, P., Biavati, B., 2010. Probiotics and prebiotics in animal feeding for safe food production. International journal of food microbiology, S15-S28 (141). Doi: https://doi.org/10.1016/j.ijfoodmicro.2010.02.031.

Genova, J. L., Melo, A. D. B., Rupolo, P. E., Carvalho, S. T., Costa, L. B., Carvalho, P. L. D. O., 2020. A summary of feed additives, intestinal health and intestinal alkaline phosphatase in piglet nutrition. Czech Journal of Animal Science, 281-294 (65). Doi: https://doi.org/10.17221/70/2020-CJAS.

Genova, J. L., Melo, A. D. B., Rupolo, P. E., Macedo, R. E. F. D., Engracia Filho, J. R., Carvalho, S. T., Faucitano, L., Costa, L. B., Carvalho, P. L. D. O., 2022. New findings of intestinal alkaline phosphatase: effects on intestinal and organ health of piglets challenged with ETEC F4 (K88). Revista Brasileira de Zootecnia, 51, p.e20210144. Doi: https://doi.org/10.37496/rbz5120210144.

Gomes, M. D. S., Duarte, M. E., Saraiva, A., de Oliveira, L. L., Teixeira, L. M., Rocha, G. C., 2023. Effect of antibiotics and low-crude protein diets on growth performance, health, immune response, and fecal microbiota of growing pigs. Journal of Animal Science, 101, skad357. Doi: https://doi.org/10.1093/jas/skad357.

Gribble, F. M., Reimann, F., 2016. Enteroendocrine cells: chemosensors in the intestinal epithelium. Annual review of physiology, 277-299(78). Doi: https://doi.org/10.1146/annurev-fisiol-021115-105439.

Grilli, E., Messina, M. R., Tedeschi, M., Piva, A., 2010. Feeding a microencapsulated blend of organic acids and nature identical compounds to weaning pigs improved growth performance and intestinal metabolism. Livestock science, 173-175 (133). Doi: https://doi.org/10.1016/j.livsci.2010.06.056.

Hanczakowska, E., Świątkiewicz, M., Natonek-Wiśniewska, M., Okoń, K., 2016. Medium chain fatty acids (MCFA) and/or probiotic Enterococcus faecium as a feed supplement for piglets. Livestock Science, 192, 1-7. Doi: https://doi.org/10.1016/j.livsci.2016.08.002.

Huang, C., Song, P., Fan, P., Hou, C., Thacker, P., Ma, X., 2015. Dietary Sodium Butyrate Decreases Postweaning Diarrhea by Modulating Intestinal Permeability and Changing the Bacterial Communities in Weaned Piglets. The Journal of Nutrition, 2774-2780 (145). Doi: https://doi.org/10.3945/jn.115.217406.

Jackman, J. A., Boyd, R. D., Elrod, C. C., 2020. Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation. Journal of animal science and biotechnology, 44 (11). Doi: https://doi.org/10.1186/s40104-020-00446-1.

Jha, R., Fouhse, J. M., Tiwari, U. P., Li, L., Willing, B. P., 2019. Dietary fiber and intestinal health of monogastric animals. Frontiers in veterinary science, 48(6). Doi: https://doi.org/10.3389/fvets.2019.00048.

Jensen, J., Larsen, M. M., Bak, J., 2016. National monitoring study in Denmark finds increased and critical levels of copper and zinc in arable soils fertilized with pig slurry. Environmental Pollution, 334-340 (214). Doi: https://doi.org/10.1016/j.envpol.2016.03.034.

Jiang, Y., Zhang, W., Gao, F., Zhou, G., 2015. Effect of sodium butyrate on intestinal inflammatory response to lipopolysaccharide in broiler chickens. Can. J. Anim. Sci. 389–395 (95). Doi: https://doi.org/10.4141/cjas-2014-183.

Jung, K., Saif, L. J., 2017. Goblet cell depletion in small intestinal villous and crypt epithelium of conventional nursing and weaned pigs infected with porcine epidemic diarrhea virus. Research in veterinary science, 12-15(110). Doi: https://doi.org/10.1016/j.rvsc.2016.10.009

Kuang, Y., Wang, Y., Zhang, Y., Song, Y., Zhang, X., Lin, Y., Fang, Z., 2015. Effects of dietary combinations of organic acids and medium chain fatty acids as a replacement of zinc oxide on growth, digestibility and immunity of weaned pigs. Animal Feed Science and Technology, 145-157(208). Doi: https://doi.org/10.1016/j.anifeedsci.2015.07.010.

Lee, J., Kim, J. W., Hall, H., Nyachoti, C. M., 2021. Effect of dietary organic acids supplementation on growth performance, nutrient digestibility, and gut morphology in weaned pigs. Canadian Journal of Animal Science 255-265(102). Doi: https://doi.org/10.1139/cjas-2021-0080.

Li, S., Zheng, J., Deng, K., Chen, L., Zhao, X. L., Jiang, X., Wu, D., 2018. Supplementation with organic acids showing different effects on growth performance, gut morphology, and microbiota of weaned pigs fed with highly or less digestible diets. Journal of Animal Science, 3302-3318(96). Doi: https://doi.org/10.1093/jas/sky197.

Liu, P. P. X. S., Piao, X. S., Thacker, P. A., Zeng, Z. K., Li, P. F., Wang, D., Kim, S. W., 2010. Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with Escherichia coli K88. Journal of Animal Science, 3871-3879 (88). Doi: https://doi.org/10.2527/jas.2009-2771.

Liu, P., Pieper, R., Rieger, J., Vahjen, W., Davin, R., Plendl, J., Zentek, J., 2014. Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets. PloS one, e91091 (9). Doi: https://doi.org/10.1371/journal.pone.0091091.

Liu, Y., Espinosa, C. D., Abelilla, J. J., Casas, G. A., Lagos, L. V., Lee, S. A., Stein, H. H., 2018. Non-antibiotic feed additives in diets for pigs: A review. Animal nutrition, 113-125(4). Doi: https://doi.org/10.1016/j.aninu.2018.01.007.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 − ΔΔCT method. Methods, 402-408(25). Doi: https://doi.org/10.1006/meth.2001.1262.

Ma, J., Piao, X., Shang, Q., Long, S., Liu, S., Mahfuz, S., 2021. Mixed organic acids as an alternative to antibiotics improve serum biochemical parameters and intestinal health of weaned piglets. Animal Nutrition, 737-749 (7). Doi: https://doi.org/10.1016/j.aninu.2020.11.018.

Ma, J., Long, S., Wang, J., Gao, J., Piao, X., 2022. Microencapsulated essential oils combined with organic acids improves immune antioxidant capacity and intestinal barrier function as well as modulates the hindgut microbial community in piglets. Journal of Animal Science and Biotechnology, 1-17(13). Doi: https://doi.org/10.1186/s40104-021-00670-3.

Mandarim De Lacerda, C. A., 1995. Métodos quantitativos em morfologia. Rio de Janeiro. Eduerj. 131p.

Marchiori, M. S., Paiano, D., Zatti, E., Tarasconi, L., Ficagna, C., Amaral, M. A., Da Silva, A. S., 2024. Butyric acid glycerides as substitutes for antibiotics as growth enhancers in the diet of nursery piglets. Research in Veterinary Science, 105110(167). Doi: https://doi.org/10.1016/j.rvsc.2023.105110.

Muniyappan, M., Palanisamy, T., Kim, I. H., 2021. Effect of microencapsulated organic acids on growth performance, nutrient digestibility, blood profile, fecal gas emission, fecal microbial, and meat-carcass grade quality of growing-finishing pigs. Livestock Science, 104658(252). Doi: https://doi.org/10.1016/j.livsci.2021.104658.

Nari, N., Ghasemi, H. A., Hajkhodadadi, I., Farahani, A. K., 2020. Intestinal microbial ecology, immune response, stress indicators, and gut morphology of male broiler chickens fed low-phosphorus diets supplemented with phytase, butyric acid, or Saccharomyces boulardii. Livestock Science, 103975(234). Doi: https://doi.org/10.1016/j.livsci.2020.103975.

Nguyen, D. H., Lee, K. Y., Mohammadigheisar, M., Kim, I. H., 2018a. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers. Poultry science, 4351-4358 (97). Doi: https://doi.org/10.3382/ps/pey339.

Nguyen, D. H., Lee, K. Y., Tran, H. N., Kim, I. H., 2018b. Effect of a protected blend of organic acids and medium-chain fatty acids on growth performance, nutrient digestibility, blood profiles, meat quality, faecal microflora, and faecal gas emission in finishing pigs. Canadian journal of animal science, 448-455 (99). Doi: https://doi.org/10.1139/cjas-2016-017.

Odle, J., 1997. New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. The Journal of nutrition, 127(6), 1061- 1067. Doi: https://doi.org/10.1093/jn/127.6.1061.

Papatsiros, V. G., Christodoulopoulos, G., Filippopoulos, L. C., 2012. The use of organic acids in monogastric animals (swine and rabbits). J. Cell Anim. Biol, 154-159 (6). Doi: https://doi.org/10.5897/JCAB11.081.

Pluske, J. R., Williams, I. H., Aherne, F. X., 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Animal Science, 131-144(62). Doi: https://doi.org/10.1017/S1357729800014417.

Pluske, J. R., Turpin, D. L., Kim, J. C., 2018. Gastrointestinal tract (gut) health in the young pig. Animal Nutrition, 187-196 (4). Doi: https://doi.org/10.1016/j.aninu.2017.12.004.

Rose, E. C., Odle, J., Blikslager, A. T., Ziegler, A. L. (2021). Probiotics, prebiotics and epithelial tight junctions: a promising approach to modulate intestinal barrier function. International Journal of Molecular Sciences, 22(13), 6729. Doi: https://doi.org/10.3390/ijms22136729.

Rostagno, H. S., Albino, L. F. T., Hannas, M. I., Donzele, J. L., Sakomura, N. K., Perazzo, F. G., Brito, C. O., 2017. Brazilian Tables for Poultry and Swine: composition of feedstuffs and nutritional requirements. UFV, Universidade Federal de Viçosa, Departamento de Zootecnia: Viçosa, Brazil. 488p.

Ruggeri, J., Foresti, F., Pavesi, R., Terrini, A., Giudici, F., Padoan, D., Alborali, G. L., 2018. The synergistic effect of organic acids, phytochemicals and a permeabilizing complex reduces

Salmonella Typhimurium 1, 4,[5], 12: i-shedding in pigs. Veterinary research communications, 209-217 (42). Doi: https://doi.org/10.1007/s11259-018-9723-3.

Satessa, G. D., Kjeldsen, N. J., Mansouryar, M., Hansen, H. H., Bache, J. K., Nielsen, M. O. (2020). Effects of alternative feed additives to medicinal zinc oxide on productivity, diarrhoea incidence and gut development in weaned piglets. Animal, 1638-1646 (14). Doi: https://doi.org/10.1017/S1751731120000154.

Slifer, Z. M., Blikslager, A. T., 2020. The integral role of tight junction proteins in the repair of injured intestinal epithelium. International Journal of Molecular Sciences, 972(21). Doi:https://doi.org/10.3390/ijms21030972.

Sun, M., Wu, W., Chen, L., Yang, W., Huang, X., Ma, C., Cong, Y., 2018. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. Nature communications, 3555(9). Doi: https://doi.org/10.1038/s41467-018-05901-2.

Tugnoli, B., Piva, A., Sarli, G., Grilli, E., 2020a. Tributyrin differentially regulates inflammatory markers and modulates goblet cells number along the intestinal tract segments of weaning pigs. Livestock Science, 103996 (234). Doi: https://doi.org/10.1016/j.livsci.2020.103996.

Tugnoli, B., Giovagnoni, G., Piva, A., Grilli, E., 2020b. From acidifiers to intestinal health enhancers: How organic acids can improve growth efficiency of pigs. Animals, 134(10). Doi: https://doi.org/10.3390/ani10010134.

Upadhaya, S. D., Lee, K. Y., Kim, I. H., 2014. Protected organic acid blends as an alternative to antibiotics in finishing pigs. Asian-Australasian journal of animal sciences, 1600(27). Doi: http://dx.doi.org/10.5713/ajas.2014.14356.

Upadhaya, S. D., Jiao, Y., Kim, Y. M., Lee, K. Y., Kim, I. H., 2020. Coated sodium butyrate supplementation to a reduced nutrient diet enhanced the performance and positively impacted villus height and faecal and digesta bacterial composition in weaner pigs. Animal feed science and technology, 114534 (265). Doi: https://doi.org/10.1016/j.anifeedsci.2020.114534.

Vahjen, W., Pietruszyńska, D., Starke, I. C., Zentek, J., 2015. High dietary zinc supplementation increases the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs. Gut pathogens, 7(1), 1-5. Doi: https://doi.org/10.1186/s13099-015-0071-3.

Valini, G. A. C., Duarte, M. S., Calderano, A. A., Teixeira, L. M., Rodrigues, G. A., Fernandes, K. M., Rocha, G. C., 2021. Dietary nucleotide supplementation as an alternative to in-feed antibiotics in weaned piglets. Animal, 100021 (15). Doi: https://doi.org/10.1016/j.animal.2020.100021.

Vinolo, M. A., Rodrigues, H. G., Hatanaka, E., Sato, F. T., Sampaio, S. C., Curi, R., 2011. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. The Journal of nutritional biochemistry, 849-855 (22). Doi: https://doi.org/10.1016/j.jnutbio.2010.07.009.

Yang, K. M., Jiang, Z. Y., Zheng, C. T., Wang, L., Yang, X. F., 2014. Effect of Lactobacillus plantarum on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic Escherichia coli K88. Journal of Animal Science, 1496-1503 (92). Doi: https://doi.org/10.2527/jas.2013-6619.

Yang, Y., Lee, K. Y., Kim, I. H., 2019. Effects of dietary protected organic acids on growth performance, nutrient digestibility, fecal microflora, diarrhea score, and fecal gas emission in weanling pigs. Canadian Journal of Animal Science, 514-520 (99). Doi: https://doi.org/10.1139/cjas-2018-0159.

Weber, T. E., Kerr, B. J., 2008. Effect of sodium butyrate on growth performance and response to lipopolysaccharide in weanling pigs. Journal of Animal Science, 442-450(86). Doi: https://doi.org/10.2527/jas.2007-0499.

Wu, W., Xiao, Z., An, W., Dong, Y., Zhang, B., 2018. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PloS one, e0197762 (13). Doi: https://doi.org/10.1371/journal.pone.0197762.

Wu, X., Tian, Z., Lv, Z., Chen, Z., Liu, Y., Yong, X., Wei, P., 2020. Effects of copper salts on performance, antibiotic resistance genes, and microbial community during thermophilic anaerobic digestion of swine manure. Bioresource technology, 122728 (300). Doi: https://doi.org/10.1016/j.biortech.2019.122728.

Zentek, J., Buchheit-Renko, S., Ferrara, F., Vahjen, W., Van Kessel, A. G., Pieper, R. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Animal Health Research Reviews, 83-93 (12). Doi: https://doi.org/10.1017/S1466252311000089.

Zentek, J., Ferrara, F., Pieper, R., Tedin, L., Meyer, W., Vahjen, W., 2013. Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive tract of weaning piglets. Journal of animal science, 3200-3210 (91). Doi: https://doi.org/10.2527/jas.2012-5673.

Zhang, W. X., Zhang, Y., Zhang, X. W., Deng, Z. X., Liu, J. X., He, M. L., Wang, H. F., 2020. Effects of dietary supplementation with combination of tributyrin and essential oil on gut health and microbiota of weaned piglets. Animals, 180(10). Doi. https://doi.org/10.3390/ani10020180.

TABELAS

Table 1- Ingredients and chemical composition of control diets fed to nursery piglets from 21 to 35 d of age (g/kg, as-fed basis).

Item[1]	
Ground corn, 7.8% CP	464.34
Soybean meal, 46.0% CP	186.97
Dried whey, 12.5% CP	140.0
Soybean micronized, 36.0% CP	100.0
Plasma protein, 78.0% CP	40.0
Sugar	28.0
Inert (kaolin)	3.00
Dicalcium phosphate	10.70
Limestone calcitic	9.64
Soybean oil	2.96
Choline chloride	1.95
L-lys, 78.0%	4.26
DL-met, 99.0%	2.06
L-thr, 98.5%	1.99
L-trp, 99.0%	0.26
L-val, 96.5%	0.75
Salt	1.61
Vitamin-mineral premix[2]	1.40
Phytase[3]	0.05
BHT	0.10
Calculated composition	
ME, kcal/kg	3.400
Crude protein, g/kg	214.00
SID[4] lys, g/kg	14.51
SID met, g/kg	4.62
SID met + cys, g/kg	8.13
SID thr, g/kg	9.72
SID trp, g/kg	2.76
SID val, g/kg	10.01
SID ile, g/kg	7.89
Available P, g/kg	5.00
Total Na, g/kg	3.20
Lactose, g/kg	107.80

[1]The feed additives were supplemented as a replacement for the inert in the diet. (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

[2]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.
[3]Natuphos®, Basf enzyme.
[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′
SMCT2	XM_003122908.1	F: 5′AGGTCTACCGCTTTGGAGCAT3’ R:5′GAGCTCTGATGTGAAGATGATGACA3’
MCT1	AM_286425.1	F: 5′GGTGGAGGTCCTATCAGCAG3’ R: 5′AAGCAGCCGCCAATAATCAT3’

[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; SMCT2, sodium-coupled monocarboxylate transporter; MCT1, monocarboxylate transporter 1.

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

Table 3 - Growth performance and diarrhea occurrence of piglets fed diets supplemented with organic acids (at 35 days-old)[1]

Item[2]	Dietary treatment[3]					SEM[4]	*P*-value			
	CON	PA	HPA	LA	HLA		CON × PA	CON × HPA	CON × LA	CON × HLA
IBW, kg	6.8	6.9	6.9	6.8	6.8	0.19	0.846	0.853	0.975	0.947
FBW, kg	9.7	10.1	9.9	10.2	9.5	0.47	0.556	0.684	0.442	0.847
ADFI, g/d	239	242	253	252	226	22.52	0.934	0.666	0.686	0.692
ADG, g/d	190	213	205	225	180	23.23	0.496	0.656	0.294	0.765
FC, g/g	1.37	1.15	1.31	1.15	1.38	0.09	0.118	0.693	0.124	0.941
Diarrhea occurrence, %	25.4	17.1	24.6	15.1	27.9	0.113	0.099	0.884	0.041	0.650

[1]Data are means of 8 pens replicates per dietary treatment and 1 piglet per pen as an experimental unit.

[2] Initial body weight (IBW), final body weight (FBW), average daily feed intake (ADFI, g/d), average daily weight gain (ADG, g/d), feed conversion ratio (FC, g/g).

[3]Dietary treatment: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

[4]Pooled standard error of the mean.

Table 4 - Blood profile of piglets fed diets supplemented with organic acids (at 35 days-old)[1]

Item	Dietary treatment[2]					SEM[3]	*P*-value			
	CON	PA	HPA	LA	HLA		CON × PA	CON × HPA	CON × LA	CON × HLA
Urea, mg/dL	14,1	13.0	12,6	13,6	13,6	1,57	0.598	0.499	0.810	0.812
Creatinine, mg/dL	0.90	0.84	0.89	0.88	0.85	0,03	0.134	0.744	0.488	0.172
IgG, mg/dL	181.1	168.0	187.8	191.8	212.4	15,07	0.530	0.749	0.608	0.129

[1]Data are means of 8 pens replicates per dietary treatment and 1 piglet per pen as an experimental unit.

[2]Dietary treatment: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

[3]Pooled standard error of the mean.

Table 5 - Intestinal pH of piglets fed diets supplemented with organic acids (at 35 days-old)[1]

Item	Dietary treatment[2]					SEM[3]	*P*-value			
	CON	PA	HPA	LA	HLA		CON × PA	CON × HPA	CON × LA	CON × HLA
pH	6.47	6.02	5.97	6.14	6.09	0.11	0.010	0.005	0.054	0.031

[1]Data are means of 8 pens replicates per dietary treatment and 1 piglet per pen as an experimental unit.

[2]Dietary treatment: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

[3]Pooled standard error of the mean.

Table 6 - Intestinal morphology of piglets fed diets supplemented with organic acids (at 35 days-old)[1]

Item	Dietary treatment[2]					SEM[3]	*P*-value			
	CON	PA	HPA	LA	HLA		CON × PA	CON × HPA	CON × LA	CON × HLA
Duodenum										
Villus height, µm	230	276	272	289	278	17.0	0.066	0.092	0.022	0.057
Crypt depth, µm	198	227	213	226	224	12.7	0.126	0.438	0.135	0.174
Villus:crypt ratio	1.17	1.23	1.29	1.28	1.25	0.05	0.509	0.159	0.197	0.375
Goblet cells, %	34.1	39.8	35.4	38.7	34.9	3.75	0.287	0.811	0.394	0.876
Jejunum										
Villus height, µm	241	240.80	259.99	246.14	244.00	15.38	0.975	0.401	0.831	0.908
Crypt depth, µm	188	195.53	210.41	185.11	187.94	9.78	0.567	0.109	0.863	0.975
Villus:crypt ratio	1.34	1.23	1.24	1.34	1.32	9.78	0.394	0.445	0.992	0.912
Goblet cells, %	20.8	25.9	28.2	25.8	28.0	1.63	0.034	0.003	0.037	0.004
Ileum										
Villus height, µm	235	250	230	238	238	13.13	0.447	0.783	0.889	0.897
Crypt depth, µm	211	178	181	182	200	107.38	0.036	0.058	0.067	0.482
Villus:crypt ratio	1.13	1.42	1.32	1.32	1.22	0.09	0.031	0.152	0.144	0.462
Goblet cells, %	29.85	36.91	36.89	34.92	37.40	1.83	0.011	0.011	0.061	0.007
Peyer's patches, n	39.4	34.3	32.3	32.6	33.8	2.71	0.191	0.073	0.088	0.153

[1]Data are means of 8 pens replicates per dietary treatment and 1 piglet per pen as an experimental unit.

[2]Dietary treatment: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON +

0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

[3]Pooled standard error of the mean.

Table 7 - Relative expression of jejunum genes of piglets fed diets supplemented with organic acids (at 35 days-old)[1]

Item[2]	Dietary treatment[3]					SEM[4]	*P*-value			
	CON	PA	HPA	LA	HLA		CON × PA	CON × HPA	CON × LA	CON × HLA
GPX	6.1	5.8	6.2	6.6	6.0	0.19	0.206	0.839	0.124	0.727
SOD	4.7	4.5	4.7	4.9	4.5	0.18	0.609	0.980	0.325	0.513
CAT	6.7	6.5	6.6	6.4	6.3	0.23	0.530	0.768	0.394	0.210
OCL	8.4	8.9	9.2	9.4	9.3	0.27	0.157	0.053	0.017	0.022
ZO-1	9.2	9.3	9.6	9.7	9.8	0.27	0.801	0.348	0.171	0.155
IFN- γ	12.8	12.9	13.2	13.2	13.0	0.33	0.917	0.444	0.411	0.748
TNF-α	12.7	12.8	13.2	12.8	12.8	0.33	0.948	0.369	0.892	0.855
IL1- β	13.6	13.6	13.6	13.6	13.6	0.30	0.841	0.834	0.890	0.988
IL-10	12.7	13.0	13.6	13.2	12.6	0.27	0.425	0.024	0.206	0.763
SMCT2	11.4	11.4	12.1	11.7	11.7	0.30	0.896	0.126	0.445	0.496
MCT1	11.0	10.7	11.4	11.6	11.0	0.26	0.672	0.345	0.122	0.957

[1]Data are means of 8 pens replicates per dietary treatment and 1 piglet per pen as an experimental unit.

[2]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; SMCT2, sodium-coupled monocarboxylate transporter; MCT1, monocarboxylate transporter 1.

[3]Dietary treatment: (1) CON: control, basal diet; (2) powder acid (PA): CON + 0.75 g of a blend of fatty acids in powder form/kg diet, (3) high powder acid (HPA): CON + 3.00 g of a blend of fatty acids in powder form/kg diet, (4) liquid acid (LA): CON + 0.50 g of a blend of fatty acids in liquid form/kg diet, (5) high liquid acid (HLA): CON + 2.00 g of a blend of fatty acids in liquid form/kg diet.

4Pooled standard error of the mean

ANEXO

UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
CEUAP/UFV

Campus Universitário – Viçosa, MG – 36570-900 – Telefone:(31) 3899.3275 – e-mail: ceuap@ufv.br – site: www.ceuap.ufv.br

Viçosa, 17 de Julho de 2023

CERTIFICADO

Certificamos que o projeto intitulado **"Blend de ácidos orgânicos para suínos em fase de creche"**, protocolo **nº 069/2023**, sob a responsabilidade de **Gabriel Cipriano Rocha** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo chordata, subfilo vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo conselho nacional de controle da experimentação animal (concea), e foi apreciado pela comissão de ética no uso de animais de produção da universidade federal de viçosa (ceuap-ufv) em reunião de **12 de Julho de 2023.**

Finalidade: **(x)Pesquisa ( )Ensino**
Vigência do Projeto: de **Julho de 2023** a **Novembro de 2023**
Espécie/linhagem: **Suíno (Sus Domesticus)** Nº de animais: **100**
Peso: **6Kg** Idade: **21 dias** Sexo: **Macho/Fêmea** Origem: **Setor Suinocultura - DZO /UFV - CNPJ/CPF: 25.944.455/0001-96**
Data de Aprovação: **17 de Julho de 2023**

CERTIFICATE

We certify that the project entitled **"Organic acid blend for nursery pigs"**, protocol **nº 069/2023**, under the responsibility of **Gabriel Cipriano Rocha** - which involves the production, maintenance and/or use of animals belonging to the phylum chordata, subphylum vertebrata (except man), for scientific research purposes (or education) - is in accordance with the law nº. 11.794, of October 8, 2008, Decree nº. 6899 of July 15, 2009, and the rules issued by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (CEUAP-UFV) in its meeting on **Jul. 12Th of 2023**.

Finality: **(x)Research ( )Education**
Duration of the Project: from **Jul. of 2023** to **Nov. of 2023**.
Species / strain: **Pig (Sus Domesticus)** Nº of animals: **100**
Weight: **6Kg** Age: **21 days** Sex: **Male/ Female** Source: **Setor Suinocultura - DZO /UFV - CNPJ/CPF: 25.944.455/0001-96**
Approval date: **Jul. 17Th of 2023**

Luciana Navajas Rennó
Coordenadora da CEUAP/UFV