UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

ÁCIDOS ORGÂNICOS COMO MELHORADORES DE DESEMPENHO PARA SUÍNOS

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Engenheira Agrônoma - UFRGS

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UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”

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IMPACTO POTENCIAL DESTA PESQUISA

Com insights derivados de uma análise sistemática de 147 artigos científicos publicados ao longo de três décadas, este estudo apresenta evidências convincentes sobre a eficácia dos ácidos orgânicos como melhoradores de desempenho para suínos. Nota-se que os benefícios dos ácidos orgânicos abrangem diferentes e importantes fases produtivas da suinocultura: gestação, maternidade, creche, crescimento e terminação. Em um cenário de restrições ao uso aos antibióticos promotores de crescimento, os ácidos orgânicos emergem como ser uma alternativa viável e eficaz para a substituição dos antibióticos na suinocultura.

POTENTIAL IMPACT OF THIS RESEARCH

Based on insights from a systematic analysis of 147 scientific articles published over three decades, this study provides compelling evidence of the effectiveness of organic acids as performance enhancers for pigs. It's worth noting that the benefits of organic acids extend across different crucial stages of pig farming: gestation, maternity, nursery, growth, and finishing phases. In a scenario where the use of antibiotics growth-promoting is restricted, organic acids emerge as a viable and effective alternative for replacing antibiotics in pig farming.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

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Dedico este trabalho à minha família, especialmente à minha mãe Maria Helena, minha grande companheira de vida, minha maior incentivadora e minha melhor amiga, aos meus irmãos Marcus e Rafael, aos meus sobrinhos Benjamin e Antonella, às minhas cunhadas Fernanda e Rosana, e à minha falecida avó Elvira (sempre nos meus pensamentos e no meu coração). Vó, a senhora foi um grande exemplo para mim. Sei que estaria orgulhosa da sua neta.

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SUMÁRIO

CAPÍTULO I **8**
INTRODUÇÃO **9**
1. REVISÃO BIBLIOGRÁFICA 11
1.1 O papel da saúde intestinal no desempenho produtivo dos suínos 11
1.2 Restrições de uso aos antibióticos promotores de crescimento 13
1.3 Alternativas aos antibióticos promotores de crescimento na suinocultura 15
1.4 Os ácidos orgânicos 17
1.5 Modos de ação dos ácidos orgânicos 19
1.6 Desempenho produtivo e reprodutivo de porcas alimentadas com ácidos orgânicos 21
1.7 Desempenho produtivo de suínos alimentados com ácidos orgânicos 22
1.8 Efeitos dos ácidos orgânicos na morfologia intestinal 24
1.9 Revisão sistemática e meta-análise na ciência animal 25
2.HIPÓTESES E OBJETIVOS **26**
2.1 Hipóteses 26
2.2 Objetivos 27
CAPÍTULO II **28**
Effects of organic acids on performance and intestinal morphology of piglets challenged by enteric bacteria: A systematic review and meta-analysis 29
Abstract 29
Implications 30
Introduction 30
Material and methods 32
Results 35
Discussion 41
Conclusion 46
CAPÍTULO III **66**
Organic acids for pigs from nursery to finishing phases: Systematic review and meta-analysis 67
Highlights 67
Abstract 67
Introduction 68

Material and methods 69
Results 72
Discussion 80
CAPÍTULO IV **115**
Meta-analysis of organic acid supplementation impact on sow productivity and reproduction 116
Highlights 116
Abstract 116
Introduction 117
Material and methods 118
Results 121
Discussion 123
Conclusion 124
CAPÍTULO V **136**
3. CONSIDERAÇÕES FINAIS 137

LISTA DE ABREVIAÇÕES

ADFI – Average daily feed intake

ADG – Average daily gain

AGP – Antibiotic growth promoter

ANT – Antibiótico/Antibiotic

APC – Antibióticos promotores de crescimento

AO – Ácido orgânico

BWG – Body weight gain

CD – Crypt depth

CON – Controle/Control

FCR – Feed conversion ratio

FE – Feed efficiency

GI – Gastrointestinal tract

OA – Organic acid

SCFA – Short-chain fatty acid

VH – Villi height

VH:CD – Villi height:Crypth depth ratio

TCA – Tricarboxylic acid

TGI – Trato gastrointestinal

LISTA DE TABELAS

CAPÍTULO I

Tabela 1. Lista dos principais ácidos orgânicos e suas propriedades. Adaptado de TUGNOLLI *et al*., 2020 .... **18**

CAPÍTULO II

Table 1. Search terms for PICo methodology: Population, Intervention and Context used in the systematic review **54**

Table 2. Meta-analysis database description **55**

Table 3. Descriptive analysis of meta-analysis database **58**

Table 4. NRC nutritional recommendations for nursery phase and nutritional meta-analysis average numbers **60**

Table 5. Performance of piglets challenged by enteric bacteria and supplemented with dietary antibiotics or organic acids **61**

Table 6. Intestinal morphology of piglets challenged by enteric bacteria and supplemented with dietary antibiotics or organic acids **62**

CAPÍTULO III

Table 1. Search terms for PICo methodology: Population, Intervention and Context used in the systematic review **103**

Table 2. Meta-analysis database description **104**

Table 3. Performance of pigs supplemented with dietary antibiotics or organic acids **110**

Table 4. Intestinal morphology of pigs supplemented with dietary antibiotics or organic acids **111**

Table 5. Digestibility coefficients (ATTD) of pigs supplemented with dietary antibiotics or organic acids **112**

CAPÍTULO IV

Table 1. Outlines the search terms employed for the PICo methodology, encompassing Population, Intervention, and Context, as utilized in the systematic review **130**

Table 2. Description of the meta-analysis database **131**

Table 3. Performance and body composition of sows supplemented with dietary organic acids **132**

Table 4. Reproductive performance of sows supplemented with dietary or organic acids **133**

LISTA DE FIGURAS

CAPÍTULO I

Figura 1 – Dois mecanismos de ação propostos para os ácidos orgânicos sobre o pH do trato gastrointestinal e sua ação direta sobre os patógenos. Adaptado de DITTOE et al. (2018). 20

CAPÍTULO II

Fig. 1. Flowchart for searching progress – PRISMA Flow Diagram...........................63

Fig. 2. Location of the studies focusing on organic acids for challenged piglets evaluation...64

Fig. 3. Performance responses of antibiotics and organic acids treatments relativized to the respective control 65

CAPÍTULO III

Figure 1. Prisma Flow Diagram of the Systematic review and selection process....113

Figure 2. Geographical distribution of the studies included in the meta-analysis 114

CAPÍTULO IV

Figure 1. Prisma Flow Diagram of the Systematic review and selection process....134

Figure 2. Geographical distribution of the studies included in the meta-analysis 135

ÁCIDOS ORGÂNICOS COMO MELHORADORES DE DESEMPENHO PARA SUÍNOS[1]

Autora: Maria Sara Cabrera Mendéz

Orientador: Luciano Hauschild – Coorientadora: Ines Andretta

Este trabalho teve como objetivos avaliar sistematicamente as informações disponíveis na literatura sobre os efeitos dos ácidos orgânicos nos desempenhos produtivo e reprodutivo de suínos nas fases de creche, crescimento, terminação e de porcas gestantes e lactantes. Três revisões sistemáticas seguidas por meta-análise foram conduzidas entre janeiro e outubro de 2022 a partir do desenvolvimento de três bancos de dados distintos. Informações de variáveis exploratórias como o desempenho produtivo (consumo de ração, ganho de peso e eficiência alimentar), o desempenho reprodutivo (número de leitões nascidos vivos, peso dos leitões ao nascimento, número de leitões desmamados e peso dos leitões ao desmame), coeficientes de digestibilidade (proteína bruta, energia metabolizável e matéria seca) e a morfologia intestinal (altura de vilosidades, profundidade de criptas e relação vilosidade:cripta) de 147 artigos científicos foram dispostas em planilhas eletrônicas para posterior análise estatística individual. Ao todo, este trabalho deu origem a três artigos científicos sintetizando dados publicados desde o ano de 1997 até o ano de 2022. Foram comparados os tratamentos dieta basal (sem suplementação), contendo antibióticos e contendo ácidos orgânicos, visando avaliar os ácidos orgânicos como potenciais substitutos aos antibióticos promotores de crescimento. O primeiro artigo avaliou o efeito dos ácidos orgânicos no desempenho produtivo e na morfologia intestinal de suínos desafiados por bactérias entéricas na fase de creche. Neste trabalho, observamos que os ácidos orgânicos aumentaram a eficiência alimentar dos leitões e auxiliaram na manutenção da morfologia intestinal mesmo sob condições de desafio sanitário. O segundo artigo avaliou o efeito dos ácidos orgânicos no desempenho produtivo, morfologia intestinal e digestibilidade de dietas em suínos nas fases de creche, crescimento e terminação. Os ácidos orgânicos melhoraram a eficiência alimentar de leitões em creche, além de beneficiar a morfologia intestinal. Adicionalmente, os coeficientes de digestibilidade da proteína bruta e do fósforo foram maiores no grupo suplementado com ácidos orgânicos. No terceiro artigo, foram avaliadas variáveis de desempenhos produtivo e reprodutivo de porcas alimentadas com ácidos orgânicos durante a gestação. Foi possível constatar que as fêmeas gestantes alimentadas com ácidos orgânicos apresentaram maior peso no pós-parto, além de um número maior de leitões desmamados, bem como leitões mais pesados ao desmame. Podemos concluir que os ácidos orgânicos são eficazes como melhoradores de desempenho para suínos sob condições de desafio sanitário, na fase de creche e na maternidade. Dessa forma, estes compostos apresentam resultados promissores em um cenário de restrição ao uso dos antibióticos promotores de crescimento.

Palavras-chave: Aditivos; Meta-análise; Promotores de crescimento; Suinocultura

[1]Dissertação de Mestrado em Ciência Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, SP, Brasil, 153 páginas, Novembro de 2023.

ORGANIC ACIDS AS PERFORMANCE ENHANCERS FOR SWINE[1]

Author: Maria Sara Cabrera Mendéz

Supervisor: Luciano Hauschild – Co-supervisor: Ines Andretta

The aim of this study was to systematically assess available literature on the effects of organic acids on the productive and reproductive performances of pigs in the nursery, growth, finishing phases, as well as pregnant and lactating sows. Three systematic reviews followed by meta-analysis were conducted between January and October 2022 using three separate databases. Data from exploratory variables such as productive performance (feed consumption, weight gain, and feed efficiency), reproductive performance (number of live piglets born, piglet weight at birth, number of weaned piglets, and piglet weight at weaning), digestibility coefficients (crude protein, gross energy, and dry matter), and intestinal morphology (villus height, crypt depth, and villous:crypt ratio) from 147 scientific articles were organized in spreadsheets for subsequent individual statistical analysis. In total, this work resulted in three scientific articles synthesizing published data from 1997 to 2022. Treatments were compared, including basal diet (no supplementation), antibiotics-containing, and organic acids-containing, aiming to assess organic acids as potential substitutes for antibiotics growth-promoting. The first article evaluated the effect of organic acids on the productive performance and intestinal morphology of pigs challenged by enteric bacteria in the nursery phase. Organic acids were found to increase piglet feed efficiency and assist in maintaining intestinal morphology even under sanitary challenge conditions. The second article assessed the effect of organic acids on the productive performance, intestinal morphology, and digestibility of diets in pigs during the nursery, growth, and finishing phases. Organic acids improved feed efficiency in nursery piglets and benefited intestinal morphology. Additionally, digestibility coefficients of crude protein and phosphorus were higher in the group supplemented with organic acids. In the third article, performance and reproductive variables of sows fed with organic acids during gestation were evaluated. It was observed that gestating females fed with organic acids had higher postpartum weight, a greater number of weaned piglets, and heavier piglets at weaning. In conclusion, organic acids are effective performance enhancers for pigs under sanitary challenge conditions, in the nursery phase, and during maternity. Thus, these compounds show promising results in a scenario of restricted use of antibiotics growth-promoting.

Keywords: Additives; Meta-analysis; Growth promoters; Swine Farming

[1]Master of Science dissertation in Animal Science, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal, SP, Brazil, 153 pages, November 2023.

CAPÍTULO I

INTRODUÇÃO

Em um cenário ideal da produção de suínos, visa-se a criação de animais saudáveis e que apresentem bons índices de desempenho produtivo. Com o avanço tecnológico da suinocultura das últimas décadas, tem-se produzido e utilizado linhagens cada vez mais precoces e as práticas de manejo possibilitam o desmame de leitões com idades entre 18 e 28 dias (CASTILLO GÓMEZ, 2006). Como resultado da precocidade, os leitões são desmamados mais leves e com um sistema digestivo bastante imaturo. Esse fato permite que os leitões fiquem mais suscetíveis a microrganismos patogênicos presentes no ambiente produtivo, acarretando desordens de origem gastrointestinal (CAMPBELL *et al.*, 2013). Nesse contexto, o uso racional de medicamentos para controlar possíveis patógenos nesses sistemas é uma prática bastante comum, tanto por questões econômicas quanto de qualidade alimentar (CANIBE *et al*., 2005).

Na produção comercial de suínos, os animais são expostos a diferentes agentes estressores, especialmente os leitões. Fatores como a mudança ambiental, separação da porca e alteração brusca da dieta contribuem para a criticidade da fase (LÓPEZ-GÁLVEZ *et al.*, 2021). Um sistema digestivo pouco desenvolvido no momento do desmame confere aos leitões uma baixa atividade de enzimas responsáveis pela digestão dos alimentos de origem vegetal (GRESSE *et al.*, 2017). Somado a isto, o trato gastrointestinal dos animais jovens apresenta menor produção de ácidos gástricos – o que compromete a digestão de diversos nutrientes, mas em especial da proteína (MAO *et al.*, 2019). Estes nutrientes não digeridos podem servir como substrato, proporcionando condições favoráveis ao desenvolvimento de microrganismos indesejáveis no trato digestório.

A fase de desmame é conhecida como uma das mais críticas nos sistemas de criação de suínos. Contudo, o desempenho produtivo nas fases de crescimento e terminação também é desafiador e importante, considerando que cerca de 60% dos animais na suinocultura brasileira se encontram nessas fases (ABCS, 2014). Além disto, fêmeas reprodutoras apresentam-se como um fator chave na busca por bons índices de produtividade. As porcas são responsáveis por originar os leitões que entrarão nas fases de crescimento e terminação – parcela mais significativa do plantel. Portanto, o número de leitões desmamados por porca e a qualidade destes animais impacta diretamente o número de animais abatidos e a quantidade de carne produzida (MIRILOVIĆ *et al.*, 2016). Dessa forma, os estados nutricional e de saúde da porca são determinantes na produção de suínos. Nesse contexto, a utilização de medicamentos para porcas gestantes e lactantes também é uma prática usual na suinocultura, contribuindo para melhor desempenho reprodutivo da fêmea e produtivo da leitegada (HAYS *et al.*, 1978). Ainda que em menor proporção, animais adultos também estão suscetíveis à ação de microrganismos indesejáveis, fato que pode acarretar importantes perdas produtivas e levar à mortalidade.

Visando reduzir perdas relacionadas a ação de patógenos no trato digestivo, a indústria animal durante décadas faz uso dos antibióticos promotores de crescimento (APC). Estes compostos nada mais são do que medicamentos fornecidos em doses geralmente sub terapêuticas durante períodos mais prolongados dentro do ciclo produtivo (MOREL *et al.*, 2019). Os APC vêm sendo utilizados há cerca de 50 anos na produção animal, garantindo melhor desempenho produtivo dos suínos, especialmente dos animais mais jovens, além de reduzir a incidência de doenças e a mortalidade (RATHNAYAKE *et al.*, 2021). O fornecimento dietético de APC para suínos contribuiu para incrementos entre 3 e 8% no ganho de peso e aumento da eficiência alimentar em cerca de 3% (FUNK *et al.*, 2001). Acredita-se que esses compostos são capazes de modular a microbiota intestinal, inibindo a proliferação de microrganismos indesejáveis. Adicionalmente, aos APC alteram positivamente a morfologia intestinal, melhorando a digestão dos nutrientes e, consequentemente, o desempenho produtivo (MARSHALL E LEVY, 2011). Devido aos seus efeitos positivos, os APC se configuram como importantes aliados, especialmente nas fases iniciais da produção de suínos.

Porém, nas últimas décadas, a utilização de antibióticos na produção animal vem sendo alvo de diversos questionamentos, os quais têm demonstrado notoriedade pública. Esse movimento teve um marco importante no ano de 2006, quando a União Europeia emitiu uma nota regulatória limitando o uso dos APC como aditivos na nutrição animal (BRIZ, 2006). No mesmo sentido, a FAO reportou a importância do uso de compostos antimicrobianos na produção animal. Contudo, reiterou em seus comunicados a discussão quanto ao desenvolvimento de resistência bacteriana como um ponto crítico, tanto no que tange a saúde animal quanto a humana (WHO, 2012). Nesse cenário, a utilização de antibióticos na produção animal tem sido amplamente questionada, especialmente no tocante da qualidade alimentar humana (VAN DEN BOGAARD E STOBBERINGH, 2000). Em meio às incertezas, diversos países optaram por limitar o uso dos APC em seus sistemas produtivos, estendendo as limitações, inclusive, para a importação de produtos de origem animal.

Considerando que o crescimento e a eficiência alimentar são fatores críticos para a produção industrial de suínos, os APC desempenharam durante décadas um papel essencial na suinocultura, reduzindo a mortalidade, melhorando a saúde intestinal e contribuindo para a manutenção de bons índices produtivos (RATHNAYAKE *et al.*, 2021). Nesse sentido, as limitações no uso destes compostos originaram uma desafiadora e importante lacuna na produção animal, abrindo espaço para a investigação e o desenvolvimento de compostos alternativos que possam atuar como promotores de crescimento, substituindo os APC na produção de suínos. Entre esses compostos, destacam-se os ácidos orgânicos, que são tema central desta dissertação.

1. REVISÃO BIBLIOGRÁFICA

1.1 O papel da saúde intestinal no desempenho produtivo dos suínos

O intestino é composto por um complexo sistema que apresenta três principais componentes: barreira intestinal (formada pela mucosa), a microbiota e o sistema imune local (ROSELLI *et al.*, 2005). A microbiota intestinal dos mamíferos desempenha diferentes papéis no hospedeiro, como participação na digestão e a fermentação de carboidratos, manutenção do funcionamento das vilosidades intestinais, produção de vitaminas, regulação do sistema imune e proteção contra microrganismos patogênicos (BUFFIE E PAMER.; KAMADA *et al.*, 2013). A porção intestinal é um dos maiores componentes do trato digestório, sendo responsável por grande parte da imunidade do organismo. O epitélio intestinal é um dos maiores órgãos imunes dos animais, comportando cerca de 70% das células imunes do organismo (VEIZAJ-DELIA *et al*., 2012). Os intestinos são responsáveis pela digestão dos alimentos, absorção dos nutrientes e proteção contra toxinas e patógenos (SUN *et al*., 2017). Portanto, a manutenção da saúde intestinal é um dos fatores chave para o sucesso da produção animal.

De acordo com BISCHOFF (2011), pode-se citar cinco como os principais critérios que poderiam definir saúde intestinal: 1) digestão e absorção eficazes, 2) ausência de desordens gastrointestinais, 3) microbioma estável e normal, 4) boa imunidade e 5) bem-estar. Nesse cenário, infecções patogênicas são responsáveis pela maior parte dos desafios que reduzem a saúde intestinal dos animais. Quando a saúde intestinal dos animais está comprometida, a digestão e absorção de nutrientes é negativamente influenciada, levando a efeitos negativos no desempenho produtivo, perdas econômicas e maior suscetibilidade a doenças (ROSELLI *et al.*, 2005). A infecção por *Escherichia coli* enterotoxigênica (ETEC) na fase de creche é responsável por diversos impactos a níveis globais na suinocultura (SUN *et al.*, 2017). A ação desta bactéria entérica culmina na Diarreia Pós-Desmame (DPD), uma desordem intestinal altamente comum e com grande capacidade de levar os leitões à morte.

A infecção por ETEC induz a um quadro de diarreia durante a primeira e segunda semanas pós-desmame, acarretando desidratação, anorexia, redução do ganho de peso e mortalidade (VERDONCK *et al.*, 2007). A severidade da DPD se relaciona a outros fatores além da infecção por ETEC. Dentre eles podem ser citados o estresse do período de desmame, a separação da porca, alterações na dieta e falhas na colostragem (FAIRBROTHER *et al.*, 2005).

Nesse contexto, a utilização dos APC na suinocultura tem sido um forte aliado no controle de bactérias entéricas como a *Escherichia coli.* Em contrapartida, as restrições de uso destes compostos podem estar relacionadas ao aumento na ocorrência de desordens gastrointestinais, especialmente em leitões desmamados (LIU *et al.*, 2018), comprometendo a sua saúde intestinal e o desempenho produtivo. No tocante das fases de crescimento e terminação, os impactos da retirada dos APC são menores em comparação a creche (WIERUP, 2001), mas ainda assim significativos.

Considerando a importância da manutenção da saúde intestinal dos suínos, diversas moléculas vêm sendo estudadas de maneira a atuarem como promotores de crescimento na produção animal, substituindo os APC. Em alguns cenários de produção, como o europeu, esses compostos são considerados também como possíveis alternativos também ao uso de óxido de zinco. Esses estudos visam encontrar compostos que contribuam para a saúde intestinal, reduzam os efeitos nocivos da infecção por patógenos entéricos e melhorem o desempenho produtivo. Dentre estes compostos alternativos podem ser citados prebióticos, enzimas exógenas, ácidos graxos de cadeia média, leveduras, aditivos fitogênicos, pós bióticos e ácidos orgânicos (RATHNAYAKE *et al.*, 2021).

De acordo com MROZ (2005), *blends* de ácidos orgânicos têm sido utilizados mundialmente e apresentado considerável sucesso na redução da severidade da DPD, bem como a melhoria de parâmetros de qualidade da carne de suínos em crescimento (MUNIYAPPAN *et al.*, 2021). No tocante das fêmeas reprodutoras, a suplementação com ácidos orgânicos contribuiu para um incremento no número de anticorpos do colostro (LIU *et al.*, 2014) e para a redução da diarreia em leitões desmamados (YANG *et al.*, 2019).

1.2 Restrições de uso aos antibióticos promotores de crescimento

O uso inicial de antibióticos na produção animal, muitos deles compartilhados por humanos e animais, se deu com a premissa de tratar doenças (RICHOU, 1952). Antibióticos vêm sendo utilizados na suinocultura para prevenir doenças e aumentar a produtividade desde a década de 40 (DIBNER E RICHARDS, 2005). A partir do uso de doses geralmente sub terapêuticas (10 a 20 vezes inferiores às terapêuticas) por períodos mais prolongados durante o ciclo produtivo, os antibióticos atuam como promotores de crescimento a partir de diferentes mecanismos (LÓPEZ-GÁLVEZ *et al*., 2021). Foram propostos alguns mecanismos de ação para essas moléculas, a partir dos quais acredita-se que esses compostos atuem como melhoradores de desempenho: a) redução do pH intestinal; b) controle da população microbiana indesejada, reduzindo a competição por nutrientes; c) redução da atividade microbiana deletéria ao animal, culminando em menor formação de aminas e d) melhoria na absorção dos nutrientes devido ao estímulo enzimático, contribuindo para uma melhor digestibilidade (HENDERICKX *et al*., 1981). Acredita-se que ao contribuir para um ambiente digestivo mais saudável, os APC favoreçam o a absorção dos nutrientes, levando a um desempenho superior.

A indústria e os produtores adotaram amplamente o uso dos antibióticos devido as melhorias no status de saúde dos animais e a redução da mortalidade, culminando em um incremento produtivo significativo (WEN *et al*., 2022). No contexto da produção animal, a suinocultura é uma das cadeias que faz uso dos APC por maior período dentro do ciclo produtivo em comparação às demais espécies de interesse zootécnico (VAN BOECKEL *et al*., 2015). Na suinocultura, os APC são comumente utilizados até a fase de crescimento em doses baixas, ou seja, até os 120 dias de idade ou até que o animal atinja cerca de 35 kg de peso vivo (EC No. 429, 2008). Contudo, há um incremento no fornecimento destas moléculas na fase de desmame, entre a segunda e a quarta semanas de vida, período crítico no qual o leitão é submetido a diferentes agentes estressores e altamente suscetível a ocorrência da DPD.

Neste cenário, nas últimas décadas, há um crescente movimento mundial que visa limitar o uso destas substâncias na produção animal. Pesquisas recentes têm associado os APC utilizados na produção animal ao aparecimento de resistência microbiana em bactérias patogênicas (HOELZER *et al*., 2017). Esses achados foram observados, inclusive, em patógenos comuns aos animais de produção e aos seres humanos (MARSHALL *et al*., 2011). No ano de 1986, a Suécia baniu o uso de APC na produção animal. Duas décadas depois, em 2006, a União Europeia realizou o mesmo movimento (DIBNER E

RICHARDS, 2005). No Brasil, no ano de 2016, o uso de Colistina como promotor de crescimento foi proibido e, posteriormente, as moléculas Tylosina, Tiamulina e Lincomicina também tiveram seu uso restringido (MAPA, 2020). Entre 2017 e 2019, os Estados Unidos restringiram o uso de diversas moléculas de APC no ambiente produtivo (ANDREI *et al*., 2019). Mais recentemente, em 2020, a China também optou por banir e restringir o uso de 11 APC na indústria animal, dentre elas a bacitracina de zinco, flavomicina, avilamicina e clorotetraciclina, moléculas comumente empregadas na produção animal (HU E COWLING, 2020).

Uma meta-análise conduzida por CARDINAL *et al*. (2021) estimou economicamente os impactos da retirada dos APC na suinocultura. De acordo com o estudo, dietas com APC contribuem para incrementos de 6% no ganho de peso dos animais na fase de creche – a mais crítica dentro do ciclo de produção. Adicionalmente, remover os APC na fase de creche leva a um aumento nos custos de produção de US$ 0,86 por animal. Já na fase de crescimento, o aumento nos custos é substancialmente superior – cerca de US$ 3,11 por animal. Dessa forma, o estudo concluiu que o uso dos APC na dieta dos suínos contribui positivamente para o desempenho produtivo, e que remover esses compostos aumenta significativamente os custos de produção.

Neste contexto, devido à importância dos APC na produção animal e à crescente restrição de uso destes compostos, o mercado da proteína animal está cada vez mais favorável a utilização de moléculas alternativas que possam substituir os antibióticos (PLUSKE *et al*., 2018).

1.3 Alternativas aos antibióticos promotores de crescimento na suinocultura

Diversas estratégias vêm sendo estudadas nas últimas décadas visando reduzir a infecção por *E. coli* e a severidade da DPD. Estratégias nutricionais como o fornecimento de dietas com baixo teor de proteína bruta ou altos teores de fibra bruta são duas das diversas alternativas. Estudos prévios demonstraram que um baixo teor de proteína na dieta reduziu significativamente a população de bactérias proteolíticas e de *E. coli* no trato digestivo dos leitões, além de reduzir os sintomas da DPD. Em contrapartida, os leitões que receberam dietas mais pobres em proteína apresentaram um desempenho inferior ao grupo que recebeu uma dieta com as recomendações adequadas para a fase (DÉGEN *et al*., 2007; HEO *et al*., 2010). No mesmo contexto, o uso de fibras na dieta pode ser uma das alternativas para melhorar a saúde intestinal dos leitões durante o período de desmame. O fornecimento de fibras insolúveis reduziu a contagem bacteriana no trato digestivo e a severidade dos sintomas da DPD em leitões desafiados por *E. coli* (MOLIST *et al*., 2010; JI *et al*., 2020). Nestes estudos, não foram observados efeitos significativos das fibras sobre o desempenho produtivo dos leitões.

Além da composição da dieta, diversas moléculas e compostos naturais também têm sido estudados como alternativas aos APC. Óleos essenciais demonstraram efeitos positivos ao reduzir os sintomas da DPD e contagem de bactérias indesejáveis no trato digestivo dos leitões (NAZZARO *et al*., 2013; ROSSI *et al*., 2020). Adicionalmente, nestes estudos, os autores observaram que a digestibilidade foi positivamente influenciada pelo fornecimento destes compostos via dieta, bem como a morfologia intestinal. Esses impactos positivos garantiram um incremento no desempenho em comparação aos leitões que não foram alimentados com óleos essenciais. O uso de extratos herbáceos ricos em polifenóis também apresentou resultados positivos na morfologia intestinal e na atividade de enzimas digestivas, além de reduzir a severidade da DPD em leitões desafiados por *E. coli* (VERHELST *et al*., 2010; DUBREUIL, 2013).

Outros aditivos alimentares como peptídeos antimicrobianos, anticorpos da gema do ovo e plasma sanguíneo também são compostos estudados visando minimizar o impacto da retirada dos APC. Contudo, apesar de contribuírem para redução dos sintomas da DPD e melhorar o desempenho produtivo, ainda existem algumas lacunas quanto a estabilidade destes compostos no trato digestório dos leitões (XIAO *et al*., 2015; WANG *et al*., 2016), seu alto custo para adoção em grande escala e a possibilidade de estarem contaminados com patógenos devido à sua origem animal (MINE *et al*., 2002). Além os aditivos alternativos supracitados, podemos citar os ácidos orgânicos. Estes compostos apresentam alta atividade antimicrobiana, reduzem o pH gástrico, aumentam a digestibilidade dos nutrientes e o desempenho produtivo, melhoram a morfologia intestinal e reduzem a severidade da DPD (TSILOYIANNIS *et al*., 2001; TUGNOLI *et al*., 2020).

1.4 Os ácidos orgânicos

Assim como os antibióticos, os ácidos orgânicos também apresentam propriedades antimicrobianas. Estas moléculas podem penetrar na parede celular bacteriana e interromper o seu funcionamento, especialmente em determinados gêneros de bactérias como *Salmonella* spp., *E. coli*, *Clostridia* spp. e *Listeria* spp. (SURYANARAYANA *et al*., 2014). Os dois primeiros, inclusive, de grande relevância para a suinocultura. Por definição, os ácidos orgânicos podem ser considerados ácidos “fracos”, compostos por no mínimo um grupo carboxílico (-COOH) e uma cadeia carbônica contendo de um a sete átomos de carbono (CANIBE *et al*., 2001). Esses ácidos compõem a categoria dos acidificantes fornecidos via dieta e apresentam excelente potencial antimicrobiano, além de características químicas específicas (GOUALIÉ *et al*., 2014; RATHNAYAKE *et al*., 2021). De acordo com a sua funcionalidade, GRILLI *et al*. 2012 dividiram os ácidos orgânicos em três principais categorias: ácidos graxos de cadeia curta (AGCC), ácidos graxos de cadeia média (AGCM) e ácidos tri carboxílicos (AT). A Tabela 1 apresenta uma síntese dos principais ácidos orgânicos dentro de seus grupos funcionais e suas características.

Há algumas décadas, os ácidos orgânicos são conhecidos devido às suas propriedades antimicrobianas. Inicialmente estes compostos foram utilizados para controlar a proliferação de bactérias e fungos em ingredientes utilizados para a alimentação animal (FRANK *et al*., 1994). Contudo, atualmente, os ácidos orgânicos vêm sendo utilizados como um aditivo na produção animal, segmento no qual têm desempenhado um papel de grande importância. Diversos estudos recentes demonstraram que esses compostos melhoram a digestão e a digestibilidade dos nutrientes, promovem a saúde intestinal e melhoram o desempenho dos animais de produção monogástricos (NDELEKWUTE *et al*., 2016; LONG *et al*., 2018).

Tabela 1. Lista dos principais ácidos orgânicos e suas propriedades. Adaptado de TUGNOLLI *et al.*, 2020

Categoria	Ácido	Fórmula molecular	Forma física	Odor	Sabor
Cadeia curta	Fórmico	CH_2O_2	Líquido incolor	Odor forte	-
	Acético	$C_2H_4O_2$	Líquido incolor	Odor forte de vinagre	Azedo e picante
	Propiônico	$C_3H_6O_2$	Líquido incolor oleoso	Odor forte e râncido	-
	Butírico	$C_4H_8O_2$	Líquido incolor oleoso	Odor râncido	Picante
Cadeia média	Capróico	$C_6H_{12}O_2$	Líquido oleoso incolor/amarelado	Odor de cabra	-
	Caprílico	$C_8H_{16}O_2$	Líquido oleoso incolor/amarelado	Odor de cabra	-
	Cáprico	$C_{10}H_{20}O_2$	Pó cristalino branco	Odor de cabra	-
	Láurico	$C_{12}H_{24}O_2$	Flocos brancos	Odor de louro	-
Tri carboxílicos	Cítrico	$C_6H_8O_7$	Pó cristalino branco/incolor	Sem odor	Picante
	Alfacetoglutárico	$C_5H_6O_5$	Pó cristalino branco	Sem odor	-
	Fumárico	$C_4H_4O_4$	Pó cristalino branco	Sem odor	Frutado
	Málico	$C_4H_6O_5$	Líquido ou pó cristalino branco	Sem odor	Maçã
Outros	Sórbico	$C_6H_8O_2$	Pó cristalino branco ou grânulos	Sem odor	Azedo e picante
	Benzóico	$C_7H_6O_2$	Pó cristalino incolor	Odor forte	Amargo
	Lático	$C_3H_6O_3$	Líquido viscoso branco/amarelado ou cristais	Sem odor	Picante

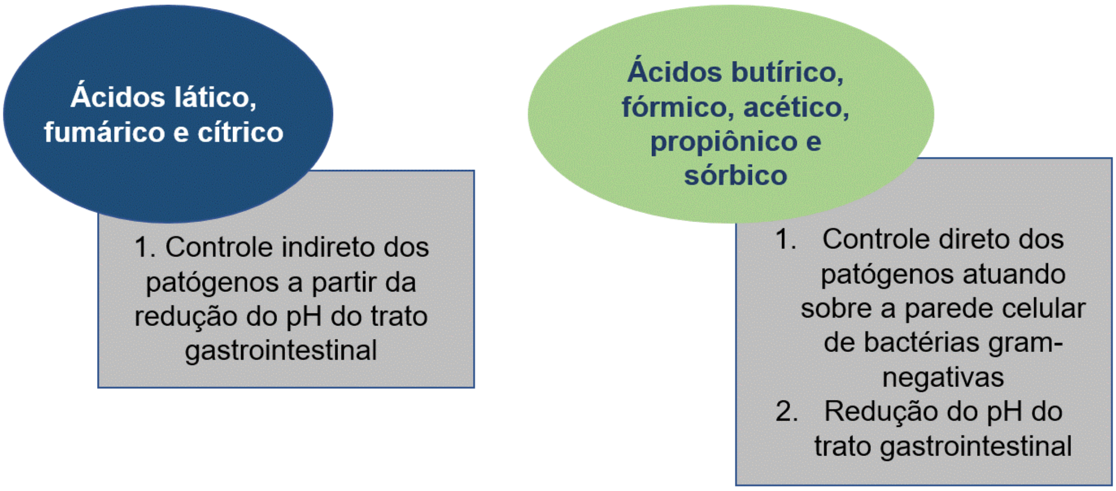
Em um cenário de substituição aos antibióticos, os ácidos orgânicos podem apresentar algumas vantagens. Estes compostos atuam inibindo, inclusive, o crescimento de bactérias multirresistentes, agindo contra a formação de biofilmes (GOUALIÉ *et al.*, 2014). Os APC comumente utilizados na produção animal são poucos eficazes – de maneira geral, contra a formação de biofilmes. Neste contexto, os ácidos orgânicos preenchem uma lacuna chave das restrições de uso dos APC – o desenvolvimento de resistência antimicrobiana a esses compostos. A bactéria *S. entérica* Typhimurium é o sorotipo mais comum encontrado em carcaças suínas na Europa, estando presente em 70% dos casos de contaminação pelo gênero (SUIRYANRAYNA E RAMANA, 2015). Este sorotipo é altamente persistente no ambiente e de difícil controle, e uma das formas de minimizar a contaminação e os riscos para os consumidores é controlar a disseminação da bactéria entre animais no ambiente produtivo.

LAN *et al*. (2018) constataram que o fornecimento de um blend de ácidos orgânicos via dieta para porcas gestantes reduziu a contagem de bactérias do gênero Enterobacteriaceae nas fezes das porcas e dos leitões logo após o nascimento e no desmame. No mesmo sentido, LYNCH *et al.,* (2017) observaram uma redução na excreção de *Salmonella* via fezes de suínos em crescimento alimentados com ácido cítrico. Estes achados demonstram que os ácidos orgânicos podem reduzir a excreção de *Salmonella* para o ambiente, minimizando a disseminação da bactéria e, consequentemente, a contaminação em larga escala.

1.5 Modos de ação dos ácidos orgânicos

Os ácidos orgânicos são os constituintes principais dos acidificantes, comumente utilizados na produção de rações para suínos, bovinos, coelhos, aves e peixes (LEVIĆ *et al*., 2007; CHWEN *et al*., 2013; LI *et al*., 2019). Estas moléculas comumente utilizadas como aditivos alimentares ocorrem naturalmente como produtos do metabolismo de plantas e organismos vivos, sendo constituintes naturais destes ou originados a partir do processo de fermentação microbiana no trato digestivo (GAUTHIER *et al*., 2002; NOWAK *et al*., 2020). Devido às suas origens, os ácidos orgânicos, de maneira geral, são muito bem tolerados e metabolizados (KIL *et al*., 2011), raramente ocasionando toxicidade aos animais. Apesar dos resultados positivos quanto à saúde e ao desenvolvimento do trato gastrointestinal como um todo (RATHNAYAKE *et al*., 2021), os mecanismos de ação dos ácidos orgânicos não foram completamente elucidados. Seus modos de ação são atribuídos a diversos fatores, como: a) redução do pH da digesta, especialmente no estômago, contribuindo para a digestão proteica; b) estímulo da produção e da atividade de enzimas endógenas e pancreáticas no intestino delgado; c) inibição do desenvolvimento de microrganismos patogênicos; d) redução da taxa de esvaziamento gástrico, promovendo maior saciedade; d) manutenção da integridade da mucosa intestinal; e f) quelação a minerais e estímulo do metabolismo intermediário (KIM *et al*., 2005; DE LANGE *et al*., 2010). Um resumo de dois diferentes mecanismos de ação propostos para os ácidos orgânicos pode ser observado na Figura 1.

Figura 1 – Dois mecanismos de ação propostos para os ácidos orgânicos sobre o pH do trato gastrointestinal e sua ação direta sobre os patógenos. Adaptado de DITTOE et al. (2018).



Alguns autores observaram que os ácidos orgânicos foram capazes de inibir o crescimento de microrganismos indesejáveis que apresentam sensibilidade à variação de pH do ambiente, como bactérias pertencentes à família Enterobacteriaceae, composta basicamente por bactérias gram-negativas como *E. coli* e *Salmonella* spp. (BARROW *et al*., 1977). No mesmo estudo, os autores constataram que os ácidos orgânicos não afetam negativamente a população microbiana benéfica do trato digestivo, como as bactérias ácidos láticas do gênero *Lactobacillus* spp. O uso de acidificantes compostos por 1% de ácido cítrico e 0,7% de ácido fumárico reduziu o pH estomacal de 4,6 para 3,5 em leitões desmamados, acidificando o ambiente e criando uma barreira para a entrada de bactérias no intestino (EASTER, 1988; PEARLIN *et al*., 2020). Nesse cenário, os ácidos orgânicos são considerados uma ferramenta interessante para controlar a disbiose intestinal ocasionada pela alta proliferação de microrganismos indesejáveis e que culmina na redução da população de lactobacilos – uma das problemáticas do período pós-desmame em suínos.

O espectro de ação e a eficácia dos ácidos orgânicos pode variar de acordo com a natureza do organismo-alvo, especificamente de acordo com a complexidade e a estrutura da parede celular e/ou membrana plasmática desse organismo (JAY *et al*., 2005). Bactérias gram-positivas como *Clostridium perfingens*, *Enterococcus* spp. e *Streptococcus* spp. são mais suscetíveis à ação de ácidos graxos de cadeia média, como os ácidos orgânicos capróico, caprílico, cáprico e láurico. Bactérias gram-negativas como *E. coli*, *Campylobacter jejuni* e *Salmonella* spp. são mais suscetíveis aos ácidos graxos de cadeia curta, como os ácidos acético, propiônico e butírico (GIOVAGNONI *et al*., KOVANDA *et al*., 2019). Nesse sentido, o entendimento de como agem os diferentes ácidos orgânicos sobre os patógenos que acometem os animais de produção torna-se fundamental. Essa compreensão irá auxiliar na tomada de decisão na escolha de um determinado composto, visando combater um patógeno específico no ambiente produtivo.

1.6 Desempenho produtivo e reprodutivo de porcas alimentadas com ácidos orgânicos

A produção moderna industrial de suínos está fortemente atrelada aos desempenhos produtivo e reprodutivo das porcas. Esses, por sua vez, determinam o número de leitões desmamados e, consequentemente, o número de animais que chegam à fase de terminação, culminando na quantidade de carne produzida (MIRILOVIĆ *et al*., 2016). Portanto, práticas de manejo, especialmente as nutricionais, que visem melhorar a performance das porcas durante a gestação e a maternidade podem ser determinantes para o sucesso de um lote (CHEN *et al*., 2019). Na alimentação de porcas gestantes, o fornecimento de ácidos orgânicos contribuiu para um maior ganho de peso diário dos leitões, determinando um maior peso ao desmame (ØVERLAND *et al*., 2009). No mesmo estudo os autores observaram que, durante a gestação, as porcas alimentadas com ácidos orgânicos ganharam espessura de toucinho em comparação ao grupo que não recebeu ácidos orgânicos, contribuindo para a manutenção do escore de condição corporal das fêmeas na lactação.

No mesmo sentido, LÜCKSTÄDT *et al*. (2011) observaram que porcas suplementadas com ácidos orgânicos apresentaram menor perda de peso durante a amamentação, além de menor perda de espessura de toucinho no mesmo período. Em contrapartida, VIEIRA *et al*. (2020) não constataram efeitos diretos do fornecimento de ácidos orgânicos sobre a condição corporal das porcas durante a gravidez e a lactação. Porém, a leitegada destas porcas apresentou tendência de ser maior (em número) e os leitões mais pesados ao desmame em comparação às porcas que não receberam o aditivo.

Achados como estes são promissores e indicam que os ácidos orgânicos apresentam potencial para contribuir com o desempenho produtivo e reprodutivo das porcas. Esses compostos podem atuar como promotores de crescimento quando fornecidos durante a gestação, auxiliando na manutenção da condição corporal das fêmeas e melhorando o desempenho produtivo dos leitões.

1.7 Desempenho produtivo de suínos alimentados com ácidos orgânicos

Diversos autores relataram que a inclusão de ácidos orgânicos e/ou seus sais na dieta de suínos foi capaz de contribuir para a melhoria do desempenho produtivo, resultados avaliados nas diferentes fases do ciclo produtivo (PARTANEN E MROZ, 1999; ALVARADO *et al*., 2013; RODRIGUES *et al*., 2020). Em contrapartida, alguns autores não observaram influência no desempenho produtivo de leitões alimentados com *blends* de ácidos orgânicos em relação ao grupo controle não alimentado (GRECCO *et al*., 2018; BOAS *et al*., 2016). Um estudo conduzido por KIARIE *et al*. (2018) comparou os efeitos do ácido benzoico com APC, tendo sido observado um efeito equivalente e estatisticamente igual entre os dois aditivos. Nesse estudo, concluiu-se que o ácido benzoico foi capaz de conferir a mesma performance em relação aos antibióticos, atuando como um promotor de crescimento no contexto experimental.

Além do desempenho produtivo dos leitões, alguns autores relataram a influência positiva do fornecimento de ácidos orgânicos sobre o escore fecal e a redução na incidência de diarreia em suínos na fase de creche. CHO *et al*. (2015) suplementaram leitões desmamados com um blend de ácidos orgânicos via dieta e observaram aumento significativo no desempenho produtivo em relação ao grupo não suplementado, além da melhoria do escore fecal dos animais.

No mesmo sentido, CALLEGARI *et al*. (2016) avaliaram o fornecimento de *blends* de ácidos orgânicos dietéticos no desempenho de leitões desmamados, não tendo sido observado efeitos do tratamento nestas variáveis. Contudo a incidência e a severidade da diarreia foram reduzidas no grupo alimentado com ácidos orgânicos. O fornecimento de ácidos orgânicos pode trazer benefícios aos suínos em diferentes fases, inclusive no período de crescimento e terminação. Alguns estudos testaram diferentes blends de ácidos orgânicos no desempenho produtivo de suínos nas fases de crescimento e terminação. A utilização desses blends foi capaz de melhorar a performance produtiva dos animais adultos (UPADHAYA *et al*., 2016; NYUGEN *et al*., 2018), inclusive melhorando a qualidade da carne (UPADHAYA *et al*., 2014) e o rendimento de carcaça (OVERLAND *et al*., 2000).

Em um cenário de restrições ao uso de APC da produção animal, alguns autores constataram que suínos alimentados com blends de ácidos orgânicos apresentaram desempenho produtivo similar aos animais que receberam antibióticos durante o período de crescimento e terminação (NGOC *et al*., 2020). No mesmo estudo foi avaliado o custo de produção atrelado ao fornecimento de ácidos orgânicos por kg de ganho de peso animal. A utilização desses compostos reduziu em US$ 0,078 os custos por kg em relação aos APC. Como conclusão tem-se que o blend de ácidos orgânicos poderia ser um substituto aos APC, reduzindo, inclusive, os custos de produção. Ainda, alguns autores discutiram que a inclusão dos ácidos fórmico e lático na dieta de suínos adultos contribuiu para o aumento da digestibilidade dos nutrientes (MROZ *et al*., 2000) e, também, para uma maior eficiência alimentar em comparação aos suínos não alimentados com ácidos orgânicos (JONGBLOED *et al*., 2000).

1.8 Efeitos dos ácidos orgânicos na morfologia intestinal

Alterações na morfologia intestinal são intrínsecas ao período de desmame para leitões. De acordo com PLUSKE *et al*. (1997) e MONTAGNE *et al*. (2004), o período de desmame é caracterizado por uma prolongada alteração estrutural e funcional do intestino delgado. Nessa fase, há encurtamento das vilosidades (atrofia) e aumento da profundidade das criptas. Estes fatores, aliados a baixa atividade de enzimas endógenas relacionadas à digestão, reduzem a capacidade digestiva, absortiva e secretória do trato gastrointestinal, contribuindo para a DPD (CAMPBELL *et al*., 2013). Além disto, metabólitos produzidos por bactérias gram-negativas, como a *E. coli*, acarretam inflamação intestinal, edemas na submucosa, necrose e ruptura das vilosidades (NOWAK *et al*., 2020), reduzindo a capacidade de absorção do intestino delgado. De acordo KAMADA (2011), quanto mais desenvolvidas forem as vilosidades intestinais, maior e mais eficiente será a absorção e a utilização dos nutrientes fornecidos via dieta. Dessa forma, o fornecimento de aditivos que possam mitigar alterações na morfologia intestinal é de extrema importância, especialmente na fase de desmame.

De acordo com DIAO *et al*. (2014), leitões alimentados com ácido benzóico apresentaram altura de vilosidades cerca de 20% superiores após 14 dias recebendo o ácido orgânico. Ainda, os autores observaram que os mesmos leitões tiveram a profundidade de cripta reduzida em 5 a 10% em comparação aos leitões não alimentados com estes compostos. Uma menor profundidade de cripta indica menor atividade mitótica na região, indicando boas condições de vilosidades, pois há menor atividade da cripta para produzir células novas visando repor o tecido das vilosidades (NABUURS *et al*., 1994). Estes resultados positivos na morfologia intestinal se relacionam com a inibição do desenvolvimento de microrganismos patogênicos no trato digestivo. Além disso, DIBNER E BUTTIN (2002) discutiram que os ácidos orgânicos podem influenciar diretamente a proliferação de células epiteliais devido à redução do pH e estímulo enzimático, aumentando a relação da altura das vilosidades e da profundidade das criptas. Estes fatores contribuem para vilosidades mais longas e funcionais, melhorando a saúde intestinal e garantindo resultados superiores no desempenho produtivo dos leitões (PLUSKE *et al*., 1997).

1.9 Revisão sistemática e meta-análise na ciência animal

Ferramentas como a revisão sistemática e a meta-análise apresentam como principais objetivos organizar e sumarizar informações de maneira a se obter respostas para um determinado problema ou questão (DOS SANTOS E CUNHA, 2013). A revisão sistemática é uma ferramenta reprodutível e de tendência imparcial. Se caracteriza por uma busca científica de metodologia e sistemática bem definidas, possibilitando a identificação de estudos publicados para responder uma questão de investigação. Nessa metodologia, avalia-se a qualidade dos trabalhos, extraindo dados e sintetizando seus resultados (FIGUEIREDO, 2014). As buscas em revisões sistemáticas de literatura são realizadas a partir de uma pergunta chave, considerando o método PICo, um acrônimo para População, Interesse e Contexto (SANTOS, 2007). A determinação destes objetos de pesquisa possibilita a execução da revisão sistemática que visa responder uma pergunta de interesse.

A fim de embasar conclusões quantitativas a respeito de uma pergunta de pesquisa, alguns pesquisadores utilizam do agrupamento de diversos estudos prévios (PEARSON, 1904). Esse tipo de metodologia iniciou no campo da medicina, se estendendo para experimentos agrícolas algumas décadas depois (YATES e COCHRAN, 1938). Atualmente, essa metodologia é denominada meta-análise. A meta-análise permite analisar estudos já realizados para se obter uma visão geral dos dados. No contexto da produção animal, possibilita a sumarização de resultados sem a necessidade da realização de um experimento in vivo, fato que se alinha às questões éticas quanto a experimentação e o uso de animais (LOVATO *et al*., 2007).

Além das questões éticas, na produção animal a meta-análise provou ser uma importante ferramenta que possibilita reavaliar dados publicados a fim de criar modelos empíricos, trazendo progresso no entendimento e na predição de diversos fatores (SAUVANT *et al*., 2020). Por ser uma ferramenta que possibilita a avaliação sistemática de dados previamente originados, a meta-análise pode incluir uma análise mais precisa dos resultados, trazendo a possibilidade da formulação de novas hipóteses que não seriam possíveis avaliando resultados de estudos individuais (LEAN *et al*., 2009).

No contexto experimental, a meta-análise é uma ferramenta importante que auxilia na otimização, direcionamento e planejamento de experimentos. Uma aplicação pode se dar a partir do levantamento dos melhores níveis de suplementação para um determinado composto a ser testado, por exemplo, direcionando o desenho experimental, dentre outros fatores pertinentes ao delineamento experimental e à otimização de recursos. No tocante da busca por alternativas aos promotores de crescimento, a utilização das ferramentas revisão sistemática e meta-análise possibilitam explorar dados disponíveis na literatura que avaliaram diferentes tipos e doses de ácidos orgânicos para suínos nas suas diferentes fases produtivas. Os achados oriundos de um estudo meta-analítico podem responder os efeitos destes compostos no desempenho produtivo e na morfologia intestinal dos animais, além de possibilitar desenhar um experimento com tipos e doses específicas de AO, melhorando o direcionamento de um experimento *in vivo*, por exemplo.

2. HIPÓTESES E OBJETIVOS

2.1 Hipóteses

a) O fornecimento de ácidos orgânicos de forma individual ou combinada melhora o desempenho produtivo de suínos nas fases de creche, crescimento e terminação;

b) O fornecimento de ácidos orgânicos de forma individual ou combinada melhora os desempenhos produtivo e reprodutivo de porcas gestantes e de suas leitegadas;

c) Os ácidos orgânicos podem atuar como promotores de crescimento para suínos de diferentes categorias, substituindo o uso dos APC.

2.2 Objetivos

Este trabalho teve como propósito avaliar sistematicamente as informações disponíveis sobre os efeitos dos ácidos orgânicos no desempenho produtivo e reprodutivo em suínos nas fases de creche, crescimento e terminação, gestação e maternidade, respectivamente.

[1]CAPÍTULO II

[1] Este capítulo corresponde ao artigo redigido e formatado no modelo da revista Animal.

Effects of organic acids on performance and intestinal morphology of piglets challenged by enteric bacteria: A systematic review and meta-analysis

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Abstract

The use of antibiotics growth promoting (AGP) in animal feed has been restricted, bringing the need for alternative substitute compounds. Organic acids (OA) have been used as substitutes for AGP, especially in the nursery phase. However, some results are still controversial. The present study was performed to evaluate the effects of OA on the performance and intestinal morphology of piglets challenged by enteric bacteria. The work consisted of a systematic review (qualitative data) followed by meta-analysis (quantitative data). Studies were searched using the keywords "*piglet*", "*pig*", "*organic acids*", "*challenge*", "*Escherichia coli*" and "*Salmonella*" in PubMed, Scopus, and Web of Science digital databases. The research question was proposed following the PICo-format (population: swine; intervention: organic acids supplementation; context: studies that evaluated enteric challenge). Obtained references (n: 1,774 from which 1,001 were duplicates) were assessed carefully considering the information presented in each title (532), abstract (195 removals) and complete article (21 removals). The final database included information collected from 24 studies published between 2001 and 2021. The impact of enteric challenge on piglet performance, as reflected in average daily feed intake (ADFI), average daily gain (ADG), and feed efficiency (FE) values, was not significant (P>0.05). However, additives demonstrated a significant influence on FE, with AO treatment exhibiting the highest FE, followed by antibiotics (ANT), while control (CON) had the lowest FE. Analysis of intestinal morphology revealed that the enteric challenge had no notable impact on duodenal parameters, but significant differences were observed among additive groups. Jejunal and ileal morphology showed significant interactions between the effects of additives and the presence or absence of enteric challenge. Specifically, the use of ANT or AO additives resulted in increased villus height (VH) in the jejunum and ileum compared to CON, indicating potential positive effects on intestinal health. The interactions between challenge and additive were significant for ileal VH and crypt depth (CD) ratio. We can conclude that OA are safe compounds and can be included in the diet of weaned piglets, both under enteric challenge and non-challenge conditions.

Keywords: Additives, Growth promoting, Intestinal Challenge, Nursery phase, Swine production

Implications

Escherichia coli and *Salmonella* spp. colonize piglet digestive tracts, causing economic losses. While antibiotics growth promoting (AGP) traditionally mitigated these bacterial impacts on animal production, restrictions on their use have emerged. Organic acids (OA), proposed as substitutes, presents some controversial results. This meta-analysis systematically examined published data to evaluate the impact of organic acids on the performance and intestinal morphology of piglets facing enteric bacterial challenges.

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) causes post-weaning diarrhoea (PWD), an intestinal disorder that has significant impacts on swine production (Li *et al*., 2020). In the same context, *Salmonella* spp. also colonizes the digestive tract of piglets and reduces their performance (Fabà *et al*., 2019). Additionally, Salmonella is a concern in human health. Several cases of human gastroenteritis are recorded every year, and many of them are related to the consumption of pork or pork products (EFSA, 2015). *Escherichia* and *Salmonella* bacteria belong to the Enterobacteriaceae (enteric bacteria) family and have several common characteristics. Among them is its ability to cause a wide range of infections in humans and animals (Carter & Chengappa, 1990).

For many years, sub-therapeutic doses of antibiotics have been administered with success to reduce the negative impact of enteric bacteria on piglet performance (Liu *et al*., 2016). In the last few decades, the use of these compounds has been called into question. In particular, because of the risk of bacterial resistance, bringing an urgent need to reduce antibiotic usage in animal production (Lynch *et al*., 2017). Therefore, alternative strategies that can promote gut health and growth performance in pigs is key.

In swine industry, alternative compounds as organic acids and its salts are used in nursery piglets' diets to replace antibiotics. These molecules have acidifying properties and diverse modes of action. Organic acids can ensure nutrient digestion by lowering the intestinal pH and counteracting the buffering capacity of diet (Heo *et al*., 2013). In addition, organic acids such as citric acid, fumaric acid, lactic acid or formic acid decrease the stomach pH and its bactericidal effect is imparted such the growth performance, gut health and intestinal morphology of pigs are significantly improved (Tsiloyiannis *et al*., 2001; Pettigrew, 2006).

Several studies to assess the effects of organic acids and its salts on piglet performance and gut health have been carried out. The results produced are different and, although they encompass a large amount of information, they are not conclusive in some respects. Therefore, more information is required to better understand the variations in those findings. In this context, a meta-analytical study was performed to investigate the effects of organic acids on productive performance and intestinal morphology of piglets experimentally challenged by enteric bacteria.

Material and methods

Search strategy and selection criteria

A database was created using data from published scientific papers detailing the incorporation of organic acids or their salts into piglet diets subjected to experimental challenges with enteric bacteria (i.e., *Escherichia coli* or *Salmonella* spp.). A secondary database was formed by selecting scientific papers that assessed both growth performance and the intestinal morphology of piglets under the same experimental conditions. Following the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement guidelines (Moher *et al*., 2009), a systematic review was conducted. Searches were performed on digital databases (PubMed, SciELO, ScienceDirect, and Scopus) between July and October 2022, utilizing combined keywords listed in Table 1. The review question formulated using the PICo strategy indicating 'Population,' 'Intervention,' and 'Context,' was defined as follows: Population - piglets, Intervention - organic acids and their salts, Context - experimental challenge by enteric bacteria. Results from each digital database were exported to the bibliographic manager EndNote X9 (Clarivate Analytics, CA) for the selection process and database construction.

The selection process, in line with PRISMA methodology, was meticulously documented, excluding duplicate records. Titles and abstracts of selected scientific papers were reviewed to identify papers for full assessment. The final database comprised complete articles meeting the specified eligibility criteria: 1) studies published in indexed journals (excluding short communications, reviews, or conference abstracts/papers); 2) *in vivo* experiments with nursery-phase pigs; 3) performance evaluation (e.g., feed intake, body weight gain, feed efficiency); 4) experimental challenge by enteric bacteria (*Escherichia coli* or *Salmonella* sp.); 5) dietary supplementation with organic acids or their salts.

Data extraction

The data from chosen scientific papers was compiled into an electronic spreadsheet. The dataset included a column for qualitative information (author, journal, year, country of origin, piglet strain, diet type, presence/absence of challenge, treatment name, type of organic acid) and columns for quantitative exploratory and descriptive variables (number of replications, number of animals, age, experiment duration, organic acid dose, performance parameters).

The methodology for constructing the database and encoding data followed established literature guidelines (Sauvant *et al*., 2008). Each selected paper was assigned a unique code (ID) as a moderating variable in the analysis, accounting for variability between scientific papers. Additional codes were assigned for qualitative comparisons, such as treatments (1 to 3) and the presence or absence of challenge (+ or -). Treatments were organized into a factorial arrangement (3x2), incorporating three additive factors (CON = control with no additive; ANT = antibiotics; AO =

organic acids) and two challenge factors (presence (+) or absence (-) of enteric bacterial challenge). The composition of experimental diets was assessed and compared with NRC recommendations (NRC, 2012) for the nursery phase, categorized into three groups for each nutrient in the database: above NRC recommendations, meeting NRC recommendations, and below NRC recommendations.

Statistical analysis

The analysed variables encompassed performance responses (average daily feed intake, average daily gain, and calculated feed efficiency ratio - ADFI, ADG, and FE, respectively) and intestinal morphology (villus height, villus crypt depth, and villus:crypt ratio - VH, CD, and V:C, respectively), considering the presence or absence of enteric challenge and additives (no additives, antibiotics, or organic acids). Data were examined in two formats: raw data (as presented in the original papers) and relativized information (responses of antibiotic and organic acid-supplemented treatments were relativized to the respective control treatment and expressed as a percentage of variation). The adoption of the second procedure aimed to mitigate variations among the raw data from different experiments included in the database. Variance analysis was conducted based on the following statistical model:

$$\boldsymbol{Y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{\tau_i} + \boldsymbol{\beta_j} + (\boldsymbol{\tau\beta})_{\boldsymbol{ij}} + \boldsymbol{\alpha_k} + \boldsymbol{\varepsilon_{ijk}}$$

in which $\boldsymbol{Y_{ijk}}$ is the response, $\boldsymbol{\tau_i}$ is the fixed effect of treatment (control or additive), $\boldsymbol{\beta_j}$ is the fixed effect of enteric challenge, $(\boldsymbol{\tau\beta})_{\mathbf{ij}}$ is the interaction effect between $\boldsymbol{\tau_i}$ and $\boldsymbol{\beta_j}$, $\boldsymbol{\alpha_k}$ is the random effect of study, and $\boldsymbol{\varepsilon_{ijk}}$ is the residual variation.

R 4.2.1 (R Core Team, 2022) statistical software was employed for the statistical procedures. Data were submitted to analysis of variance using lme4 (Fitting Linear Mixed-effects models), lmerTest (ANOVA Tables for Linear Mixed Models), and emmeans (Estimated Marginal Means for Linear, Generalized Linear, and Mixed Models) packages for linear mixed-effects models. Interaction between factors (additive and challenge) was assessed using General Linear Mixed Models (GLMM). Statistical models comprised fixed effects of the challenge factor (presence or absence) and additive factor (control, antibiotic, organic acid), along with the random effect of the study (based on the single code ID). The choice of linear mixed model methodology was based on its suitability for developing empirical modelling on biological responses in experimental designs. Analysis of variance relied on p-values, with a significance criterion set at p-value < 0.05 for each variable. When p-values fell between 0.05 and 0.10, it was considered that the effect tended to be significant.

Results

Study selection process

A total of 1,774 scientific papers were retrieved from the digital search into four different databases: Web of Science (69 papers), Scopus (1,002 papers), Pubmed (689 papers) and SciELO (14 papers). Starting the selection process, 1,001 scientific papers were removed before screening due to duplicity, 773 studies were screened by title, 241 studies were screened by abstract, and 46 studies were assessed by a full-text reading, i.e., they met the eligibility criteria. Details from systematic review and PRISMA flow diagram are shown in Figure 1. During the full-text analysis, 21 scientific papers were excluded by different reasons such as no performance information, results showed in charts and the presence of other additives combined with organic acids. As the result, a total of 24 scientific papers were selected to compose the meta-analysis performance database. Of these 24 papers, 18 evaluated the intestinal morphology of piglets supplemented with organic acids and challenged by enteric bacteria, which were selected to compose a second database analysed in the intestinal morphology meta-analysis.

Database description

Table 2 presents key data points extracted for the database, such as first author, publication year, country, challenge type, number of animals, organic acid or salt type, dosage, presence or absence of antibiotics, and form of organic acid or salt administration. The database comprises 24 scientific papers published between 2001 and 2021, distributed across 15 peer-reviewed journals. Predominant publication journals include the Journal of Animal Science (28% of papers), Animal Feed Science and Technology (12%), Asian-Australasian Journal of Animal Sciences, Archives of Animal Nutrition, Journal of Animal Physiology and Animal Nutrition, and Animals (8% each). Other contributions came from journals like Translational Animal Science, Polish Animal Science, Preventive Veterinary Medicine, and Livestock Science. The geographic distribution of studies can be seen in Figure 2, with a notable concentration in China (20% of studies), followed by Brazil and Korea (12% each), and contributions from Italy and Australia (8%). Other countries, including Spain, United States, Canada, Ireland, Greece, Netherlands, and Denmark, collectively contributed 40%.

The database, detailed across 192 lines in the electronic spreadsheet, encompasses a total of 4,333 piglets. Genetic lines were predominantly Landrace x Large-White (40%), with DanBred x PIC 337 accounting for 34%, and the remaining 26% representing various genetic lines. Unsexed piglets were employed in the majority of studies (45%), while 44% used males, and 11% did not specify the gender. The average age of piglets at the beginning of experiments was 23 days, though 31% of articles did not specify the age of the animals. The primary organic acid or salt types comprised mixtures or blends (52%), benzoic acid (24%), sodium butyrate (12%), and other variations such as potassium and calcium diformate, citric acid, fumaric acid, lactic acid, and formic acid (12% collectively). Table 3 presents a descriptive analysis of the database, indicating an average organic acid dosage of 0.22%, ranging from 0.1% to 2%. Solid administration was prevalent (76%), with liquid or a combination of liquid and solid present in only 8% of papers each. Antibiotics were omitted in most of the treatments (56%), with 44% comparing these compounds with organic acids. Chlortetracycline was the most frequently used antibiotic (20%), followed by Apramycin, Carbadox, Colistin, Kitasamycin, and Zinc Bacitracin (10% each), while a variety of other antibiotics and combinations constituted the remaining 30%.

Common ingredients in experimental diets included corn (99%), soybean meal (93%), porcine plasma spray (63%), and whey powder (46%). Alternative ingredients like barley meal (23%), fish meal (19%), and wheat (17%) were observed in varying proportions. Approximately 7% of studies did not disclose diet composition. Dietary nutrient content, including metabolizable energy, amino acids, calcium, and phosphorus, was examined, with only 11% of studies presenting laboratory-analysed values. As such, comparisons were based on calculated or included nutrient levels. Table 4 shows NRC recommendations for the nursery phase with the average composition of experimental diets. Metabolizable energy fell below NRC recommendations in 55% of studies, while 45% exceeded them. Amino acids lysine, methionine + cysteine, and threonine were predominantly below NRC recommendations (47%, 61%, and 63%, respectively), whereas methionine and tryptophan exceeded recommendations in 72% and 63% of studies. Mineral levels, including total phosphorus, available phosphorus, and calcium, generally surpassed NRC recommendations (60%, 80%, and 49% of studies, respectively). Only 9% of studies presented diets aligning with NRC recommendations, indicating a prevailing deviation from the established nutritional guidelines.

Performance responses

Figure 3 illustrates the percentage variations in ADFI, ADG, and FE concerning the control group among challenged piglets receiving antibiotics or organic acids. Notably, improvements in ADFI were observed in 71% and 66% of studies for the ANT and AO treatments, respectively, compared to the CON group. ADG exhibited an increase in 71% and 73% of observations relative to CON for ANT and AO treatments. Finally, FE showed improvement in 79% and 78% of observations when comparing CON to ANT and AO, respectively.

Table 5 provides a concise overview of the impact of enteric challenge, antibiotics, or organic acids on piglet performance. Importantly, there is no significant difference in ADFI, ADG, and FE values between piglets subjected to bacterial challenge (CON+) and those not challenged (CON-) (C; p = 0.5734, p = 0.6073, and p = 0.7422, respectively). Additives demonstrated no significant influence on ADFI (A; p = 0.7651), although a trend towards increased ADG was observed (A; p = 0.0850), and a significant impact on FE was evident (A; p = 0.0374). Notably, AO treatment exhibited the highest FE, regardless of the challenge, followed by ANT treatment, with CON presenting the lowest FE. No significant interactions were noted between the challenge and additive in the evaluation of ADFI, ADG, and FE (CxA; p = 0.8970, p = 0.9049, p = 0.9812, respectively).

Effects on intestinal morphology

The analysed segments include the duodenum, jejunum, and ileum. Table 6 summarizes these results. The enteric challenge had no notable impact on duodenal villus height (VH), crypt depth (CD), and villus-to-crypt (VC) ratio (C; p = 0.3478, p = 0.7381; p = 0.1934, respectively). In contrast, variations across all morphological parameters (VH, CD, VC ratio) were observed among the additive groups (CON, ANT, OA), with consistently low p-values ($p < 0.05$). VH was notably higher in the ANT and OA groups compared to the CON group (A; p = 0.0043). CD, similarly, to VH, demonstrated significant additive-related effects, with piglets in the OA group showing a desired lower CD compared to the CON and ANT groups (A; p = 0.0169). The VC ratio exhibited significant differences among additives, with the OA group leading to a higher VC ratio compared to the ANT group (A; p = 0.0084). The interaction between enteric challenge and additives (CxA) did not show significant effects on VH, CD, or VC ratio (CxA; p = 0.8371, p = 0.7653, p = 0.6682, respectively).

Jejunal VH exhibited no significant difference between CON- and CON+ groups (C; p = 0.4575). A notable decrease in CD was observed when piglets were challenged, with the CON- group presenting higher CD values compared to CON+ (C; p = 0.0281). The VC ratio showed a tendency to be influenced by the challenge (C; p = 0.0584). The incorporation of additives (ANT and OA) resulted in a significant increase in VH compared to CON (A; p = 0.0281). No significant difference in CD was noted between groups with different additives (A; p = 0.6496). However, the use of ANT or OA additives did not lead to significant differences in VC ratio compared to the CON group (A; p = 0.1931). The interaction between challenge and additive on VH and VC ratio was not significant (CxA; p = 0.1491, p = 0.5486, respectively). Conversely, when evaluating CD, a tendency of significant interaction between these factors was observed (CxA; p = 0.099). These findings suggest that the impact of additives on jejunal morphology may vary depending on whether piglets are challenged with enteric bacteria or not.

The ileal VH values indicate a significant difference between CON+ and CON- groups (C; p = 0.0023), as well as between different additives. The challenged groups (CON+ and ANT+) show a lower VH compared to non-challenged groups (CON- and ANT-). There is a significant difference in CD between the challenged and non-challenged groups (C; p = 0.0418). The challenged groups have a higher CD compared to the non-challenged groups. The VC ratio shows no significant difference between the challenged and non-challenged groups (C; p = 0.3911). Additives significantly affected VH (p = 0.004), with the AO- group exhibiting the highest VH value. CD exhibited a tendency to be influenced by additives (A; p = 0.0617), while the VC ratio values showed no significant influence from additives (A; p = 0.1659).

Significant interactions (CxA; $p < 0.001$; $p = 0.0226$) were noted in VH and VC ratio, indicating that the effects of various additives on these parameters are influenced by the presence or absence of enteric challenge. Conversely, the interaction between challenge and additive in CD exhibited a trend towards significance but did not reach statistical significance (CxA; $p = 0.0846$).

Discussion

Performance and feed efficiency improvement

Organic acids are commonly used for newly weaned piglets due to their ability to reduce coliform counts and increase *Lactobacillus* in the gastrointestinal tract (Namkung *et al*., 2004; Gong *et al*., 2008), promoting a balanced microbiota. These findings are particularly relevant, highlighting the antibacterial efficacy of these compounds, especially in the context of exposure to enteric bacteria and health challenges. In our meta-analysis of 24 scientific papers, we observed that enteric challenges did not impact the feed efficiency of piglets compared to the unchallenged group. This contrasts with findings from other studies indicating that sanitary challenges from *Escherichia coli* can increase feed intake and reduce piglet weight gain, consequently decreasing feed efficiency (Canibe *et al*., 2008; Han *et al*., 2020). In our study, both average daily feed intake and average daily gain remained unaffected by the challenge or the presence of additives. These results align with prior research (Silveira *et al*., 2018; Fabà *et al*., 2019), where, despite the absence of statistically significant differences between treatments, piglets supplemented with antibiotics or organic acids demonstrated numerically superior ADFI and ADG compared to the control group. This observed trend in our meta-analysis reinforces these previous findings.

When examining the feed efficiency of piglets, both in unchallenged and challenged groups, a notable trend emerges favouring organic acids over antibiotics. This aligns with the findings reported by Gebru *et al*. (2010), who similarly observed enhanced feed efficiency with organic acids in piglets. Notably, in the context of enteric challenge, antibiotics and organic acids demonstrated comparable effectiveness. In contrast, the same study revealed that average daily feed intake and average daily gain were influenced by additives, with antibiotic treatments and organic acids exhibiting equivalent impacts. These results differ from the outcomes revealed in our meta-analysis. In a study conducted by Lynch *et al*. (2017), the comparison among control, antibiotics, and organic acids did not yield significant differences in ADFI or feed conversion ratio (FCR) among challenged piglets. However, additive effects were evident in ADG, with superior outcomes observed in the antibiotic treatment group.

While not impacting ADFI or ADG regardless of the challenge scenario, it is evident that organic acids have the potential to match or even surpass the efficacy of antibiotics in ensuring feed efficiency, even in the presence of enteric challenges. Moreover, the absence of a significant reduction in ADFI in the organic acid treatment suggests that these compounds do not compromise the palatability of the feed. These observations agree with the findings discussed by Jessica *et al*. (2018), who, in their evaluation of palatability and feed intake with different organic acid supplements, similarly reported no significant impact on the ADFI of piglets, aligning with our results. In this meta-analysis, most studies (52%) administered organic acid blends to piglets. Individual analyses of each organic acid blend were constrained due to variations in their proportions and compositions. The diverse composition of the blends posed challenges for a detailed compositional analysis. Nevertheless, multiple studies indicate that the synergistic combination of organic acids is more effective than individual components (Li *et al*., 2008; Upadhaya *et al*., 2016; Lei *et al*., 2017).

Variability in the performance outcomes of challenged piglets, as revealed in this meta-analysis, can be attributed, in part, to the diverse experimental factors across the included studies. These factors encompass variations in the type of organic acid employed, dosage levels, assorted combinations, and other variables. A more comprehensive discussion of these issues will be provided in the limitations section of the paper.

Assessing the impacts on intestinal morphology

Intestinal morphology plays a crucial role in animal health, serving as a significant mechanical defence for intestinal barrier functions (Montagne *et al*., 2004). In this current investigation, the enteric challenge significantly impacted villus height (VH), crypt depth (CD), and the villus-to-crypt (VC) ratio across various intestinal segments. These observations are consistent with earlier research that underscored the detrimental effects of an *Escherichia coli* challenge on analogous intestinal segments (Silveira *et al*., 2018). As noted by Swidsinski *et al*. (2002), enteric bacteria, including *Escherichia coli*, produce toxins that compromise the morphological integrity of the small intestine, resulting in detrimental effects on intestinal morphology. In this context, the challenge also influenced CD in the jejunal and ileal segments. The observed increase in CD across various small intestine segments during the enteric challenge in our study corresponds with findings discussed in previous studies by other authors (Silveira *et al*., 2018; Han *et al*., 2020). Therefore, our results support the existing body of evidence on the impact of enteric challenges on intestinal morphology.

Alterations in the intestinal morphology of weaned piglets are a common occurrence and may be linked to the natural aging process characterized by periodic renewals of the intestinal epithelium (Rodrigues *et al*., 2020). In our study, the challenged group receiving antibiotics or organic acids exhibited the highest VH values, surpassing even the control groups. Notably, these groups exceeded the VH of the unchallenged control group. According to Bosi *et al*. (2007), the stress associated with weaning leads to a reduction in the intestinal morphology of piglets. This reduction is attributed to decreased food consumption in the initial post-weaning weeks, resulting in diminished VH and increased CD. In our study, the enteric challenge had a negative impact on intestinal morphology. However, in the challenged groups receiving additives, whether antibiotics or organic acids, the detrimental effects were mitigated. The presence of additives proved effective in countering the challenges to the extent that villus height exceeded that of the non-challenged control group. In this context, dietary additives demonstrated a positive influence on the epithelial development of the intestine, regulating the reproduction of pathogenic microorganisms and mitigating the adverse effects of enteric bacteria proliferation in the region (Rodrigues *et al*., 2020).

Database limitations

Limited information availability prevented certain additional analyses. Approximately 52% of the studies included diverse combinations of organic acids, known as blends. Due to significant variability in the proportions and combinations of these compounds, assessing the effects solely through blend comparisons proved unfeasible. Furthermore, the variety of antibiotics used posed challenges for direct comparisons with organic acids. Understanding the impact of different doses and types of organic acids is crucial, but only a few studies have explored this aspect concerning the productive performance and intestinal morphology of challenged piglets. The distinct chemical properties of organic acids contribute to the complexity of comparisons, making it challenging to analyse blends or individual compounds. Additionally, responses to different doses are not consistently predictable, as noted by Ferronato *et al*. in 2020. Including various doses in studies could facilitate regression analysis, providing valuable dose-response data for these compounds. Finally, the inoculation dose of enteric bacteria during the challenge varied significantly among studies, with many failing to report this information. These information gaps present challenges in conducting more in-depth analyses to comprehensively understand how these components influence performance and intestinal morphology in the context of enteric challenges.

Organic acids as an alternative to antibiotics: productive performance

Similar to antibiotics, organic acids exhibit antimicrobial activities. Due to the increasing restrictions on the use of AGP in animal production, compounds such as organic acids are increasingly employed to achieve optimal production rates in swine farming. Previous studies have suggested that organic acids are comparable to antibiotics in evaluating variables such as ADFI, ADG, and FCR (Weber *et al*., 2007; Bhandari *et al*., 2008; Choi *et al*., 2020). Notably, neither the antibiotics nor the organic acids groups differed significantly from the control in the studies under consideration. These findings align with our work, as no discernible differences were observed between treatments when assessing ADFI and ADG.

However, the treatments did impact FE. In our study, the FE of organic acids exceeded that of antibiotics, consistent with previously published findings (Tsiloyiainnis *et al*., 2002; Gebru *et al*., 2010). Therefore, in the context of this study, organic acids demonstrated effectiveness in replacing antibiotics under conditions of enteric challenge and in the absence of challenge, ensuring efficient feed utilization.

Conclusion

Organic acids are added to piglet diets to mitigate the negative effects of post-weaning diarrhea (PWD). Their effects and mechanisms are varied, including the reduction of digesta and gastric pH, as well as the inhibition of the proliferation of pathogenic microorganisms. These interactions collectively improve feed efficiency and piglet intestinal morphology, even in the presence of prevalent sanitary challenges in the production environment. Consequently, organic acids show promise as alternatives to antibiotic growth promoters (AGP).

Data and model availability statement

Data and models related to this published article are not deposited in any official repository. Information may be made available by the authors upon request.

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Table 1

Search terms for PICo methodology: Population, Intervention and Context used in the systematic review

Acronym	Search string
Population (P)	swine OR pig OR pigs OR piglet OR piglets OR "weaned pig" OR "weaned piglet" OR suckling OR "sucking pig" OR "suckling piglets" gilt OR gilts OR barrow OR barrows
Intervation (I)	"organic acid" OR "organic acids" OR "organic acid salt" OR "acetic acid" OR "sodium acetate" OR "formic acid" OR "sodium formate" OR "propionic acid" OR "sodium propionate" OR "butyric acid" OR "sodium butyrate" OR "malic acid" OR "sodium malate" OR "lactic acid" OR "sodium lactate" OR "citric acid" OR "sorbic acid" OR "sodium sorbate" OR "potassium sorbate" OR "calcium sorbate" OR "fumaric acid" OR "sodium fumarate" OR "benzoic acid" OR "sodium benzoate"
Context (Co)	salmonella OR escherichia OR "escherichia coli" OR ETEC OR challenge OR challenged

Table 2

Meta-analysis database description

Reference	First author	Year	Country	Challenge	Animals (n)	Organic acid/salt	Dosage (%)	Antibiotic	Form
1	Li	2008	China	Escherichia coli	120	blend, potassium diformate	0.5	yes	solid
2	Bosi	2007	Italy	Escherichia coli	60	calcium formate	1.2	no	solid
3	Halas	2009	Australia	Escherichia coli	48	benzoic acid	0.5	no	solid
4	Walsh	2012	USA	Salmonella sp.	88	blend	2.58 mL/L	yes	liquid
5	Ahmed	2014	Korea	Escherichia coli	60	blend, citric acid	0.5	yes	solid
6	Silveira	2018	Brazil	Escherichia coli	224	benzoic acid	0.5 to 0.75	no	solid
7	Rodrigues	2020	Brazil	Escherichia coli	270	benzoic acid	0.5	yes	solid
8	Barba-Vidal	2017	Spain	Salmonella sp.	78	sodium butyrate	0.21	no	solid
9	Bhandari	2008	United States	Escherichia coli	108	blend	-	yes	-

10	Choi	2020	Canada	Escherichia coli	24	blend	2	yes	solid
11	Mohana Devi	2015	Korea	Escherichia coli	120	blend	0.2	yes	solid
12	Fabà	2019	Netherlands	Salmonella sp.	32	blend	0.4	no	solid
13	Gebru	2010	Korea	Salmonella sp.	108	blend	0.2	no	-
14	Han	2020	China	Escherichia coli	150	blend	0.2 to 0.8	yes	solid
15	Lei	2017	Korea	Escherichia coli	30	blend	0.2 to 0.3	no	solid
16	Lynch	2017	Ireland	Salmonella sp.	50	sodium butyrate, benzoic acid, blend	0.3 to 0.4	no	solid
17	Matsumoto	2021	China	Escherichia coli	108	fumaric and lactic acid	1	no	-
18	Pu	2018	China	Escherichia coli	30	benzoic acid	0.3	yes	solid
19	Ruggeri	2017	Italy	Salmonella sp.	1373	blend	1	no	liquid/solid
20	Pluske	2021	Australia	Escherichia coli	72	blend	1.2	no	solid
21	Tsiloyiannis	2001	Greece	Escherichia coli	240	citric and lactic acid	1.5 to 1.6	yes	-

22	Weber	2008	United States	Escherichia coli	108	sodium butyrate	0.2	yes	solid
23	Weber	2014	United States	Escherichia coli	448	butyric acid	2	no	solid
24	Canibe	2008	Denmark	Escherichia coli	160	formic acid	0.2	no	solid

* - no information

Table 3

Descriptive analysis of meta-analysis database

Item	n	Min	Max	Mean	SD
Piglets	192	24	1.373	162	260
Organic acid dose, %	145	0.10	2.00	0.22	0.44
Ingredient composition of diets					
Barley meal, %	44	10.00	31.17	19.89	8.07
Corn, %	190	8.20	65.5	41.71	13.91
Fish meal, %	36	2.67	8.41	5.17	1.66
Spray porcine plasma, %	122	0.00	15.00	4.05	4.22
Soybean meal, %	178	5.00	30.03	19.02	5.92
Wheat, %	32	4.80	69.40	35.42	24.35
Whey powder, %	89	3.03	25.00	10.69	6.09
Nutrient composition of diets					
Crude protein, %	189	14.30	24.00	19.83	2.20
Metabolizable energy, kcal/kg	176	2,425	4,206	3,365	330
Lysine, %	187	0.78	1.63	1.30	0.21
Methionine, %	187	0.30	0.70	0.49	0.12

Methionine + Cysteine, %	176	0.48	1.00	0.70	0.14
Threonine, %	101	0.50	1.08	0.79	0.15
Tryptophan, %	98	0.17	0.32	0.25	0.04
Performance					
Average daily feed intake, g/day	192	204	798	597	42.18
Average daily gain, g/day	192	117	582	356	18.29
Feed efficiency, g/g	192	0.37	0.89	0.67	0.28
Intestinal morphology					
Villi height, µm	134	233.10	512.00	334.92	63.61
Crypt depth, µm	134	229.91	355.17	243.13	86.62
Villi : Crypt ratio, µm	134	0.76	1.90	1.28	0.54

Table 4

NRC nutritional recommendations for nursery phase and nutritional meta-analysis average numbers

Nutrient	NRC recomendation	Studies average	Below average	Above average
Metabolizable energy, kcal/kg	3,400	3,365	3,182	3,571
Lysine, %	1.35	1.30	1.14	1.45
Methionine. %	0.39	0.49	0.35	0.54
Methionine + Cysteine. %	0.74	0.70	0.62	0.78
Threonine. %	0.79	0.79	0.66	0.86
Tryptophan. %	0.22	0.25	0.19	0.25
Total phosphorus. %	0.65	0.67	0.55	0.71
Available Phosphorus. %	0.40	0.51	0.37	0.51
Calcium. %	0.80	0.81	0.67	0.88

Table 5

Performance of piglets challenged by enteric bacteria and supplemented with dietary antibiotics or organic acids

	Treatment										
Bacterial challenge[1] (C)	-	+	-	+	-	+	*SEM²*	*P-value*[*]			R^2
Additive (A)	Control		Antibiotic		Organic acid			C	A	C x A	
Average daily feed intake, g/day	622	629	600	661	643	596	19.27	0.5734	0.7651	0.8970	60%
Average daily gain, g/day	413	327	397	373	410	364	29.33	0.6073	0.0850	0.9049	59%
Feed efficiency, g/g	0.57 b	0.51 b	0.64 ab	0.62 ab	0.73 a	0.69 a	0.07	0.7422	0.0374	0.9812	63%

[1]Weaned pigs under enteric bacterial challenge with *Escherichia coli* or *Salmonella spp*.

[4]Standard Error of the Mean (SEM)

[*]Probability of fixed effetcs: presence or absence of challenge/additive and its interaction.

Means followed by different letters differ statistically at the level 5% of significance.

R^2: Coefficient of determination of the General Linear Model of Mixed Effetcs

Table 6

Intestinal morphology of piglets challenged by enteric bacteria and supplemented with dietary antibiotics or organic acids

	Bacterial challenge[1] (C)	-	+	-	+	-	+	*SEM*[2]	*P-value*[*]			R^2
	Additive (A)	Control		Antibiotic		Organic acid			C	A	C x A	
Duodenum	Villi height, µm	444 b	415 b	497 a	461 a	482 a	459 a	65.58	0.3478	0.0043	0.8371	94%
	Crypt depth, µm	329 a	463 b	343 ab	444 b	287 a	291 a	44.43	0.7381	0.0169	0.7653	98%
	Villi : Crypt ratio, µm	1.65 a	0.91 b	1.46 ab	1.08 b	1.63 a	1.52 ab	0.09	0.1934	0.0084	0.6682	91%
Jejunum	Villi height, µm	321 ab	301 b	332 a	343 a	337 a	345 a	14.81	0.4575	0.0281	0.1491	83%
	Crypt depth µm	337 ab	332 a	341 ab	325 a	366 b	329 a	16.09	0.0007	0.6496	0.0099	96%
	Villi : Crypt ratio, µm	1.01	0.93	0.99	1.08	0.96	1.05	0.13	0.0584	0.1931	0.5486	93%
Ileum	Villi height, µm	243 b	239 b	266 b	262 b	303 a	254 b	9.34	0.0023	0.0004	<0.001	67%
	Crypt depth, µm	250 B	238 A	247 B	228 A	246 B	235 A	31.36	0.0418	0.0617	0.0846	94%
	Villi : Crypt ratio, µm	0.98 b	1.01 b	1.09 ab	1.18 ab	1.25 a	1.11 ab	0.27	0.3911	0.1659	0.0226	96%

[1]Weaned pigs under enteric bacterial challenge with *Escherichia coli* or *Salmonella spp*.

[4]Standard Error of the Mean (SEM)

[*]Probability of fixed effetcs: presence or absence of challenge/additive and its interaction.

Means followed by different letters differ statistically at the level 5% of significance.

R^2: Coefficient of determination of the General Linear Model of Mixed Effetcs

Fig. 1. Flowchart for searching progress – PRISMA Flow Diagram

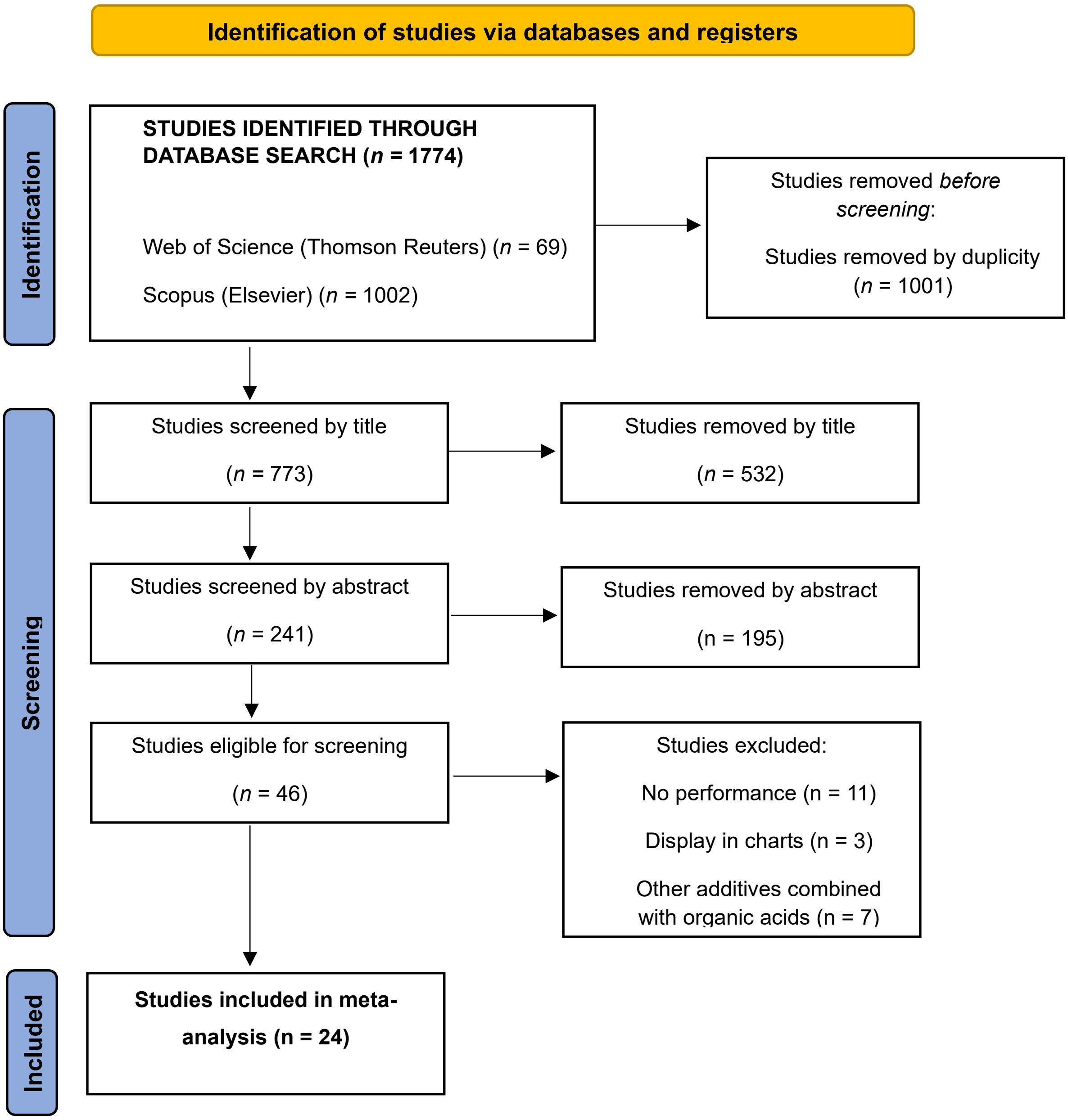


Fig. 2. Location of the studies focusing on organic acids for challenged piglets evaluation

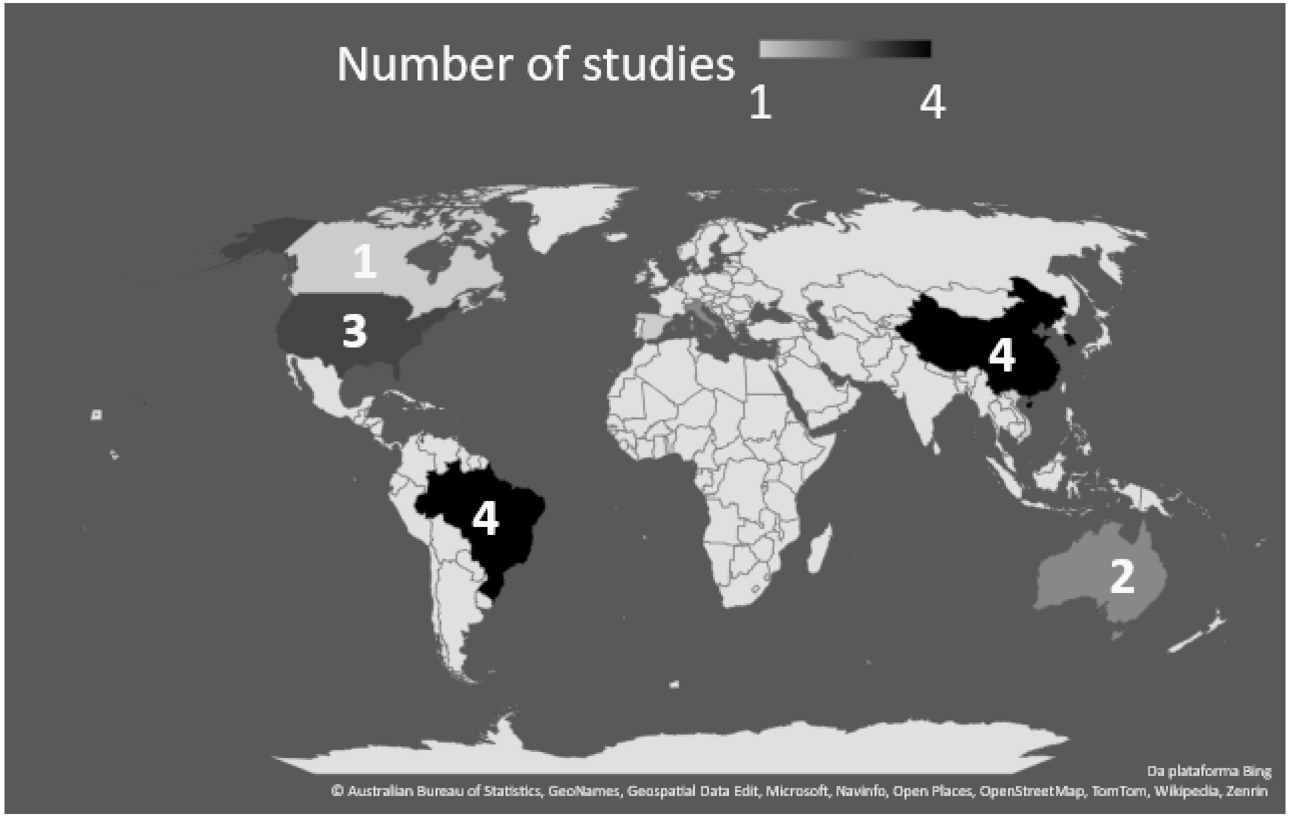
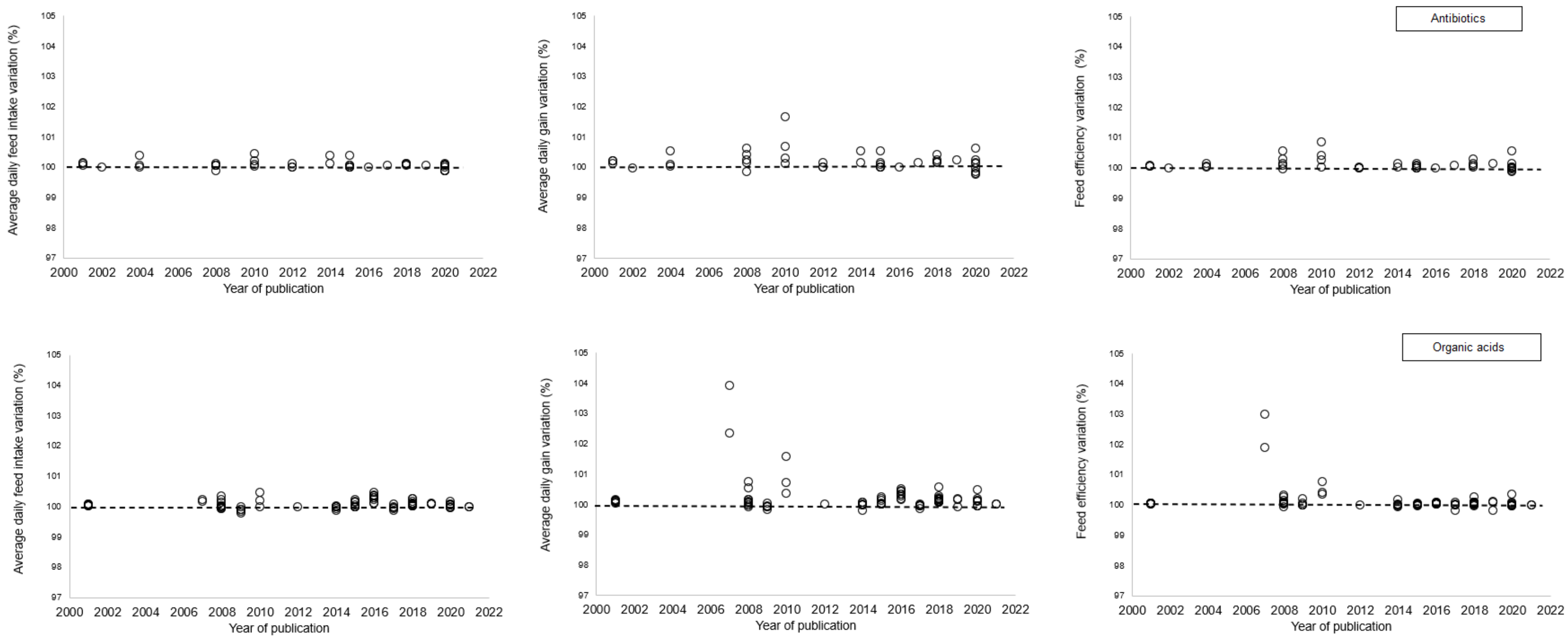


Fig. 3. Performance responses of antibiotics and organic acids treatments relativized to the respective control



CAPÍTULO III[2]

[2] Este capítulo corresponde ao artigo redigido e formatado no modelo da revista Livestock Science.

Organic acids for pigs from nursery to finishing phases: Systematic review and meta-analysis

M.S.C. Mendéz, I. Andretta, L. Hauschild

Highlights

Organic acids contributed to higher ADG and FE, comparing favorably to antibiotics.

Organic acids positively influenced intestinal morphology, varying among the different intestinal segments evaluated.

Digestibility coefficients are higher in the weanling phase compared to the growing and finisher phases.

Organic acids have a positive impact on the digestibility coefficient of the nutrients.

Organic acids are safe compounds with the potential to replace antibiotics growth-promoting.

Abstract

The reduction in the use of antibiotic growth promoters (AGP) in swine farming has led to an interest in alternative solutions. Organic acids (OA) have emerged as potential substitutes, demonstrating the ability to enhance swine health and performance. This meta-analysis aimed to comprehensively assess the effects of dietary OA supplementation on swine, focusing on intestinal morphology, nutrient digestibility, and productive performance across weaning, growing, and finishing phases. A systematic review identified 104 articles from diverse sources published between 1980 and 2021. These studies involved 19,268 pigs, with a predominant focus on the nursery phase (75%). The results indicate that the phase significantly influenced average daily feed intake (ADFI) and average daily gain (ADG), with the growing-finishing phase showing higher values. However, feed efficiency (FE) remained consistent across phases. Organic acids demonstrated potential benefits in ADFI and ADG, with the greatest impact observed during the weaning phase. Assessments of intestinal

morphology revealed phase-related impacts on villi height (VH) and the villi:crypt ratio (V:C). VH was higher in the organic acid group during the weaning phase, while the V:C ratio was influenced by phase and additive, with the weaning phase showing greater effects. Nutrient digestibility coefficients (ATTD) were affected by phase, with the weaning phase exhibiting higher values. Organic acids had potential positive impacts on crude protein (CP) and gross energy (GE) digestibility, particularly during the weaning phase. The phase did not affect phosphorus (P) digestibility, and additives consistently influenced CP, GE, and P digestibility. Organic acids (OA) have proven to be a safe additive in all phases of swine rearing, without compromising the health of the animals. Additionally, these compounds can enhance performance, nutrient digestibility, and intestinal morphology.

Keywords: Additives, animal nutrition, growth promoters, meta-analysis, swine.

Introduction

In recent decades, the use of antibiotic growth promoters (AGP) has seen a reduction in swine farming practices, driven by various factors, including concerns regarding the potential development of cross-resistance between pathogens affecting both animals and humans (Fang *et al*., 2013). For many years, AGP have played a pivotal role in enhancing swine performance from the weaning to finishing phases (Mroz, 2005). Consequently, there is an imperative need to identify viable substitutes for AGP. Organic acids (OA) have garnered recognition as potential alternatives to AGP in diets designed for weaning, growing, and finishing pigs (Morel *et al*., 2019; Xiang *et al*., 2021). OA have shown the capacity to reduce the incidence of diarrhea, enhance nutrient digestibility, and contribute to improved productive performance among piglets (Long *et al*., 2017). These advantages are primarily attributed to OA ability to positively modulate the intestinal microbiome and enhance overall intestinal health in animals.

Furthermore, Diao *et al*. (2016) conducted studies that observed positive effects associated with dietary OA supplementation on the intestinal morphology of piglets. In the context of diets for growing pigs, the inclusion of OA has resulted in greater slaughter weights (Ngoc *et al*., 2020) and increased digestibility of key nutrients such as dry matter, protein, and gross energy (Thacker *et al*., 2009). Despite these promising findings supporting OA as viable AGP substitutes, it is worth noting that certain results remain contentious. Consequently, additional research is necessary to gain a more comprehensive understanding of the underlying factors contributing to the discrepancies observed in these findings.

Considering these considerations, the current study employs a meta-analytical approach to comprehensively assess the effects of dietary OA supplementation on parameters such as intestinal morphology, nutrient digestibility, and productive performance in pigs, encompassing weaning, growing, and finishing phases.

Material and methods

Literature search strategy and study selection criteria

A systematic literature review was conducted in accordance with Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement guidelines (Moher *et al*., 2009). An electronic search was carried out in digital databases (PubMed, Scielo, ScienceDirect and Scopus) between August and October 2022 using keywords combined to identify relevant scientific articles to compose a database. The keywords used in the search are presented in Table 1. The review question was proposed using PICo strategy which indicates ‘Population’, ‘Intervention’, and ‘Context’. It was defined as Population: pigs, Intervention: organic acids or salts of organic acids, Context: productive performance. All results found in each digital database were imported to the reference manager to facilitate the selection process of articles.

The selection process was meticulously documented according to the PRISMA methodology, in which duplicate records excluded. Initially, each title and abstract of the chosen scientific papers were carefully screened to identify those eligible for full-text assessment. The final database was compiled using complete articles that met the predefined eligibility criteria: a) our study incorporated research published in indexed journals while excluding short communications, reviews, and conference abstracts/papers; b) the research focused on *in vivo* experiments involving from nursery to growing-finishing pigs; c) the assessment encompassed performance variables, such as feed intake, body weight gain, and feed efficiency; d) the study investigated dietary supplementation with organic acids or its salts.

Data extraction and database construction

Data from "Material and Methods" and "Results" sections of each scientific paper selected were transferred to an electronic spreadsheet. This dataset was structured with a column dedicated to qualitative information, including details such as authorship, journal, publication year, country of origin, pig strain, sex, diet type, phase, treatment name, and the type of organic acid used. Additionally, the dataset included columns for various quantitative exploratory and descriptive variables, such as the number of replications, the quantity of animals, duration of the experiment, organic acid dosage, and performance parameters.

The database construction and data encoding methodology followed to the principles outlined in prior literature (Sauvant *et al*., 2008). Each selected paper was assigned a unique code (ID) to serve as a moderating variable in our analysis, accounting for the variations among scientific papers, thereby influencing the statistical model's outcomes. Additionally, the database featured other codes for qualitative comparisons, including treatments labeled as 1 to 3 and the phase, W for weaning and GF for growing-finishing phase based on initial body weight (BW) in each experiment: weaning from 5 kg BW and growing-finishing from 25 kg BW.

To provide further details on the treatment arrangement, we structured it as a 3x2 factorial design. This design consisted of three primary additive factors: CON (Control): This group represents the baseline condition with no additional substances or additives introduced; ANT (Antibiotics): This category involved the inclusion of antibiotics as an additive, exploring the effects of antibiotic treatment; AO (Organic Acids): Within this category, organic acids were introduced as additives, examining their impact on the studied variables. Moreover, we factored in two phase-related variables: weaning (W) and growing-finishing (GF). This structured approach allowed for a comprehensive investigation of the effects of additives and phases on the studied parameters. In addition to the performance data, two supplementary databases were constructed using the information extracted from the selected studies concerning intestinal morphology and nutrient digestibility. These two datasets served as valuable resources for conducting further analyses under the same experimental conditions as those applied to assess productive performance variables.

Statistical analyses

The variables under analysis comprised performance variables such as average daily feed intake (ADFI), average daily gain (ADG), and calculated feed efficiency ratio (FE). Additionally, we investigated intestinal morphology, considering the parameters villi height (VH), villi crypt depth (CD), and the villi:crypt ratio (V:C). Additionally, we assessed the digestibility coefficients (Apparent Total Tract Digestibility, ATTD) for the following nutrients: Crude Protein (CP, %), Dry Matter (DM, %), Gross Energy (GE, %), and Phosphorus (P, %). All the data were assessed in two ways: first, as raw data, which were presented as they are presented in the original papers. Second, as relativized information, where the responses of antibiotic and organic acid-supplemented treatments were compared to their respective control treatments and expressed as a percentage of variation. This second approach was chosen to minimize variations among the raw data from different experiments included in the database. Variance analysis was performed using the following statistical model:

$$\boldsymbol{Y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{\tau_i} + \boldsymbol{\beta_j} + (\boldsymbol{\tau\beta})_{\boldsymbol{ij}} + \boldsymbol{\alpha_k} + \boldsymbol{\varepsilon_{ijk}}$$

in which $\boldsymbol{Y_{ijk}}$ is the response, $\boldsymbol{\tau_i}$ is the fixed effect of treatment (control or additive), $\boldsymbol{\beta_j}$ is the fixed effect of the phase (W or GF), $(\boldsymbol{\tau\beta})_{\boldsymbol{ij}}$ is the interaction effect between $\boldsymbol{\tau_i}$ and $\boldsymbol{\beta_j}$, $\boldsymbol{\alpha_k}$ is the random effect of study, and $\boldsymbol{\varepsilon_{ijk}}$ is the residual variation.

Statistical analyses were performed using R 4.2.1 (R Core Team, 2022) statistical software. Data were submitted to analysis of variance with the utilization of the lme4 (Fitting Linear Mixed-effects Models), lmerTest (ANOVA Tables for Linear Mixed Models), and emmeans (Estimated Marginal Means for Linear, Generalized Linear, and Mixed Models) packages for linear mixed-effects models. To assess interactions between factors (additive and phase), General Linear Mixed Models (GLMM) were employed. The statistical models included fixed effects of the phase factor and the additive factor, as well as the random effect of the study, identified by a unique code ID.

The linear mixed model methodology was chosen because it is well-suited for developing empirical models for biological responses in experimental designs. The analysis of variance was based on p-values, with a significance criterion set at $p < 0.05$ for each variable. In cases where p-values were between 0.05 and 0.10, it was considered the effect tended to significance.

Results

Systematic review and database description

The findings from the systematic review, conducted using the PRISMA methodology, are presented in Figure 1. Initially, 389 articles were identified. After eliminating duplicate entries and articles that didn't meet the predetermined inclusion criteria, we selected a final set of 104 articles, which constituted 27% of the initial search, for inclusion in this study. The primary reasons for excluding articles included: a) mixing AO with other compounds; b) literature reviews; c) experiments involving species different from the target; d) full articles not available in the selected databases; e) non-English publications; f) data presented in graphical form for performance results.

The database encompasses 113 scientific papers, published between 1980 to 2021 and disseminated across 41 peer-reviewed journals. Table 2 summarize relevant selected studies information. These studies predominantly appeared in specific journals: Journal of Animal Science (15%), Canadian Journal of Animal Science (8%), Livestock Science (7%), Journal of Animal Physiology and Animal Nutrition, and Animal Feed Science and Technology (6% each), and Journal of Animal and Feed Sciences (5%). Furthermore, 4% of the studies were found in Archives of Animal Nutrition and Arquivo Brasileiro de Medicina Veterinária e Zootecnia. Additionally, 45% of studies were distributed across various other scientific journals.

Figure 2 illustrates the geographic distribution of these studies. Most of the research was conducted from Brazil (17%), followed by Korea (14%), China (13%), the United States (9%), and Italy (5%). The remaining 42% was contributed by various other countries, including Canada, Australia, Colombia, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Mexico, Netherlands, New Zealand, Norway, Poland, Slovenia, Spain, Switzerland, Taiwan, Thailand, and Vietnam.

In the 113 studies, a performance evaluation was conducted on a total of 19,268 pigs, with 75% in the nursery, 18% in the growing, and 7% in the finishing phase. However, due to the limited representation of finishing pigs, this category has been included into the growing phase, resulting in a combined growth-finishing phase analysis. The database consisted of 466 lines in the electronic spreadsheet, where attributes like sex and genetic lineage were examined. In the most of studies, experiments were conducted with unsexed pigs (83%), while 16% exclusively involved males, and 1% did not specify the sex of the animals. In the studies included, the piglets had an average age of 29 days at the beginning of weaning experiments. Approximately 22% of the articles did not specify the age of the piglets and this information was unspecified for the growing and finishing phases. The average initial body weight (BW) at the weaning phase was 6 kg, in the growing phase it was 27 kg, and at the finishing phase, it averaged 56 kg. Moreover, around 34% of the studies did not provide information of the initial BW at the start of the experiments.

The study examined common dietary supplements of OA, including their dosages and administration methods. Predominantly, blends of organic acids in varying combinations and proportions were used in 35% of the studies. Benzoic acid was observed in 22% of studies, followed by formic acid (6%), sodium butyrate (5%), potassium diformate and fumaric acid (4% each). The remaining 24% encompassed diverse compounds such as acetic acid, calcium anarcadate, calcium formate, citric acid, gluconic acid, lactic acid, malic acid, phenyllactic acid, propionic acid, sodium benzoate, and sorbic acid. The average OA dosage was 0.43%, ranging from 0.08% to 9%. Solid administration was most prevalent (63%), while 19% utilized liquid or a combination of liquid and solid forms. The form of OA administration was unreported in 18% of studies.

Regarding antibiotics, most studies excluded them (72%), while 28% made comparisons with organic acids. The prevalent antibiotics were Carbadox, Apramycin, and Chlortetracycline, found in 53% of studies. Colistin, Aureomycin, and Zinc Bacitracin were present in 11% of studies each. Other antibiotics and combinations, including Lincospectin, Tiamulin, Penicillin, and Enrofloxacin, constituted 14% of the total antibiotic usage.

Performance results

Table 3 provides a summary of these findings. The phase had a significant impact on ADFI (P, p <0.001). As expected, ADFI values were highest during the GF phase and lowest during the WN phase. The additives (CON, ANT, and OA) produced nearly identical results in ADFI, with no significant impact on this variable (A, p = 0.3426). Similarly, we found no interaction between the phase and additive (PxA, p = 0.4597). Similarly, to ADFI, phase had a significant impact on ADG (P, p <0.001). The GF phase recorded the highest ADG values, followed by the WN phase. In the WN phase, the OA group had the highest ADG (510 g/day), followed by ANT (503 g/day) and CON (489 g/day), p<0.001. Similarly, in the GF phase, the OA group also had the highest ADG (738 g/day), with ANT (710 g/day) and CON (691 g/day) showing similar results, p<0.001. The interaction (PxA) is not statistically significant (p = 0.4597). In contrast to ADFI and ADG, the FE variable showed no significant phase-related influence (P, p = 0.1431). Both WN and GF phases exhibited similar values for this variable. When examining the different additives groups, FE was only affected during the WN phase (A, p = 0.011). In this phase, the highest FE values were observed in the ANT and OA treatments (0.58 both), followed by CON (0.56). In the GF phase, the FE values were similar. Like ADFI and ADG, the interaction between phase and additive was not statistically significant (PxA, p = 0.1369). No significant interaction between treatment and phase was found in any performance variables, indicating that treatment effects remain consistent across both phases.

Intestinal morphology assessment

Intestinal morphology results are shown in Table 4. The phase had no significant influence on duodenal VH, with similar values observed between the WN and GF phases (P, p =0.1008). Different treatments had a significant impact on duodenal morphology. In the WN phase, VH showed the higher value in the OA group (348 µm), with CON (329 µm) and ANT (324 µm) closely behind, p = 0.0150. In the GF phase, VH presented similar values between treatments, without any notable differences. The phase exhibited a tendency to impact CD values (P, p = 0.0671). However, no significant effects were detected for this factor. The additive only had an impact on CD in the GF phase (A, p = 0.0029). However, in the WN phase, the different treatments exhibited comparable values. In contrast, during the GF phase, CD was highest in the CON group (363 µm), followed by ANT (345 µm) and OA (330 µm), p = 0.0029. No interaction was detected between phase and additive factors (PxA, p = 0.2067). The V:C ratio was affected by the phase (P, p = 0.00362). The WN phase had the highest V:C ratios, and the GF phase had the lowest. The additive only had an impact during the WN phase. ANT had the highest V:C ratio at 1.95, followed by OA at 1.51, and CON at 1.28, p = 0.0463. However, during the GF phase, the results were similar across all treatments. Importantly, there was no significant interaction observed between the phase and additive (PxA, p = 0.1975).

The assessment of jejunal morphology, VH also do not demonstrated a significant effect of the phase (P, p = 0.5706). WN and GF phases presented very similar values for this variable. On the other hand, the different treatments had a significant influence on VH. AO exhibited the highest VH values at 371 µm and 348 µm, followed by ANT with values of 355 µm and 317 µm, and CON with values of 337 µm and 292 µm (p = 0.0029). The phase had a significant impact on CD, with higher values observed in the GF phase compared to WN (P, p < 0.001). The additives had an impact on CD, but this effect was only observed in the WN phase. The CON group exhibited the highest CD at 285 µm, while the ANT group had the lowest at 252 µm. The AO group's CD values were intermediate, with a value of 265 µm, p = 0.0012. The V:C ratio was affected by the phase (P, p = 0.0018), with the highest ratios in the W phase and the lowest in the GF phase. The additive had a potential impact on the V:C ratio (A, p = 0.0646). Nevertheless, the results were consistent across all treatments, and there was no significant interaction between the phase and additive (PxA, p = 0.9377).

Finally, ileal VH showed significant effects across phases. VH was significantly higher in the GF phase compared to WN (P, p = 0.0028). Additionally, the additive had an impact on VH, but this effect was only observed in the WN phase. Among the treatment groups, the OA group exhibited the highest VH value (396 µm), followed by ANT (388 µm), with CON (278 µm) having the lowest value, p = 0.0233. In the GF phase, VH values were similar across all three treatments. There was no significant interaction between the phase and the additive (PxA, p = 0.1881). In contrast, CD was not influenced by the phase (P, p = 0.1838), and both WN and GF had similar values for this variable. CD was also not affected by the different treatments (A, p = 0.8658). CON, ANT, and OA had very similar CD values. Likewise, there was no significant interaction between phase and additive in CD (PxA, p = 0.3115). V:C ratio was also not influenced by the phase (P, p = 0.3761), with similar values in WN and GF. The different additives significantly influenced V:C in the WN phase. The highest V:C ratio occurred in ANT (1.58 µm), and the lowest in CON (1.18 µm). OA had intermediate values of V:C ratio, between the ANT and CON groups (1.29 µm), p = 0.0358. V:C ratio was similar among the three treatments in the GF phase. There was no interaction between the phase and the additive in

V:C ratio (PxA, p = 0.7149). No significant interactions were observed in any of the evaluated variables, a pattern that repeated across all three intestinal segments investigated (duodenum, jejunum, and ileum). These findings suggest that the effects of the phase and additives are consistent and independent of each other.

Effects on digestibility coefficients

The results for digestibility coefficients (ATTD) can be found in Table 5. The phase had a significant effect on the DM digestibility coefficient (P, p<0.001). The highest digestibility values were observed in the WN phase, and the lowest in the GF phase. There was a tendency for treatments to influence DM digestibility (A, p = 0.0733). However, values remained similar among the CON, ANT, and AO groups. No significant interaction was observed between phase and additive in the DM digestibility coefficient (PxA, p = 0.8960). The phase significantly impacted the CP digestibility coefficient (P, p<0.001). The WN phase exhibited the highest digestibility values, while the GF phase had the lowest. The additive also influenced the CP digestibility coefficient (A, p<0.001) in both the WN and GF phases. In the WN phase, the highest digestibility coefficients were observed in the ANT (84.8%) and OA (84.0%) treatments, while the lowest were in CON (82.7%). The same pattern was observed in the GF phase, where OA and ANT exhibited the highest coefficients (71.4% and 70.7%), and the lowest were found in CON (68.3%). No significant interaction between phase and additive in CP digestibility was observed (PxA, p = 0.9172).

The phase factor also influenced the digestibility coefficient of GE (P, p<0.001). The highest coefficients were observed in the WN phase, and the lowest in GF. Different treatments also significantly influenced the GE digestibility (A, p = 0.0115), but only in GF. In the WN phase, the coefficients had similar values regardless of the treatment. In contrast, in GF, OA and ANT had the highest coefficients (76.2% and 75.8%, respectively), and CON had the lowest (72.9%). No significant interaction was observed between phase and additive for GE (PxA, p = 0.4955). Finally, the phase factor had an impact on the P digestibility coefficient (P, p = 0.0256). The WN phase showed the highest coefficients, while GF had the lowest. The three treatments also significantly affected P digestibility (A, p<0.001), but exclusively in WN phase. During the WN phase, OA presented the highest P coefficient (61.5%), while CON and ANT exhibited the lowest P digestibility coefficients (53.8% and 52%, respectively). In the GN phase, the coefficients remained consistent across treatments. There was no significant interaction between phase and additive for P digestibility (PxA, p = 0.3270). Significant interactions were not found among any of the coefficients assessed for digestibility. These results indicate that the effects of the phase and additives are consistently independent of each other.

When evaluating digestibility coefficients over the entire period (5 kg to 135 kg), we observed that different treatments significantly influenced all the coefficients. The DM digestibility coefficient was seen in the ANT treatment (82.2%), while the lowest was in CON (78.5%). OA had an intermediate value between ANT and CON (79.6%), p = 0.0304. The CP digestibility coefficient was also influenced by the additives. The ANT and OA groups had the highest CP coefficients (79.7% and 78.1%, respectively), with the lowest observed in the CON group (76.4%), p = 0.0047. Similar results to DM were observed in the digestibility coefficients of GE, with the ANT group having the highest (79.6%) and CON the lowest (78.4%). Like DM, in GE, OA had an intermediate value between ANT and CON (79.4), p = 0.0125. Finally, the treatments also influenced the P digestibility coefficient. The highest coefficient was observed in the OA group (55%), while CON and ANT had similar values (48.6% and 45.9%, respectively), p<0.001.

Discussion

Performance assessment

Based on the provided results, the phase of the study had a significant impact on performance variables, including ADFI, ADG, and FE. These findings are in line with the research conducted by Hanczakowska *et al*. (2014) and other similar studies in the field of animal science. The phase of the study had a significant influence on ADFI, because ADFI values were highest during the GF phase and lowest during the WN phase. This observation aligns with the general understanding that animals typically consume more feed during the growth phase when their nutritional demands are higher. The additives (CON, ANT, and OA) did not significantly impact ADFI, suggesting that these additives did not alter the overall feed intake of the animals. This fact was confirmed both in the individual analysis of the phases and their interaction with the additives, as well as in the evaluation of the entire experimental period (from 5 kg to 135 kg). These findings are consistent with previously published studies in which did not show differences among the CON, ANT, and AO treatments in the nursery, growing or finishing phases (Lawlor *et al*., 2006; Tutida *et al*., 2021).

Similar to ADFI, the phase of the study had a significant impact on ADG. The GF phase recorded the highest ADG values, followed by the WN phase. These responses are expected because in the GF phase, there is higher ADFI, leading to higher ADG. In the WN phase, the OA group had the highest ADG, followed by ANT and CON, with statistically significant differences. These findings differ from previously published data, where no difference in piglet ADG was observed between the CON and OA treatments (Lee *et al*., 2007; Ferreira *et al*., 2017). Similarly, in the GF phase, the OA group also had the highest ADG, with ANT and CON showing equivalent results. Nguyen *et al.* (2018) found similar results when providing dietary AO to pigs in the finishing phase. Like the findings in this study, the authors observed higher ADG in the AO treatment compared to the CON. These findings indicate that the growth phase and the type of additive used can influence ADG of the animals.

Unlike ADFI and ADG, the FE variable showed no significant phase-related influence, indicating that feed efficiency remained relatively stable across both the WN and GF phases. However, FE was affected by additives during the WN phase, where the ANT and OA treatments resulted in the highest FE values. These results are inconsistent from previous findings where the comparison between CON, ANT, and OA showed no significant differences in the G:F variable (Walsh *et al*., 2007; Luise *et al*., 2017). In the GF phase, FE values were similar across all additive groups. Similar results were found by other authors in the same comparison (Cho *et al*., 2014).

In conclusion, the results of this study are consistent with several previous studies and support the notion that the phase plays a crucial role in influencing performance variables in pigs, including ADFI, ADG, and FE. Additionally, the type of additives used can impact these variables, particularly during specific phases, as seen in the case of FE during the WN phase. Furthermore, in our study, the interaction (PxA) between phase and additive did not reach statistical significance, indicating that the effects of additives on ADFI, ADG, and FE remained consistent across the phases.

Intestinal morphology

Duodenal VH was not significantly affected by the phase (WN vs. GF). However, different treatments had a significant impact, with the OA group exhibiting the highest VH in the WN phase. These findings differ from prior published data evaluating piglets, in which various treatments including CON, ANT, and OA did not show a significant impact on VH (Braz *et al*., 2011; Ma *et al*., 2021). This suggests that dietary supplementation with OA may promote better duodenal VH in certain conditions. Duodenal CD showed no significant effect of the phase, but the additive had an impact in the GF phase, with the CON group having the highest CD. Non-significant effects of the additives were also observed in piglets in previous studies, in which OA and CON showed similar CD values (Grecco *et al*., 2018). In the GF phase, unlike the findings of this study, Giannenas *et al*. (2016) did not observe differences in the CD of growing pigs when comparing CON and OA groups. Considering that crypt depth is related to intestinal mucosa regeneration and cell turnover, and villi height to nutrient absorption capacity, the smaller the crypts, the lower the regenerative activity, and the taller the villi, the greater the absorption capacity (Johnson, 2016). Hence, treatments with lower CD values demonstrated superior efficacy. The V:C ratio was significantly affected by the phase, with the highest ratios in the WN phase. Additive also had an impact during the WN phase, with ANT resulting in the highest V:C ratio. This suggests that dietary interventions may be more effective in influencing V:C ratios during certain phases.

Jejunal VH was not significantly affected by the phase, indicating that the transition from the WN phase to the GF phase did not have a substantial impact on this parameter. However, different treatments significantly influenced VH, with AO resulting in the highest values. Morel *et al*. (2019) observed discrepant effects compared to this study, not finding significant differences in the VH of the jejunum between the CON and AO treatments. CD was significantly influenced by the phase, with higher values in the GF phase. The additive also had an impact, with the CON group showing the highest CD in the WN phase. This suggests that dietary additives can affect CD, especially in specific phases. The lowest CD values were observed in the ANT and OA treatments, indicating lower regenerative activity and better intestinal health. Similar to the duodenum, the V:C ratio was significantly affected by the phase, with the highest ratios in the WN phase. However, there was no significant additive effect in this case, suggesting that the phase plays a more critical role in influencing V:C ratios in the jejunum. These results align with an earlier study by Xu *et al*. (2017), which reported that the provision of AO had no significant impact on the jejunal V:C ratio of piglets compared to the CON group.

Ileal VH was significantly higher in the GF phase compared to the WN phase, indicating that the phase transition had a notable impact on ileal VH. In the WN phase, the additive (OA) had a significant influence on VH, with the highest values observed in the OA group. Deng *et al*. (2021) also observed an increase in the ileum VH of piglets that received OA compared to the CON group. The non-significant differences observed in ileal VH of GF pigs in this study are consistent with previous findings in which the provision of OA also did not significantly influence this variable (Morel *et al*. 2019). CD in the ileum was not significantly affected by the phase or the different additives, suggesting that this parameter remained relatively consistent across conditions. Lee et al. (2021) reported data that corroborate with the findings of this study, in which CD was not influenced by the different additives compared to the CON group in piglets. The V:C ratio in the ileum was not influenced by the phase but was significantly affected by the additives in the WN phase, with ANT resulting in the highest ratio. Some authors previously reported findings very similar to those of this study, in which CD showed no differences in the comparison between CON, ANT, and OA. However, the V:C ratio was influenced by the treatment. In contrast to this study, the authors observed a higher V:C ratio in the OA group compared to CON and ANT groups (Ma *et al*., 2021). This indicates that dietary additives can affect the V:C ratio in the ileum, particularly in the WN phase.

In summary, these findings underscore the importance of considering both the phase and additives in understanding their impact on intestinal morphology. This knowledge can have implications for optimizing nutritional strategies and enhancing gut health in swine production systems. In this context, OA yielded significant results and proved to be an interesting alternative to replace AGP for improving intestinal morphology in both the WN and GF phases.

Digestibility coefficients

The phase (WN vs. GF) had a significant impact on the digestibility coefficients of DM, CP, GE, and P. In general, the WN phase showed higher digestibility coefficients compared to the GF phase for all nutrients assessed. These findings are consistent with the basic understanding of swine nutrition (NRC, 2012), from which is understood that diets for younger animals (WN) should exhibit higher digestibility in contrast to those designed for older animals (GF). Furthermore, the higher digestibility coefficients observed during the WN phase are in line with the nutritional needs and physiological development of piglets during this period.

Lee *et al*. (2021) carried out experiments to evaluate the ATTD of pigs in the WN and GF phases. Similarly, their treatments involved CON, ANT, and AO, just as in the present study. The authors did not observe effects of the additives in the different phases evaluated, concluding that there was no influence of the diet on the digestibility coefficients. However, unlike their findings, the authors of this study observed additive effects in the various phases, indicating that the digestibility coefficients of all assessed nutrients were influenced by the additives, in addition to the phase effect. The results indicate that during the WN phase, both the ANT and OA treatments exhibited trends towards higher digestibility coefficients for CP and P compared to the CON group. This suggests that these additives may play a beneficial role in improving nutrient utilization in weanling piglets. Valencia *et al*. (2002) reported very similar results, showing that when compared to the CON group, both ANT and OA led to increased digestibility coefficients of DM, CP, and P in weaned piglets. The improved CP and P digestibility could be of particular significance during this critical phase when piglets are transitioning to solid feed and might struggle with nutrient absorption. In the GF phase, the consistent elevation of CP and GE digestibility coefficients in the OA and ANT treatments compared to CON is notable. This implies that these additives may exert a more pronounced positive influence on nutrient utilization in older pigs.

These findings contradict earlier published data, as a prior study (Devi *et al*., 2015) found no increase in the digestibility coefficients of DM, CP, and GE when comparing CON and OA. The present study suggests that as pigs mature and their digestive systems become more efficient, additives like OA and ANT could enhance the digestibility of essential nutrients, including protein and energy, which are crucial for growth and performance. There was no significant interaction detected between the phase and additives concerning any of the digestibility coefficients. This absence of interaction suggests that the influences of the growth phase and additives on nutrient digestibility operate independently of each other. In simpler terms, the effects of additives remain relatively consistent across various growth phases.

When evaluating digestibility coefficients over the entire rearing period (5 kg to 135 kg), the treatments continued to significantly influence all the coefficients. The ANT treatment consistently showed higher digestibility coefficients for DM, CP, GE, and P, while CON consistently had lower coefficients. OA had intermediate values between ANT and CON for DM and GE digestibility. Xu *et al*. (2017) provided supporting evidence for these results. Nevertheless, in their study, ANT and OA presented closely matching digestibility coefficients for DM, CP, GE, and P, while CON showed significantly lower coefficients in comparison to the other treatments. These results suggest that the additives, particularly ANT and OA, can have a sustained positive impact on nutrient digestibility being effective both in the weaning and growing, and finishing phases.

In summary, this comprehensive analysis demonstrates the relationship between rearing phases and dietary additives in swine nutrition. The influence of the phase is clearly reflected in performance variables, such as ADFI, ADG, and FE, reaffirming the established principle that pig nutritional demands are different across the time. Notably, ANT and OA additives demonstrated phase-specific effects, enhancing our understanding of their influence. This study deviates from previous research by revealing additive effects that interact with the growth phase. It implies that the choice of additives and their timing in the diet can significantly impact nutrient utilization, subsequently affecting pig growth and performance.

Regarding intestinal morphology, the findings highlight the complex interaction between phase and additive. OA supplementation holds promise for elevating duodenal villus height, while variations in crypt depth and villus height ratios depend on rearing phase and additive. This knowledge is priceless for optimizing nutritional strategies in swine production systems to promote gut health. In the context of digestibility coefficients, this study encompasses the dynamic nature of nutrient utilization throughout the phases, emphasizing the importance of tailored diets. Significantly, ANT and OA consistently improved digestibility coefficients for various nutrients, with a sustained positive impact across weaning, growing, and finishing phases.

The lack of significant interactions between growth phase and additives implies that these effects operate independently. In essence, this study emphasizes the need for a nuanced approach to swine nutrition, recognizing the intricate interplay of growth phase and dietary additives as critical factors in maximizing performance and gut health while ensuring efficient nutrient utilization across various stages of pig development. These findings establish the groundwork for further research and practical applications in swine nutrition science. This may encompass investigations into optimal OA supplementation dosages, diverse modes of action of these compounds, and a deeper understanding of their mechanisms.

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Table 1. Search terms for PICo methodology: Population, Intervention and Context used in the systematic review

Acronym	Search string
Population (P)	swine OR pig OR pigs OR piglet OR piglets OR "weaned pig" OR "weaned piglet" OR suckling OR "sucking pig" OR "suckling piglets" gilt OR gilts OR barrow OR barrows
Intervation (I)	"organic acid" OR "organic acids" OR "organic acid salt" OR "acetic acid" OR "sodium acetate" OR "formic acid" OR "sodium formate" OR "propionic acid" OR "sodium propionate" OR "butyric acid" OR "sodium butyrate" OR "malic acid" OR "sodium malate" OR "lactic acid" OR "sodium lactate" OR "citric acid" OR "sorbic acid" OR "sodium sorbate" OR "potassium sorbate" OR "calcium sorbate" OR "fumaric acid" OR "sodium fumarate" OR "benzoic acid" OR "sodium benzoate"
Context (Co)	performance OR “body weight” OR “average daily gain” OR “weight gain” OR “average daily feed intake” OR “feed intake” OR “feed consumption” OR “feed conversion” OR “feed to gain” OR “feed : gain” OR “feed efficiency” OR “gain to feed” OR “gain : feed” OR ADFI OR ADG OR BW OR FCR

Table 2. Meta-analysis database description

Reference	First author	Year	Country	Organic acid/salt	Dosage (%)	Antibiotic	[1]Performance	[1]Intestinal morphology	[1]Digestibility coefficients
1	Thacker	1980	Canada	propionic acid	3.00 - 9.00	no	NS	-	-
2	Thacker	1981	Canada	propionic acid / calcium propionate	3.50 - 7.00	no	(-)	(+)	-
3	Giesting	1991	USA	fumaric acid	2.00 - 3.00	no	(+)	-	-
4	Risley	1991	USA	citric and fumaric acids	1.50	no	(+)	-	-
5	Radcliffe	1998	USA	citric acid	1.50 - 3.00	no	(+)	-	-
6	Li	1999	China	lactic acid	0.50	no	(-)	-	-
7	Paulicks	2000	Germany	potassium diformate	1.80	no	(+)	(+)	-
8	Overland	2000	Norway	potassium and calcium diformate	0.8	no	(+)	-	-
9	Mroz	2000	Netherlands	formic and fumaric acids / calcium benzoate	1.28 - 2.67	no	NS	-	(+)
10	Jongbloed	2000	Netherlands	latic and formic acids	0.80 - 3.20	no	(+)	-	(+)
11	Tsiloyiannis	2001	Greece	citric formic, fumaric, lactic, malic and propionic acids	1.00 - 1.60	yes	(+)	(+)	-
12	Frankiewicz	2001	Poland	blend	0.2 - 1.0	yes	NS	-	NS
13	Partanen	2002	Finland	lactic and formic acids / calcium formate and sodium benzoate	0.80	no	NS	-	(+)
14	Partanen	2002	Finland	formic acid, blend	0.80	yes	NS	-	-
15	Mroz	2002	Netherlands	blend	0.35 - 1.00	no	NS	(+)	NS

16	Piva	2002	Italy	blend / sodium butyrate	0.80	no	(-)	-	-
17	Valencia and Chavez	2002	Canada	acetic acid	1.00	no	NS	-	(+)
18	Namkung	2004	Canada	blend	1.00 - 1.10	yes	NS	-	-
19	Ettle	2004	Germany	formic and sorbic acids / potassium diformate	1.20 - 2.40	no	NS	-	-
20	Pierce	2005	Ireland	latic acid	1.60	no	(+)	NS	(+)
21	Mroczek	2005	Poland	blend	0.30	yes	(+)	-	-
22	Lawlor	2005	Ireland	fumaric acid	0.20 - 0.30	no	(+)	-	(+)
23	Canibe	2005	Denmark	formic acid	1.80	no	NS	-	-
24	Kluge	2006	Germany	benzoic acid / potassium diformate	0.50 - 1.20	no	(+)	-	-
25	Kil	2006	Korea	fumaric and latic acids	0.20	no	(+)	(+)	NS
26	Freitas	2006	Brazil	latic acid	0.60 - 0.90	no	NS	(-)	-
27	Biagi	2006	Italy	gluconic acid	0.30 - 1.12	no	NS	-	-
28	Partanen	2006	Finland	formic acid, blend	0.30 - 1.20	yes	NS	-	-
29	Bühler	2006	Switzerland	benzoic acid	1.00	no	NS	-	(+)
30	Lawlor	2006	Ireland	fumaric acid / calcium formate	1.50 - 2.00	no	(+)	-	-
31	Walsh	2007	USA	blend	0.40	yes	(+)	-	-
32	Walsh	2007	USA	blend	0.40	yes	NS	-	NS
33	Piva	2007	Italy	blend	0.10 - 1.10	yes	NS	(-)	(+)

34	Partanen	2007	Finland	formic acid, blend	0.30	yes	(+)	-	-
35	Lee	2007	Taiwan	blend	0.60	no	NS	-	-
36	Guggenbuhl	2007	France	benzoic acid	0.50	no	(+)	-	(+)
37	Gottlob	2007	USA	blend	0.06 - 0.45	yes	NS	-	-
38	Gomes	2007	Brazil	butyric, formic and fumaric acids, blend	0.10 - 0.50	no	(+)	(+)	-
39	Eisemann and Heugten	2007	USA	formic acid / ammonium formate	0.60 - 1.20	no	(+)	-	(+)
40	Overland	2007	Norway	benzoic, formic and sorbic / calcium butyrate	0.85 - 1.20	no	NS	-	-
41	Biagi	2007	Italy	sodium butyrate	0.10 - 0.40	no	NS	NS	-
42	Torrallardona	2007	Spain	benzoic acid	0.50	no	(+)	-	-
43	Silva	2008	Brazil	blend	0.80 - 1.00	no	NS	-	(+)
44	Overland	2008	Norway	benzoic, formic and sorbic / calcium butyrate	0.85 - 1.20	no	(+)	-	-
45	Lu	2008	China	sodium butyrate	0.50 - 1.00	no	(+)	(+)	-
46	Wang	2009	Korea	phenyllactic	0.10 - 0.30	yes	(+)	-	(+)
47	Gheler	2009	Brazil	benzoic acid	0.25 - 0.75	no	(+)	(+)	NS
48	Thacker and Haq	2009	Canada	blend	0.10	no	(-)	-	-
49	Jia	2010	China	blend	0.03 - 0.10	no	(+)	(+)	-
50	Halas	2010	Australia	benzoic acid	0.50	no	(+)	(+)	-
51	Stukejl	2010	Slovenia	blend	0.30	no	NS	-	-

52	Grilli	2010	Italy	blend	0.30	no	(+)	-	-
53	Poeikhampha and Bunchasak	2011	Thailand	potassium diformate	0.25 - 0.80	no	(+)	(+)	-
54	Papatsiros	2011	Greece	benzoic acid	0.50	no	(+)	-	(+)
55	Luo	2011	China	sorbic acid	0.05 - 0.40	no	(+)	-	-
56	Gomes	2011	Brazil	fumaric and latic acids, blend / calcium propionate	0.50 - 1.00	no	NS	NS	-
57	Braz	2011	Brazil	blend	2.50 - 7.00	yes	(+)	(+)	-
58	Graber	2012	Germany	benzoic acid / sodium benzoate	0.20 - 0.50	no	(+)	-	(+)
59	Htoo and Molares	2012	Spain	potassium formate	1.20 - 1.56	no	(+)	-	-
60	Zentek	2013	Germany	blend	0.30 - 0.40	no	NS	(-)	-
61	Alvarado	2013	Mexico	benzoic acid	0.50	yes	(+)	-	-
62	Upadhaya	2014	Korea	blend	0.10 - 0.20	no	(+)	-	-
63	Cho	2014	Korea	blend	0.30 - 0.50	no	(+)	-	(+)
64	Fang	2014	China	sodium butyrate	0.10	no	NS	-	-
65	Hanczakowska	2014	Poland	sodium butyrate	0.30	no	(+)	(+)	-
66	Cho and Kim	2015	Colombia		0.05 - 0.20	yes	(+)	-	-
67	Cho	2015	Korea	benzoic acid	0.50	no	NS	(+)	(+)
68	Devi	2015	Korea	benzoic acid	0.50	no	NS	-	-
69	Sbardella	2015	Brazil	propionic acid	0.10 - 0.30	no	NS	-	-

70	Diao	2016	China	benzoic acid	0.50	no	(+)	(+)	(+)
71	Callegari	2016	Brazil	phosphoric acid, blend / salts calcium	0.20 - 0.85	no	NS	(+)	-
72	Boas	2016	Brazil	blend / sodium butyrate	0.10	no	NS	-	-
73	Upadhaya	2016	Korea	blend	0.10 - 0.40	no	(+)	-	-
74	Giannenas	2016	Greece	benzoic acid	0.50	no	NS	NS	-
75	Zhan	2017	China	benzoic acid	0.30 - 0.50	no	(+)	-	-
76	Papadopoulos	2017	Greece	blend	0.30	no	(+)	(+)	NS
77	Matos	2017	Brazil	calcium anacardate	0.40 - 1.20	yes	NS	NS	-
78	Luise	2017	Italy	formic acid	0.14 - 0.64	no	(+)	-	-
79	Hosseindoust	2017	Korea	blend	0.20	no	(+)	-	(+)
80	Ferreira	2017	Brazil	blend	0.35	yes	NS	NS	-
81	Chen	2017	China	benzoic acid	0.20 - 0.50	no	(+)	(+)	-
82	Lynch	2017	Ireland	citric, formic and benzoic acids / sodium butyrate	0.30 - 0.50	no	(+)	NS	-
83	Xu	2018	China		0.15	yes	(+)	(+)	-
84	Kiarie	2018	Canada	benzoic acid	0.50	yes	(+)	(+)	-
85	Grecco	2018	Brazil	fumaric acid, blend	0.20 - 0.80	yes	NS	(+)	-
86	Yang	2019	Korea	blend	0.20	no	(+)	-	(+)
87	Patino	2019	Colombia	blend	0.40	yes	(+)	-	-

88	Oh	2019	Korea	blend	0.10 - 0.20	no	(+)	(+)	-
89	Nguyen	2019	Korea	blend	0.10 - 0.20	no	(+)	-	NS
90	Morel	2019	New Zealand	benzoic acid, blend / sodium butyrate	0.15 - 0.50	no	NS	NS	-
91	Yang	2019	Korea	blend	0.10 - 0.20	no	(+)	-	(+)
92	Ngoc	2020	Netherlands	blend	0.20	yes	(+)	(+)	-
93	Silva	2020	Brazil	sodium butyrate	0.25 - 0.50	yes	(+)	(+)	-
94	Lin	2020	China	sodium butyrate	0.15 - 1.00	no	(+)	-	-
95	Sun	2020	China	sodium butyrate	0.20	yes	(-)	-	-
96	Upadhaya	2020	Korea	sodium butyrate	0.05 - 0.30	no	(+)	(+)	-
97	Ma	2021	China	blend	0.30 - 0.70	yes	NS	-	(+)
98	Dahmer and Jones	2021	USA	blend	0.25 - 0.50	yes	(-)	-	-
99	Deng	2021	China	citric acid	0.30	no	NS	(+)	-
100	Lee	2021	Canada	blend	0.55 - 1.00	yes	NS	(+)	-
101	Lee	2021	Korea	blend	0.20 - 0.30	no	(+)	-	-
102	Muniyappan	2021	Korea	blend	0.05 - 0.10	no	(+)	-	-
103	Tutida	2021	Brazil	blend	0.10	yes	NS	-	-
104	Xiang	2021	China	blend	0.20	no	(+)	NS	(+)

[1] NS = no significant effects; (+) OA positive significant effects; (-) OA negative significant effects

- no information

Table 3. Performance of pigs supplemented with dietary antibiotics or organic acids

	Treatment										
Phase[1] (P)	WN	GF	WN	GF	WN	GF	*SEM*[2]	*P-Value*[3]			R^2
Additive (A)	Control		Antibiotic		Organic acid			P	A	P x A	
Average daily feed intake, g/day	579 B	1,330 A	601 B	1,337 A	584 B	1,392 A	70.09	<0.001	0.3426	0.4597	96%
Average daily gain, g/day	489 Bb	691 Ab	503 Bab	710 Aab	510 Ba	738 Aa	22.36	<0.001	<0.001	0.4064	92%
Feed efficiency	0.56 b	0.57	0.58 a	0.58	0.58 a	0.59	0.07	0.1431	0.011	0.1369	88%

Overall (5 to 135 kg)	Treatment					
Additive	Control	Antibiotic	Organic acid	*SE4*	*P-value**	R^2
Average daily feed intake, g/day	1,083	1,089	1,096	45.77	0.7784	95%
Average daily gain, g/day	525 b	541 ab	550 a	21.17	<0.001	90%
Feed efficiency, g/g	0.56 b	0.58 a	0.58 a	0.09	0.003	89%

[1]WN = weaned pigs (5 to 25 kg BW), GF = growing-finishing pigs (25 to 135 kg BW)

[2]Standard Error of the Mean (SEM)

[3]Probability of fixed effetcs: additive/phase and its interaction. Means followed by different letters differ statistically at the level 5% of significance.

R^2: determination coefficient of Generalized Linear Mixed Effects model

Table 4. Intestinal morphology of pigs supplemented with dietary antibiotics or organic acids

		Treatment										
	Phase (P)[1]	W	GF	W	GF	W	GF	*SEM²*	*P-Value³*			R²
	Additive (A)	Control		Antibiotic		Organic acid			*P*	A	PxA	
Duodenum	Villi height, µm	329 b	294	324 ab	293	348 a	310	14.9	0.1008	0.0150	0.9395	89%
	Villi crypt, µm	317	363 b	275	345 ab	301	330 a	17.3	0.0671	0.0029	0.2067	90%
	Villi : Crypt ratio, µm	1.28 Ab	0.87 B	1.95 Aa	0.90 B	1.51 Aab	0.98 B	0.19	0.0362	0.0463	0.1975	72%
Jejunum	Villi height, µm	337 b	292 b	355 ab	337 ab	371 a	348 a	19.9	0.5706	<0.001	0.4126	92%
	Villi crypt, µm	285 Ab	436 B	252 Aa	403 B	265 Aab	413 B	16.2	<0.001	0.0012	0.9821	92%
	Villi : Crypt ratio, µm	1.60 A	0.66 B	1.68 A	0.85 B	1.59 A	0.84	0.13	0.0018	0.0646	0.9377	69%
Ileum	Villi height, µm	278 B	342 Ab	308 B	388 Aab	283 B	396 Aa	19.2	0.0028	0.0233	0.1881	93%
	Villi crypt, µm	292	309	268	310	268	320	22.6	0.1838	0.8658	0.3115	79%
	Villi : Crypt ratio, µm	1.18 b	1.42	1.58 a	1.59	1.29 ab	1.54	0.09	0.3761	0.0358	0.7149	97%

[1]WN = weaned pigs (5 to 25 kg BW), GF = growing-finishing pigs (25 to 135 kg BW)

[2]Standard Error of the Mean (SEM)

[3]Probability of fixed effetcs: additive/phase and its interaction. Means followed by different letters differ statistically at the level 5% of significance.

R^2: determination coefficient of Generalized Linear Mixed Effetcs model

Table 5. Digestibility coefficients (ATTD) of pigs supplemented with dietary antibiotics or organic acids

	Treatment										
Phase[1] (P)	W	GF	W	GF	W	GF	*SEM*[2]	*P-Value*[3]			R^2
Additive (A)	Control		Antibiotic		Organic acid			P	A	P x A	
Dry Matter ATTD, %	83.2 A	72.5 B	85.6 A	70.1 B	83.9 A	73.4 B	2.02	<0.001	0.0733	0.8960	87%
Gross Energy ATTD, %	81.2 A	72.9 Bb	82.3 A	75.8 Ba	82.1 A	76.2 Ba	1.48	0.003	0.0115	0.4955	94%
Overall (5 to 135 kg)	Treatment										
Dry Matter ATTD, %	78.5 b		82.2 a		79.6 ab		1.67	0.0304			77%
Crude Protein ATTD, %	76.4 b		79.7 a		78.1 a		1.55	0.0047			81%
Gross Energy ATTD, %	78.4 b		79.6 a		79.4 ab		1.37	0.0125			94%
Phosphorus ATTD, %	48.6 b		45.9 b		55.0 a		6.22	<0.001			93%

[1]WN = weaned pigs (5 to 25 kg BW), GF = growing-finishing pigs (25 to 135 kg BW)

[2]Standard Error of the Mean (SEM)

[3]Probability of fixed effetcs: additive/phase and its interaction. Means followed by different letters differ statistically at the level 5% of significance.

R^2: determination coefficient of Generalized Linear Mixed Effets model

ATTD: Apparent Total Tract Digestibility

Figure 1. Prisma Flow Diagram of the Systematic review and selection process

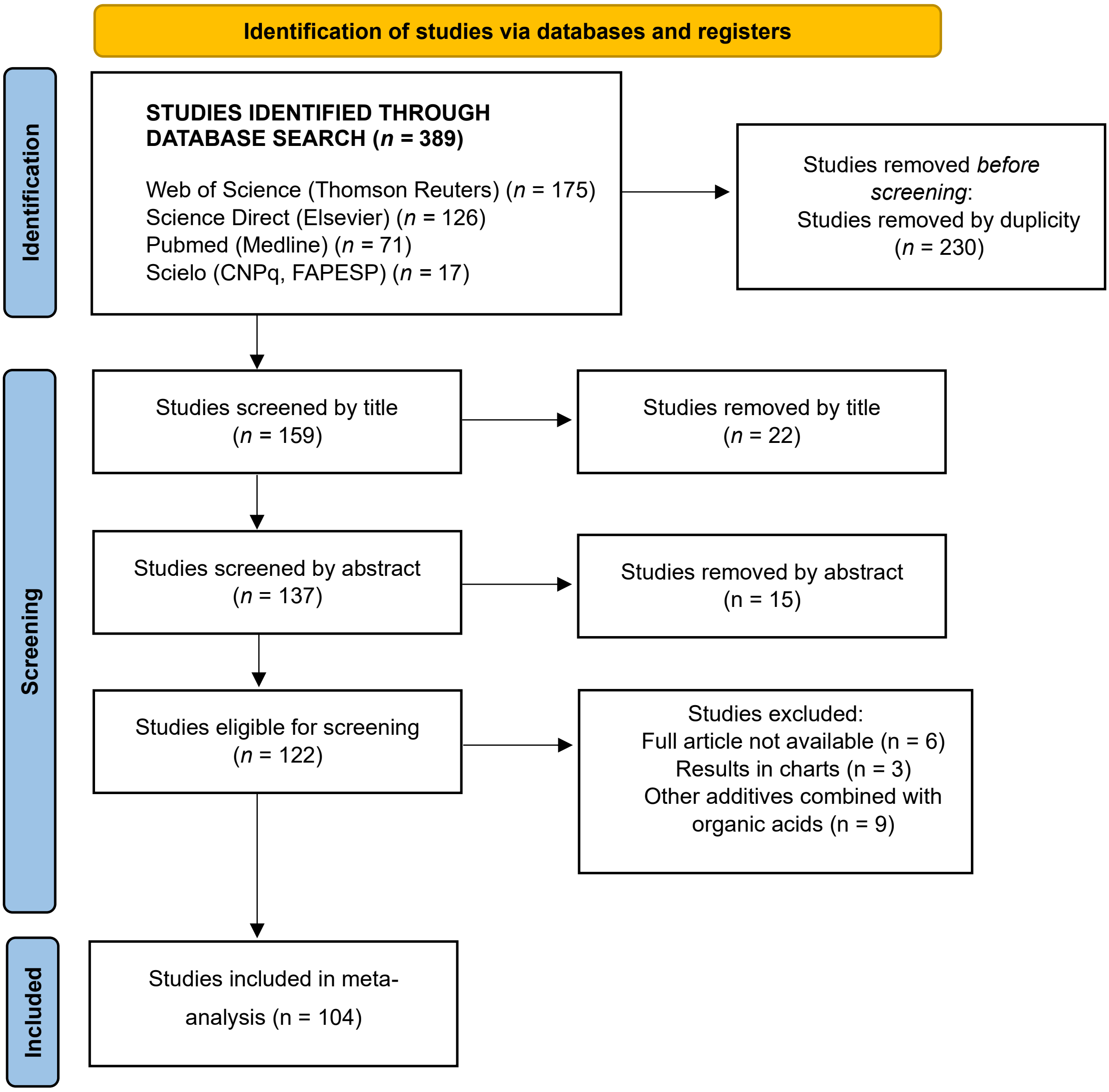
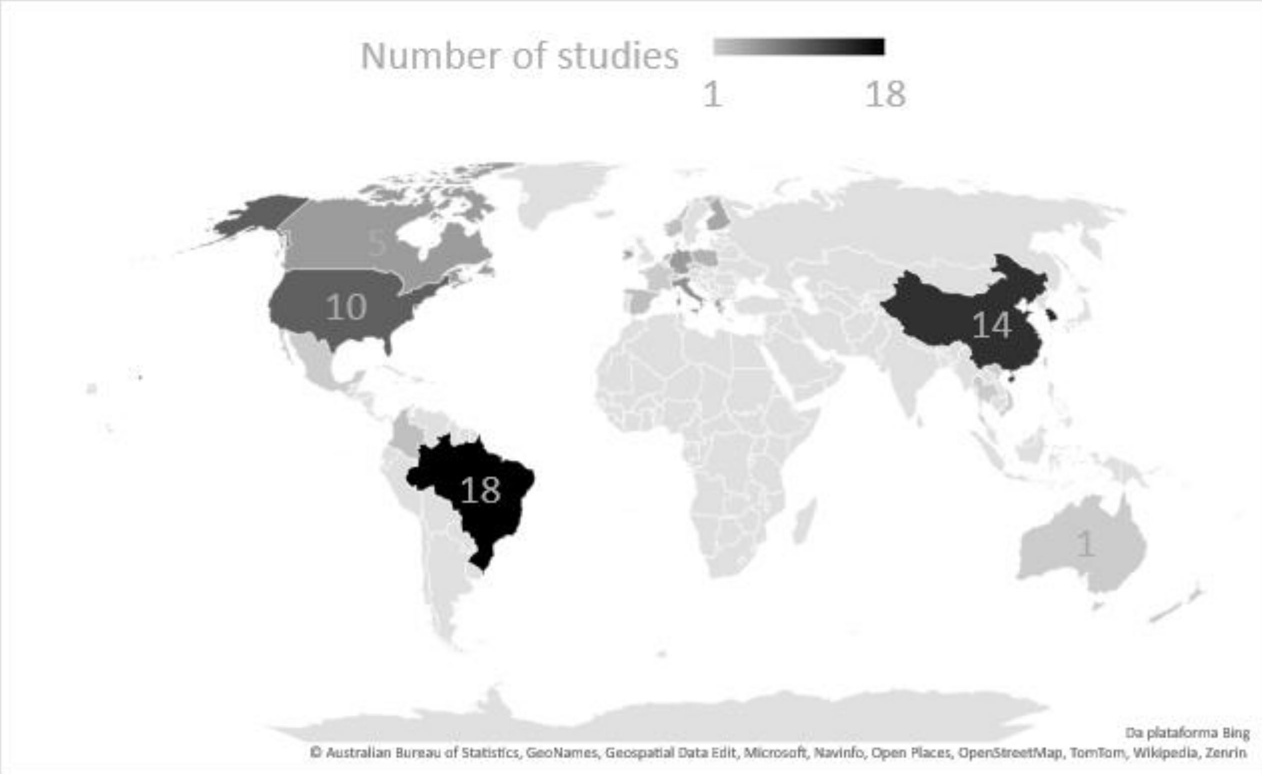


Figure 2. Geographical distribution of the studies included in the meta-analysis



CAPÍTULO IV[3]

[3] Este capítulo corresponde ao artigo redigido e formatado no modelo da revista Livestock Science.

Meta-analysis of organic acid supplementation impact on sow productivity and reproduction

M.S.C. Mendéz, I. Andretta, L. Hauschild

Highlights

In the meta-analysis involving 13 studies, a comprehensive performance assessment was conducted, covering 818 sows and 9,868 piglets

Sodium butyrate emerged as the predominant organic acid used in experiments, featured in 38% of the studies

Organic acids play a role in preserving the body condition of sows during the pos-farrowing period

The number of live births indicates a positive impact of supplementing sows with organic acids

Piglets born to sows supplemented with organic acids exhibit reduced mortality rates and higher weaning weights

Abstract

Swine industry close ties to sow productivity and reproductive performance underscore the importance of factors like the number of weaned piglets and overall meat production. Considering reduced antibiotic growth promoter (AGP) use, organic acids (OA), renowned for their antimicrobial properties and intestinal health benefits, have emerged as alternatives. Despite limited studies, gestational organic acid supplementation has demonstrated positive effects on sow productivity and reproduction. This study conducts a meta-analysis of 13 relevant studies published between 2009 and 2020. Evaluating 818 sows and 9,868 piglets across diverse organic acid types and dosages, sodium butyrate featured in 38% of studies, while blends were observed in 31%. Supplementation with organic acids during gestation and lactation had no significant impact on sow average daily feed intake (ADFI) or body weight (BW) pre-farrowing. However, OA group exhibited a notable 6% elevation in BW (204.36 vs 192.42 kg, P = 0.0266) and a substantial 32% reduction in body weight loss (BWL) compared to the control (CON) group (P = 0.0779). Backfat thickness remained unaffected. Regarding reproductive outcomes, OA group showed a significant 5% increase in weaned piglets (10.83 vs 10.36) and a higher weaning weight (6.15 vs 6.97). Although piglet daily weight gain was not significantly influenced, these results highlight the potential positive impact of organic acids on sow reproductive performance. These findings offer valuable insights into the potential of organic acids as gestational growth promoters, emphasizing their benefits for maintaining sow body condition and enhancing piglet productivity.

Keywords: Additives, growth promotors, maternity, reproduction, swine.

Introduction

Modern pig production is closely linked to sow productivity and reproductive performance, influencing the number of weaned piglets and, consequently, the meat quantity produced (Mirilovic et al., 2016). Management practices, particularly in nutrition, are crucial during gestation and lactation for batch success (Chen et al., 2019). Pigs in production face various stressors, with intestinal health playing a vital role in immunity and resistance to infections. Compromised intestinal health affects nutrient digestion, reducing productivity and increasing susceptibility to diseases (Roselli et al., 2005). With the reduction in antibiotics growth promoters (AGP) in animal production, organic acids, known for their antimicrobial properties, have emerged as alternatives to AGP. These acids create a favorable intestinal environment, promoting beneficial microorganisms associated with improved nutrient digestibility and reduced diarrhea (Liu et al., 2018). Supplementation with organic acids enhances pig productivity and overall health, showing positive results in both productivity and reproduction, even during gestation (Kluge et al., 2010; Devi et al., 2016).

Organic acids, employed in animal nutrition for decades, exhibit a strong antibacterial effect against gram-negative bacteria, contributing to a balanced gastrointestinal tract and greater resistance to intestinal diseases (Ferket, 2003). Supplementation with organic acids during gestation contributes to higher average daily gain in piglets and increased weaning weight (Overland et al., 2009). Sows supplemented with organic acids show less weight loss and backfat thickness reduction during weaning, indicating potential benefits for maintaining sow body condition and improving piglet productivity (Luckstadt et al., 2011). While some studies report conflicting results on the direct effects of organic acids on sow body condition during pregnancy and lactation (Mroz et al., 1998), there is a tendency for larger litters and higher piglet weights at weaning.

These findings highlight the potential of organic acids as growth promoters during gestation, aiding in maintaining sow body condition and enhancing piglet productivity. However, the actual influence of organic acids on these zootechnical variables remains controversial. This study aims to conduct a meta-analysis to evaluate the influence of organic acids on sow productive and reproductive performance, as well as their impact on supplemented sow piglet performance.

Material and methods

Literature Search Strategy and Criteria for Study Selection

A systematic literature review following to the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement (Moher et al., 2009) was conducted. An electronic search was performed on digital databases (PubMed, Scielo, ScienceDirect, and Scopus) from January to March 2023, utilizing a combination of keywords to identify pertinent scientific articles for database construction (see Table 1 for the keywords used). The review question was formulated using the PICo strategy, specifying 'Population,' 'Intervention,' and 'Context.' Population: pregnant sows, Intervention: organic acids or salts of organic acids, Context: productive and reproductive performance. All retrieved results from each digital database were imported into a reference manager to streamline the article selection process.

The selection procedure was meticulously documented following the PRISMA methodology, with duplicate records excluded. Initially, each title and abstract of the selected scientific papers were careful screened to identify those eligible for full-text assessment. The final database consisted of complete articles that met predefined eligibility criteria: a) our study included research published in indexed journals, excluding short communications, reviews, and conference abstracts/papers; b) the research focused on in vivo experiments involving pregnant sows; c) the assessment covered reproductive and productive variables, including live births, weaned piglets, birth and weaning weights of the piglets, and sow average daily feed intake, live weight and backfat thickness; d) the dietary supplementation involved organic acids for sows during gestation.

Extraction and compilation of data

Information extracted from the "Material and Methods" and "Results" sections of each chosen scientific paper was input into an electronic spreadsheet. The dataset was organized with a column dedicated to qualitative details, encompassing information such as authorship, journal, publication year, country of origin, sow strain, treatment designation, and the specific organic acid utilized. Furthermore, the dataset incorporated columns for various quantitative exploratory and descriptive factors, such as the number of replications, quantity of animals, experiment duration, organic acid dosage, and zootechnical parameters.

The methodology for constructing the database and encoding data adhered to principles established in previous literature (Sauvant et al., 2008). To account for variations among scientific papers and influence the statistical model's outcomes, each selected paper was assigned a unique code (ID) as a moderating variable. As a concluding step in database construction, all productive and reproductive variables were standardized concerning the control group (non-supplemented with organic acids). This allowed for the monitoring and analysis of percentage variations in the different variables relative to the control (CON) group. The methodology entailed assigning a value of 100% to each variable for the control group, while the other group (OA) received varied percentages (either positive or negative) relative to this control (CON) group. This approach aimed to minimize variability in responses across studies in the database, fostering discussion and analysis when comparing different groups.

Statistical Analysis

The examination of variables encompassed key productivity indicators, such as the average daily feed intake (ADFI) during gestation and lactation, pre- and post-farrowing body weight (BW), and pre- and post-farrowing backfat thickness (BT). Regarding piglets, the focus was on performance variables such as live births (LBP), weaned piglets (WP), birth weight (BWP), weaning weight (WWP), and average daily gain (ADGP). Additionally, a comparative analysis was conducted in a relative context, wherein the outcomes of treatments supplemented with organic acids were juxtaposed with their corresponding control treatments, expressed as a percentage of variation. This approach aimed to mitigate variations across raw data from diverse experiments integrated into the database.

The statistical model employed for variance analysis utilized the R 4.2.1 (R Core Team, 2022) statistical software. The data underwent analysis of variance using the lme4 (Fitting Linear Mixed-effects Models), lmerTest (ANOVA Tables for Linear Mixed Models), and emmeans (Estimated Marginal Means for Linear, Generalized Linear, and Mixed Models) packages designed for linear mixed-effects models. General Linear Mixed Models (GLMM) were applied to evaluate treatment effects, with fixed effects incorporating the additive factor (control or organic acid) and random effects accounting for study variations identified by a unique code ID. Below is the presentation of the statistical model employed in the analysis:

$$\boldsymbol{Y_{ijk} = \mu + \tau_i + \alpha_k + \varepsilon_{ijk}}$$

in which $\boldsymbol{Y_{ijk}}$ is the response, $\boldsymbol{\tau_i}$ is the fixed effect of treatment (control or organic acid), and $\boldsymbol{\beta_j}$, $\boldsymbol{\alpha_k}$ is the random effect of study, and $\boldsymbol{\varepsilon_{ijk}}$ is the residual variation.

The selection of the linear mixed model methodology was based on its suitability for constructing empirical models in experimental designs related to biological responses. Significance in the analysis of variance was determined by p-values, with a criterion set at p < 0.05 for each variable. For cases where p-values fell between 0.05 and 0.10, the effect was considered tendency to significance.

Results

Systematic review and database description

The findings from the systematic review, conducted using the PRISMA methodology, are presented in Figure 1. Initially, 157 articles were identified. After eliminating duplicate entries and articles that didn't meet the predetermined inclusion criteria, we selected a final set of 13 articles. The primary reasons for excluding articles included: a) mixing AO with other compounds; b) literature reviews; c) experiments involving species different from the target; d) full articles not available in the selected databases.

The database encompasses 13 scientific papers, published between 2009 to 2020 and disseminated across 9 peer-reviewed journals. Table 2 summarize relevant selected studies information. These studies predominantly appeared in specific journals: Livestock Science (31%), Animal Science Journal (15%), Italian Journal of Animal Science, Tropical Animal Health, and Production, Revista Brasileira de Zootecnia, Animal, Animal Feed Science and Technology, Journal of Animal Science, and Veterinary Research (8% each), comprising 56%. Figure 2 illustrates the geographic distribution of these studies. Most of the research was conducted from China (46%), followed by Korea and Germany (15% each), Netherlands, United States and Brazil (8% each), totalizing 24%.

In the 13 studies, a performance evaluation was conducted on a total of 818 sows, and 9,868 piglets. The database consisted of 43 lines in the electronic spreadsheet, where attributes like organic acid type and zootechnical variables were evaluated. The study examined common dietary supplements of OA, including their dosage. Predominantly, sodium butyrate was the most common organic acid used in the experiments (38% of the studies). Blends of organic acids were observed in 31% of studies, followed by the salt potassium formate (15%), citric acid, and benzoic acid (8% each). The average OA dosage was 0.40%, ranging from 0.10% to 2.00%.

Sow performance and body composition

Table 3 provides a summary of these findings. No significant distinction was observed in the ADFI and ADFI variation between the CON group and the OA group during gestation and lactation periods (P = 0.8606 and P = 0.3391, respectively). While supplementing with OA during pre-farrowing, there was no significant impact on the BW of sows (P = 0.1028). In contrast, the OA group displayed a notable 6% elevation in body weight (204.36 kg) compared to the CON group (192.42 kg), P = 0.0266. These results showcased a substantial 32% reduction in body weight loss (19.26 kg) relative to the CON group (28.42 kg). Although P = 0.0779 approached significance, the magnitude of the reduction is noteworthy. Backfat thickness (BT) remained unaffected by the supplementation of organic acids, both before and after farrowing (P = 0.1898 and P = 0.6563, respectively). Similarly, there was no significant variation in BT during the two periods (P = 0.2065).

Reproductive outcomes

Table 4 offers a concise overview of these results. The trend in the number of live births piglets suggested a positive influence of OA, although not statistically significant (P = 0.0977). In the OA group, the number of weaned piglets (10.83) demonstrated a noteworthy 5% increase compared to the CON group (10.34), with a statistically significant difference (P = 0.0377). Piglet birth weight exhibited a tendency to be higher in the group of sows supplemented with OA (P = 0.0771). Notably, piglets in the OA group had a weaning weight of 6.15 kg, reflecting a 3% increase compared to the CON group (5.97 kg), with statistical significance (P = 0.0339). However, the average daily weight gain of piglets was not influenced significantly by the supplementation of sows with OA (P = 0.5847).

Discussion

The present findings are in line with earlier research indicating that piglets from sows supplemented with organic acids tend to have higher weights at weaning (ØVERLAND et al., 2009; BALAMURALIKRISHNAN et al., 2016; LAN et al., 2018) compared to those from non-supplemented sows. However, a study by LIU et al. (2014) reported no discernible differences in the weaning weight of piglets from sows supplemented with organic acids, contrasting our results. Nonetheless, an examination of colostrum composition revealed a higher percentage of immunoglobulins compared to sows in the control group (without organic acids). While no significant effects of supplementation were observed for other variables analyzed in the meta-analysis, including postpartum weight variation, backfat thickness average daily feed intake live births, piglet birth weight, and piglet average daily gain (kg/d), organic acid (OA) supplementation did lead to less weight loss in pos-farrowing sows, contributing to the maintenance of body condition. These results are consistent with prior studies indicating reduced postpartum weight loss (BALAMURALIKRISHNAN et al., 2016) and a lesser reduction in backfat thickness during the weaning period (LÜCKSTÄDT et al., 2011). Interestingly, our study diverges, as backfat thickness was not influenced by OA supplementation.

Observations by CHEN et al. (2019) indicating a lower incidence of diarrhea in piglets from sows supplemented with organic acids suggest that such supplementation during gestation may enhance intestinal health at birth, subsequently improving productive performance and reducing mortality. This observation aligns with our meta-analysis results, revealing a significantly higher number of weaned piglets in sows supplemented with organic acids compared to those without supplementation. Additionally, piglets from sows that received OA during gestation exhibited higher weights at weaning. While not the primary focus of this study, it's noteworthy that other researchers have documented that organic acid supplementation increased nutrient digestibility in sows (KLUGE et al., 2010) and elevated immunoglobulin levels in colostrum (ØVERLAND et al., 2009; LIU et al., 2014). These findings highlight the significance of organic acid use in pig farming, especially during the critical weaning period for piglets, contributing positively from birth onward.

Conclusion

In conclusion, organic acids emerge as promising substitutes for growth-promoting antibiotics in pig farming, particularly during the nursery, growing, and finishing phases. Their application offers significant advantages in terms of enhancing both productive performance and intestinal health in swine. Despite the relatively limited number of studies available, organic acid supplementation demonstrates a clear potential to exert positive influences on key productive and reproductive variables in sows. This includes improvements in the quantity and weight of weaned piglets, coupled with a noteworthy reduction in postpartum and weaning weight loss. As the industry seeks sustainable and effective alternatives to traditional antibiotics, the encouraging findings surrounding organic acids underscore their role as an asset in advancing swine nutrition and production practices. Further research and exploration of the nuanced impacts of organic acids are warranted to unlock their full potential and contribute to the continued evolution of modern pig farming practices.

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Table 1. Outlines the search terms employed for the PICo methodology, encompassing Population, Intervention, and Context, as utilized in the systematic review

Acronym	Search string
Population (P)	"suckling pig" OR "suckling piglets" gilt OR gilts OR sow OR sows OR "reproducing sows" OR "reproducing sow" OR "lactating sow" OR "lactating sows" OR "pregnant sow" OR "pregnant sows"
Intervation (I)	"organic acid" OR "organic acids" OR "organic acid salt" OR "acetic acid" OR "sodium acetate" OR "formic acid" OR "sodium formate" OR "propionic acid" OR "sodium propionate" OR "butyric acid" OR "sodium butyrate" OR "malic acid" OR "sodium malate" OR "lactic acid" OR "sodium lactate" OR "citric acid" OR "sorbic acid" OR "sodium sorbate" OR "potassium sorbate" OR "calcium sorbate" OR "fumaric acid" OR "sodium fumarate" OR "benzoic acid" OR "sodium benzoate"
Context (Co)	"reproductive performance" OR lactation OR pregnancy OR reproduction OR "sow performance" OR "sow litter" OR "sow litters" OR progeny OR "sow progeny" OR offspring

Table 2. Description of the meta-analysis database

Code	First author	Year	Country	Sows (n)	Organic acid/salt	Dosage (%)	Sow	Piglet
1	Øverland	2009	Netherlands	156	potassium diformate	0.80 - 1.20	(+)	(+)
2	Liu	2014	China	48	citric acid	0.50 - 1.50	NS	NS
3	Balasubramanian	2016	Korea	60	blend	0.50 - 1.00	(+)	(+)
4	Lan	2018	China	36	blend	0.10 - 0.20	NS	(+)
5	Chen	2019	China	24	sodium butyrate	0.10	(+)	(+)
6	Devi	2016	Korea	12	blend	0.10 - 0.20	NS	(+)
7	Kluge	2010	Germany	28	benzoic acid	0.50 - 2.00	(+)	NS
8	Lückstädt	2011	Germany	15	potassium diformate	0.80	(+)	NS
9	Chen	2018	China	112	blend	-	(-)	(+)
10	He	2016	China	40	sodium butyrate	0.50	NS	(+)
11	Jang	2017	United States	43	sodium butyrate	0.50 - 1.00	NS	NS
12	Vieira	2020	Brazil	192	sodium butyrate	0.10 - 0.20	(+)	(+)
13	Wang	2014	China	52	sodium butyrate	0.60	(+)	(+)

[1] NS = no significant effects; (+) OA positive significant effects; (-) OA negative significant effects

- no information

Table 3. Performance and body composition of sows supplemented with dietary organic acids

Sow assessment	Treatment			SEM[2]	*P-Value*[3]	R^2
	Control	Organic acids	Δ[1]			
Gestation						
Average daily feed intake, g/d	2,467	2,472	0%	147,12	0,8606	74%
Lactation						
Average daily feed intake, g/d	5,625	5,735	2%	232,52	0,3391	85%
Variation, g/d	3,158	3,265	3%	132.39	0,3866	83%
Pre-farrowing						
Body weight, kg	220.83	222.81	1%	18.14	0,1028	98%
Pos-farrowing						
Body weight, kg	192.42 b	204.36 a	6%	12.51	0,0266	78%
Body weight loss, kg	28.42	19.26	-32%	3.85	0,0779	21%
Pre-farrowing						
Backfat thickness, mm	19.89	19.52	-2%	4.69	0,1898	89%
Pos-farrowing						
Backfat thickness, mm	15.80	15.54	-2%	5,82	0,6563	69%
Backfat thickness loss, mm	3.72	4.34	17%	0,65	0,2046	67%
n[4]	28	36				

[1]Variaton from control group not supplemented with organic acids

[2]Standard Error of the Mean (SEM)

[3]Probability of fixed effetcs: treatments control or organic acid supplementation during sow gestation.

[3]Means followed by different letters differ statistically at the level 5% of significance.

[4]Number of observations in each treatment

Table 4. Reproductive performance of sows supplemented with dietary or organic acids

Piglets assessment	Treatment			SEM[2]	*P-Value*[3]	R^2
	Control	Organic acids	Δ[1]			
Live births, n	12.06	12.48	3%	0.31	0.0977	62%
Weaned, n	10.34 b	10.83 a	5%	0.33	0.0307	76%
Birth weight, kg	1.46	1.49	2%	0.03	0.0771	81%
Weaning weight, kg	5.97 b	6.15 a	3%	0.92	0.0039	95%
Average daily gain, kg/d	0.22	0.22	1%	0.06	0.5847	84%
n[4]	25	29				

[1]variaton from control group not supplemented with organic acids

[2]Standard Error of the Mean (SEM)

[3]Probability of fixed effetcs: treatments control or organic acid supplementation during sow gestation.

[3]Means followed by different letters differ statistically at the level 5% of significance.

[4]Number of observations in each treatment

Figure 1. Prisma Flow Diagram of the Systematic review and selection process

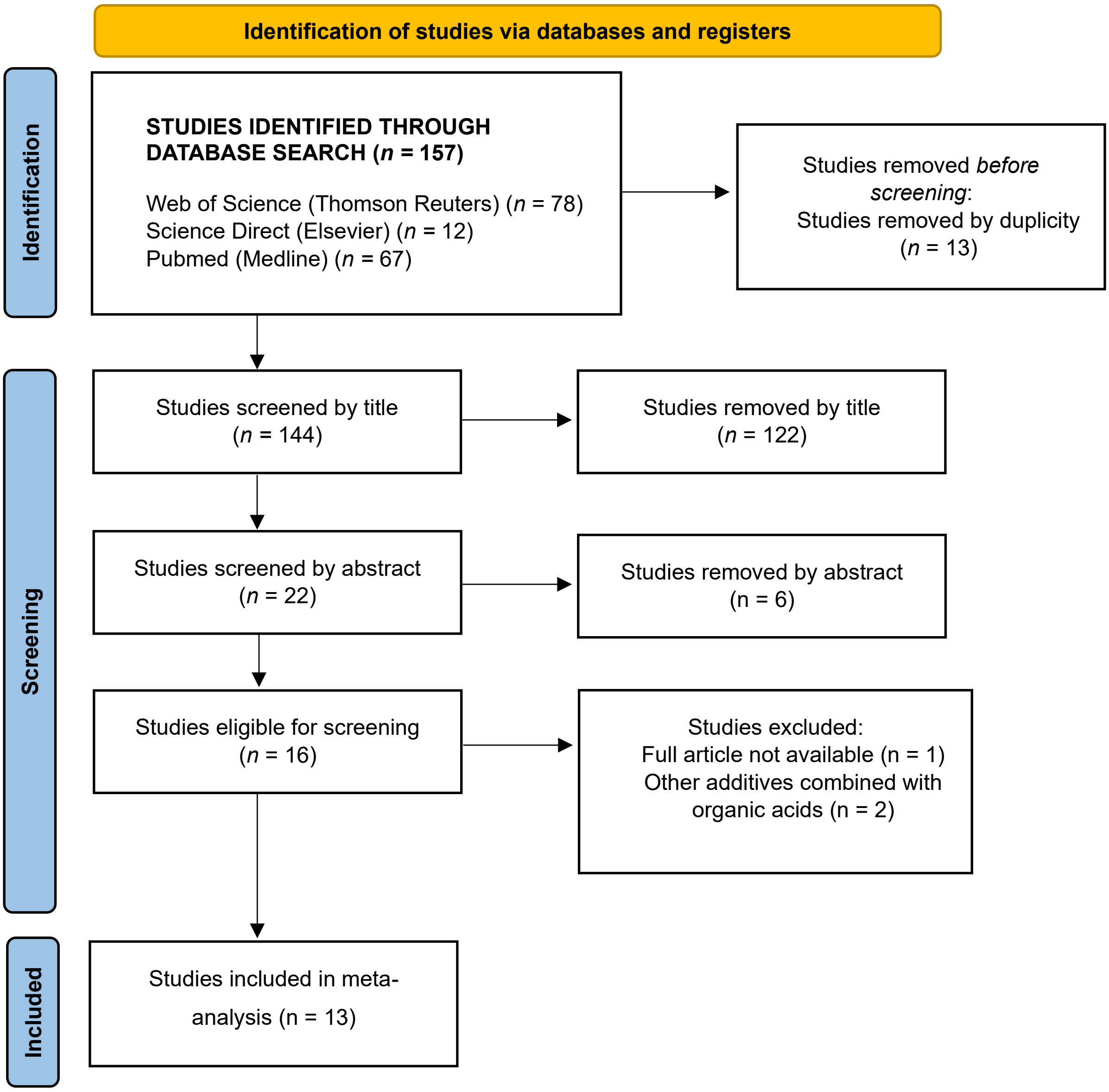
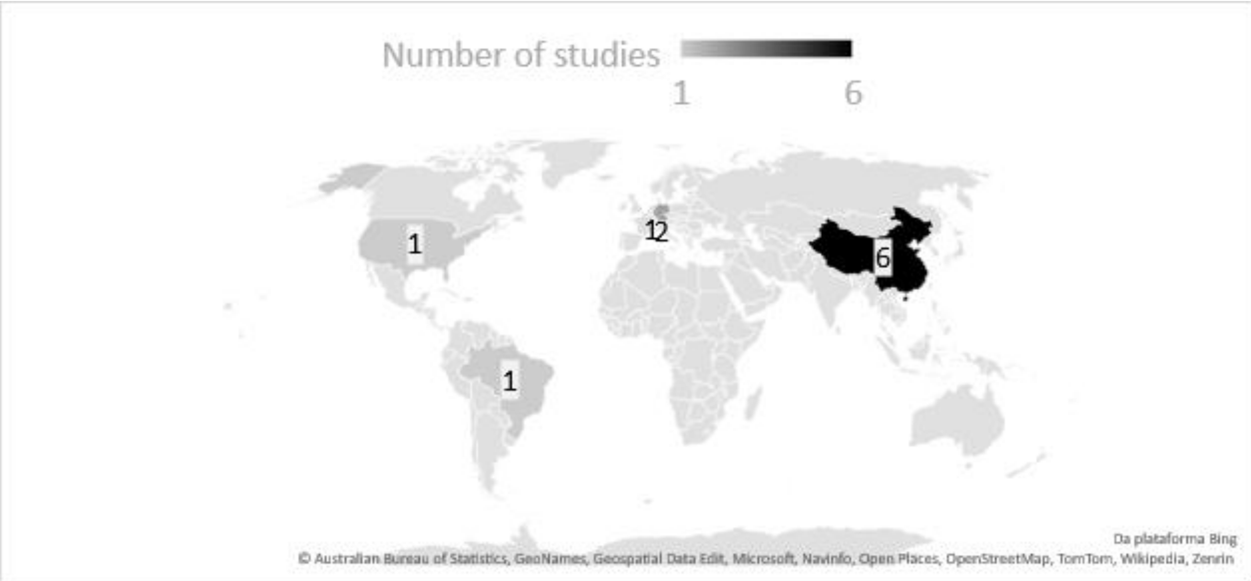


Figure 2. Geographical distribution of the studies included in the meta-analysis



CAPÍTULO V

3. CONSIDERAÇÕES FINAIS

As restrições de uso aos antibióticos promotores de crescimento na produção animal é uma questão que tem ganhado cada vez maior notoriedade. Estas questões vêm sendo amplamente discutidas pelo mercado consumidor, por grandes investidores e pelos setores produtores e exportadores de proteína animal, fomentando maiores discussões nos setores ligados à pesquisa e tecnologia. Diante disto, considerando o importante papel que os APC desempenharam, e desempenham ainda, na produção animal, encontrar alternativas eficazes ao uso destes compostos é de suma importância para a manutenção da saúde animal nas granjas e, consequentemente, a manutenção de bons índices de performance produtiva.

Neste cenário, revisões sistemáticas e meta-análises se demonstram como ferramentas bastante úteis no levantamento de informações relevantes para a produção animal. A realização de um estudo meta-analítico se apresenta como uma alternativa economicamente viável e cientificamente útil ao reanalisar dados previamente publicados em pesquisas, além da otimização no uso de recursos financeiros. Estes fatores se somam às limitações éticas e práticas que englobam a realização de experimentos *in vivo*, possibilitando a geração de dados relevantes para a produção animal a partir de estudos previamente publicados.

Esta longa revisão sistemática buscou explorar diferentes categorias e fases do ciclo produtivo de suínos: gestação, maternidade, creche, crescimento, terminação. Além disto, dentro da fase de creche, um estudo foi realizado visando avaliar esta fase crítica junto a condição de desafio sanitário experimental, visando explorar dados da literatura que buscassem retratar a exposição a patógenos do trato digestivo no período pós-desmame – situação muito comum no ambiente produtivo. Por meio da meta-análise foi possível demonstrar que os ácidos orgânicos são uma alternativa promissora à substituição dos APC na suinocultura, podendo contribuir desde o aumento no número de leitões desmamados na maternidade até o aumento da eficiência alimentar nas fases de crescimento e terminação.

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