

Article - Human and Animal Health

Implication of Dietary Acidifiers for Growth Performance and Intestinal Morphometry in Nursery Piglets

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Editor-in-Chief: Paulo Vitor Farago

Associate Editor: Paulo Vitor Farago

Received: 16-Aug-2023; Accepted: 06-May-2024

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HIGHLIGHTS

- Sanitary challenge negatively affects nursery piglets performance.
- Acidifiers for nursery piglets improved feed conversion ratio and change intestinal morphology.
- Organic acids and blends are more effective in nursery piglets.

Abstract: This study was conducted to assess the use of dietary acidifiers and their effects on the growth performance and intestinal morphometry of challenged and non-challenged nursery piglets. A total of 18,597 piglets distributed across 1,300 experimental groups from 128 papers published between 1984 and 2020 were included in the meta-analysis. All treatments were categorized as negative control (CON), organic acid (OAC), salts of organic acids (SAL), and blends of acidifiers (BLE). The presence (+) or absence (-) of health challenges in each study was also considered. The meta-analysis was conducted sequentially via graphical, correlation, and variance-covariance analyses. Piglets weighed between 8.4 and 15.8 kg and were assessed at 29.4–48.3 days of age. The addition of OAC, BLE, and SAL to the diets improved the feed conversion ratio (FCR) of piglets ($P < 0.001$) by 5.3%, 3.6%, and 3.6%, respectively, compared to CON. Challenged piglets consumed 7.7% less feed ($P < 0.05$) than the non-challenged piglets. Addition of OAC to diets reduced stomach pH by 8.6% ($P < 0.05$) compared to CON piglets. The OAC and BLE diets reduced jejunum pH by 2.7% and 2.1% ($P < 0.05$), respectively, compared to CON piglets. Challenged piglets had a 14.8% lower ($P < 0.01$) villus height in the ileum compared non-challenged piglets. Acidifiers reduced the crypt depth in the jejunum of piglets by 17.4% ($P < 0.05$) compared to the CON group. Acidifiers in the diet of nursery piglets improve performance by reducing pH in the gastrointestinal tract and indirectly improving intestinal integrity. Blends improved the performance of nursery piglets.

Keywords: organic acids; meta-analysis; sanitary challenge.

INTRODUCTION

The weaning of piglets can be challenging in intensive pig production systems and is associated with abrupt changes in the environment and diet as well as emotional stress in piglets. Weaning is typically performed before the gastrointestinal system is fully developed. The digestive and absorption capacity of the intestinal system is limited by insufficient production of hydrochloric acid and pancreatic enzymes [1]. Piglets are predisposed to a greater susceptibility to diseases caused by pathogens, resulting in diarrhea and desquamation of the intestinal epithelium [2]. This desquamation shortens the villi and increases crypt depth, thereby compromising nutrient absorption capacity. This is particularly true for piglets housed under poor sanitary conditions [3]. One way to control this situation is to supplement the diet of nursery piglets with acidifiers.

Acidifiers reduce gastric pH, resulting in a more conducive environment for digestive enzymes, especially proteases that require a low pH for zymogen activation [4]. Furthermore, this reduction in pH induces microbial selectivity as it inhibits the growth of pathogenic bacteria that colonize the gastrointestinal tract in more alkaline environments [5]. At the intestinal level, the morphometry of the epithelium was shown to be enhanced either by better nutrient utilization, by the trophic effect on the villus caused by the acidifier, or through colonization by beneficial bacteria [6]. Overall, these effects have been reported to improve the growth performance of piglets.

However, the response to acidifiers in *in vivo* assays varies depending on the type and level of acidifiers and experimental factors affecting the response of the animal, such as the age at weaning, housing type, sanitary challenge, and feed quality. Moreover, the integration of this information is challenging. In this context, a meta-analytic approach is the most suitable method for collating and synthesizing previously published results on a subject with novel conclusions [7]. Therefore, the present study aimed to estimate the performance and intestinal morphometry in challenged and non-challenged nursery piglets feed acidifiers using a meta-analysis approach.

MATERIAL AND METHODS

Selection of articles and elaboration of the database

Indexed publications based on *in vivo* experiments involving nursery piglets fed diets supplemented with acidifiers were selected from the search engines Elsevier, ScienceDirect, SciELO, and Google Scholar (Figure 1). Only papers reporting the performance and intestinal morphometry of nursery piglets and experiments applying different types and supplementation levels of acidifiers were retained. The selected manuscripts were critically evaluated in terms of quality and their relevance to the objectives of the study, experimental design, treatments, variables, and data analysis. The eligibility criteria were post-weaned and nursery piglets, results for dietary acidifiers containing a negative control without additives, with or without sanitary or environmental challenges, performance, and intestinal morphometry results. The outcome of a single study (i.e., whether acidification was beneficial) was not considered a criterion for inclusion in this database. The reasons for excluding the publications were as follows: results were shown as graphics or images; is outside the objective of this meta-analysis, such as a pig at the end of its growth period; and being published without any evaluation criteria. To be considered a sanitary challenge, the experiment should have been carried out on commercial farms (such as those with or without a previous history of sanitary problems), poor housing (as described in the study), or when pathogens such as strains of *Escherichia coli* were added intentionally to the environment or supplied directly to the piglets. Bacterial challenge information, such as strain type, concentration, challenge time, and number of challenged animals, were tabulated in the database. No study indicated ambient temperature as a challenge effect.

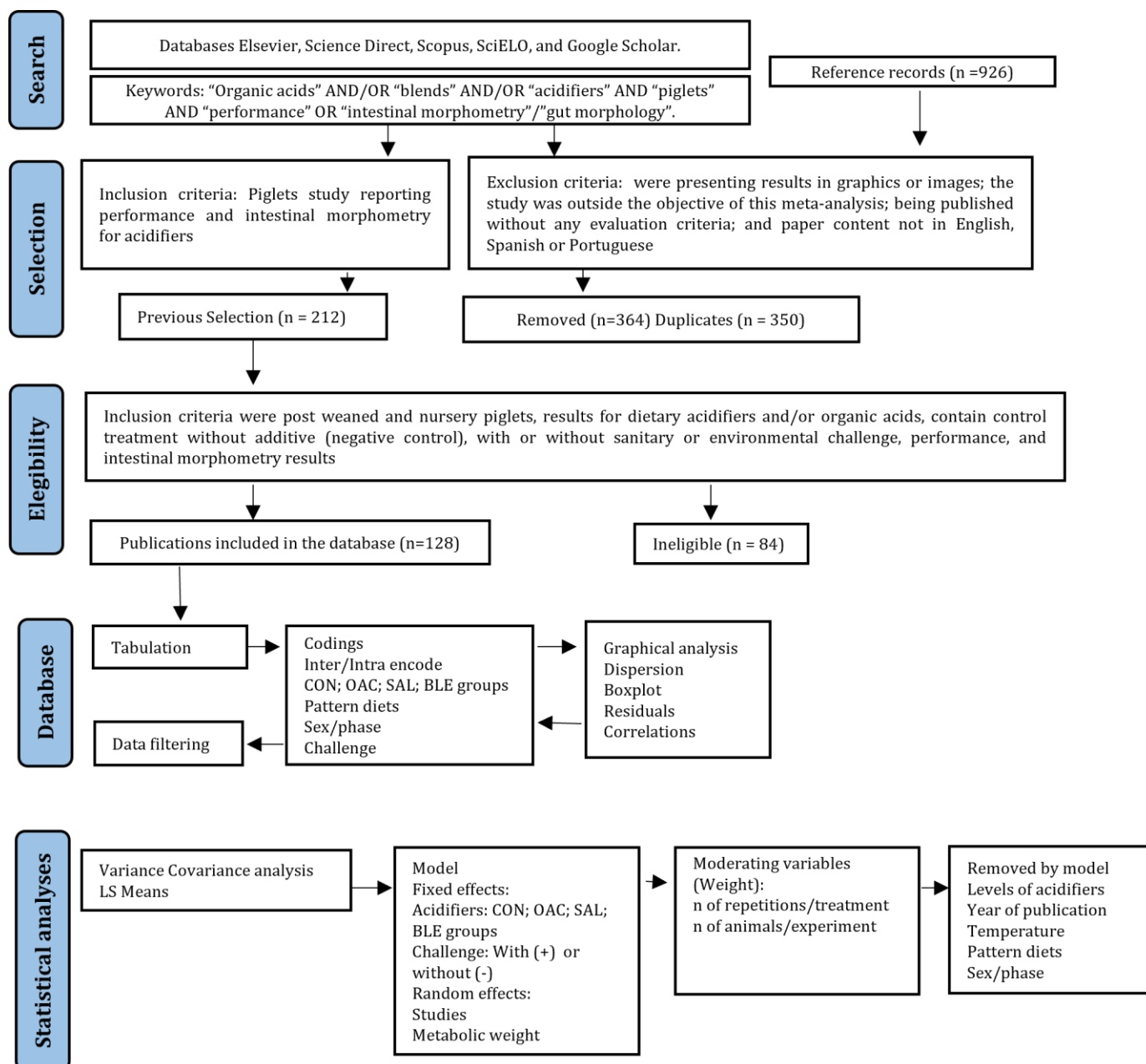


Figure 1. Flow diagram of the applied methodology

Tabulation and coding

A database with information specific to each selected paper was created using Microsoft Excel (2013). The tabulated data included bibliographic aspects (authors, year, journal, country, and institution of origin), experimental characteristics (experimental design, dietary ingredients, type and form of acidifiers, acidifiers inclusion levels in the diet, nutritional composition, ambient temperature, age and weight of piglets, and sanitary challenge) (Table 1), the variables evaluated (growth performance in terms of average daily feed intake, average daily weight gain, and feed conversion ratio), and the intestinal morphometry and pH content of gastrointestinal tract.

Graphical analysis was conducted to explore the distribution of the data and obtain a global view of the coherence and heterogeneity of the data. Through this analysis, hypotheses were established, and the statistical model was defined [8]. The dependent and independent variables were defined, and the data were codified for the analysis of inter- and intra-experimental effects based on [8,9] methods. Briefly, sequential numbers were used to encode each paper (general encoding), each treatment within an experiment (inter encoding, i.e., each treatment received a sequential number and was concatenated to the previously given paper code or when a paper had more than one experiment in the same form), and repeated measures for different time intervals or dose when available (intra encoding). Additional encodings were conducted to facilitate the graphical and statistical analysis of the database.

Table 1. Descriptive statistics of nursery piglets submitted or not to a sanitary challenge and receiving diets supplemented with acidifiers.

Variables	n	Mean	SE	SD	Min	Max
Piglets						
Initial Age, d	1396	29.5	0.283	10.5	4	38
Final Age, d	1396	48.4	0.357	13.3	17	60
Initial Body weight, kg	1483	8.42	0.092	3.56	2.60	10.1
Final Body weight, kg	1483	15.8	0.178	6.74	4.50	17.5
Calculated composition diet						
Energy metabolizable, kcal/kg	627	3,360	6.61	176	3,081	4,323
Crude protein, %	1220	20.0	0.050	1.66	15.5	28.8
Lysine T, %	1251	1.29	0.005	0.19	0.82	1.93
Methionine T, %	604	0.44	0.005	0.12	0.26	0.91
Threonine T, %	630	0.79	0.007	0.17	0.21	1.30
Tryptophan T, %	465	0.25	0.002	0.05	0.12	0.54
Calcium T, %	1184	0.82	0.010	0.36	0.12	1.10
Phosphorus T, %	1187	0.63	0.004	0.14	0.07	1.33
Acidifier Groups						
OAC, % diet	363	1.16	0.047	0.89	0.05	6.00
SAL, % diet	116	0.67	0.069	0.63	0.02	1.80
BLE, % diet	440	0.88	0.042	0.76	0.03	3.00

n, number of treatments; SE, standard error of mean, T, expressed as Total, OAC, organic acids; SAL, salts of organic acids; BLE, blend of acidifiers.

Description of the database

The database included 128 papers published in international journals from 1984 to 2020 (mode: 2006), comprising 18,597 piglets (data available upon request from the authors). The data were distributed across 1,493 rows and 181 columns. Antibiotic-containing treatments were excluded. Most papers were published in Europe (29%), North America (27%), and Brazil (22%). The most widely used acidifiers in the selected papers were organic acid (OAC; 43%), blends of acidifiers (BLE; 38%), and salts of organic acids (SAL; 10%). The most used compounds were fumaric acid (9%), benzoic acid (7%), sodium butyrate (4%), citric acid (4%), and formic acid (3%). The majority (59%) of the papers used hybrid piglets from crossbreeds, 2% used pure breeds, and 39% did not report the genetic lineage. Barrow piglets accounted for 42% of the piglets, female piglets accounted for 37%, and 20% of the papers did not report any gender information.

Statistical analysis

Analysis of variance was conducted by applying a generalized linear model with covariate adjustment (LS-means). In all analysis was considered the significance level at 5%. Initial body weight was examined as a covariate using the Fisher test ($P < 0.05$) and included in the statistical model. The effects of the type of acidifier (CON, control; OAC; SAL; and BLE), sanitary challenge (challenged versus non-challenged piglets), and the interaction between the type of acidifier and sanitary challenge were tested using the LSD Fisher test. Information on previous history of sanitary problems on commercial farms, poor housing, and bacterial challenge (species, strain, concentration, challenge time) were grouped together as sanitary challenges. Individually, these factors were not measured and could not be included in the statistical model owing to limited data availability. Additionally, interactions for intestinal morphology, pH, and sanitary challenges were not tested owing to limited data availability.

Moderating variables, such as the number of repetitions per treatment and the number of animals per experiment, were used to weight the analysis of variance. Prediction equations were established to evaluate the relationship between ADFI (average daily feed intake) and sanitary challenges using the variance-covariance method. The intercepts of the equations were associated with maintenance requirements, and the slopes were associated with changes in the daily feed intake. The adjusted R^2 was the criterion used for selecting the best models. All analyses were performed using MINITAB 18 software (Minitab Inc., State College, PA, USA).

RESULTS

In the variance analysis, metabolic weight was the most factor that affected ($P < 0.001$) the model (Table 2). In terms of performance, there was no interaction ($P > 0.05$) between the sanitary challenge and acidifiers. Addition of an acidifier did not affect ($P > 0.05$) the ADWG (average daily weight gain) and ADFI of piglets. The addition of OAC, BLE, and SAL improved the feed conversion ratio (FCR) of piglets ($P < 0.001$) by 5.3%, 3.6%, and 3.6%, respectively, compared with the CON group. Non-challenged pigs had a higher ADFI ($P < 0.05$) compared to piglets subjected to sanitary challenge. Sanitary challenge resulted in a significant decrease (-5.5%) in daily feed intake compared to non-challenged piglets. Additionally, the equations implied that the same ADFI for challenged piglets had lower nutrient availability for body weight at maintenance requirement ($BW^{0.60}$).

Table 2. Growth performance of nursery piglets submitted or not to a sanitary challenge and receiving diets supplemented with acidifiers.

Item		ADFI, kg/d		ADWG, kg/d		FCR, kg/kg	
		n	LS-means	n	LS-means	n	LS-means
Treatment							
Acidifier	CON	383	0.573	388	0.352	371	1.67 ^a
	OAC	360	0.568	368	0.363	355	1.58 ^b
	SAL	116	0.571	117	0.360	114	1.61 ^b
	BLE	441	0.568	440	0.360	428	1.61 ^b
Challenge	-	361	0.593 ^a	352	0.366	352	1.64
	+	939	0.547 ^b	961	0.352	916	1.60
Acidifier xChallenge	CON -	102	0.596	98	0.357	98	1.71
	CON +	281	0.550	290	0.347	273	1.63
	OAC -	112	0.588	109	0.367	109	1.61
	OAC +	248	0.549	259	0.358	246	1.57
	SAL -	41	0.591	40	0.370	40	1.60
	SAL +	75	0.552	77	0.351	74	1.63
	BLE -	106	0.597	105	0.368	105	1.65
	BLE +	335	0.540	335	0.351	323	1.57
P-values							
Acidifier		0.924		0.308		<0.001	
Challenge		0.031		0.330		0.385	
Acidifier x Challenge		0.878		0.909		0.247	
Metabolic weight ($BW^{0.6}$)		<0.001		<0.001		<0.001	
SD		0.09		0.06		0.21	
R ²		0.90		0.84		0.55	

n, number of treatments. ($BW^{0.6}$ metabolic body weight). P-value: probability at 5%. LS-means, least-squares means (Initial body weight as covariate). SD, standard deviation. R², coefficient of determination. Different letters within treatment represent a significant difference by the Fisher LSD test ($P < 0.05$); ns: not significant. Without sanitary challenge (-); With sanitary challenge (+). CON: Control (without addition of acidifiers), OAC: organic acids, BLE: blend of acidifiers, SAL: salts of organic acids. ADWG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio. Challenge (-): $ADFI = -0.5373 + 0.2538 \text{ kg } BW^{0.6}$; $R^2 = 73.9\%$. Challenge (+): $ADFI = -0.5074 + 0.2397 \text{ kg } BW^{0.6}$; $R^2 = 76.1\%$.

Compared to CON diets, the addition of OAC in diets reduced stomach pH by 8.64% ($P < 0.05$) and jejunum by 2.73% ($P < 0.05$), and the addition of BLE in diets reduced jejunum pH by 2.09% ($P < 0.05$) (Table 3). BLE in diets reduced colon pH by 3.9% ($P < 0.05$) compared with CON diets. The addition of salts did not significantly affect the pH value. The pH of the duodenum, ileum, cecum, and rectum of nursery piglets was not affected by any acidifiers.

Table 3. Mean pH values for the different segments of the gastrointestinal tract (GIT) of nursery piglets receiving diets supplemented with acidifiers.

GIT segment	CON		OAC		BLE		SAL		P-value	R ²
	n	pH	n	pH	n	pH	n	pH		
Stomach	27	3.59 ^a	17	3.28 ^b	24	3.44 ^{ab}	11	3.49 ^{ab}	0.042	0.87
Duodenum	15	5.66	13	5.52	22	5.64	2	5.84	0.431	0.78
Jejunum	13	6.21 ^a	10	6.04 ^b	14	6.08 ^b	4	6.19 ^{ab}	0.034	0.95
Ileum	18	6.57	9	6.39	14	6.38	10	6.60	0.062	0.89
Cecum	15	5.93	5	5.95	11	5.83	11	5.94	0.908	0.87
Colon	11	6.34 ^a	3	6.33 ^a	11	6.09 ^b	6	6.31 ^{ab}	0.026	0.91
Rectum	13	6.50	5	6.54	19	6.47	-	-	0.962	0.50

n, number of treatments. -¹ no data. P-value: probability at 5%. Different letters in the same row represent a significant difference by the Fisher LSD test (P <0.05). ns: not significant. R², coefficient of determination. CON: Control (without addition of acidifiers), OAC: organic acids, BLE: blend of acidifiers, SAL: salts of organic acids.

Diets containing OAC, BLE, or SAL did not significantly (P>0.05) affect the villus height of nursery piglets (Table 4). Crypt depth of jejunum was 19.8%, 17.4% and 15.2% lower (P<0.05) in piglets that received BLE, SAL, and OAC diets compared to piglets that received CON diets. Challenged piglets had a 14.8% lower (P<0.01) villus height in the ileum compared to non-challenged piglets. However, sanitary challenge did not alter the crypt depth of nursery piglets.

Table 4. Intestinal morphometry of nursery piglets submitted to a sanitary challenge or not receiving diets supplemented with acidifiers.

Effects		Villus height, μm			Crypt depth, μm		
		Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Acidifiers	CON	296.4	304.8	260.2	291.8	289.0 ^a	242.8
	OAC	299.1	294.9	283.7	271.7	238.8 ^b	252.6
	SAL	296.6	311.6	259.6	280.4	245.0 ^b	250.0
	BLE	312.7	325.8	273.6	292.5	231.7 ^b	235.1
Challenge	-	321.0	290.6	290.8 ^a	- ¹	254.7	250.8
	+	296.2	328.0	247.8 ^b	-	247.5	239.4
Model		Fixed effects probabilities					
Acidifiers		0.595	0.327	0.191	0.515	0.032	0.595
Challenge		0.166	0.619	0.005	-	0.270	0.166
Metabolic weight (BW ^{0.6})		0.780	0.905	0.456	0.600	0.995	0.780
SD		23.98	24.85	16.61	15.98	20.84	15.92
R ²		0.97	0.95	0.94	0.99	0.98	0.97

n, number of treatments. (BW^{0.6} metabolic body weight). -¹ no data. P-value: probability at 5%. SD, standard deviation. R², coefficient of determination. Different letters in the same column represent a significant difference by the Fisher LSD test (P <0.05). ns: not significant. Without sanitary challenge (-); With sanitary challenge (+). CON: Control (without addition of acidifiers), OAC: organic acids, BLE: mixture of acidifiers, SAL: salts of organic acids.

DISCUSSION

As ADFI and ADWG were not altered when acidifiers were added to the diet, feed conversion was better in piglets fed diets containing acidifiers, especially OAC. Diet palatability can be influenced by the type of acidifier, with SAL being tasteless and not affecting feed intake. Piglets do not respond positively to the use of dietary acidifiers and are commonly fed dairy ingredients and highly digestible diets with few anti-nutritional factors [10]. Moreover, highly digestible diets can reduce the effectiveness of acidifiers as piglets adapt quickly to diets [11]. The better feed conversion of piglets that received acidifiers can be explained by a

favorable set of factors such as increased villus height, better nutrient absorption capacity, longer gastric retention time, and greater effectiveness of pancreatic and enteric enzymes. This indicates that organic acids systemically affect animal performance [12]. Moreover, younger piglets respond better to acidifiers than pigs in the growth and finishing phases. This response to the use of acidifiers is more pronounced in the first 2 weeks after weaning [13]. A meta-analysis performed by Wang and coauthors (2022) [14] in growing pigs indicate that acidifiers, specially blends of acids improving growth of pigs. In addition, factors related to effective dose and growth stage of pigs affect efficacy of acids.

We found that the use of OAC and BLE decreased the pH of the stomach and jejunum of nursery piglets. The reduction in pH in the gastrointestinal tract favors the activation of gastric enzymes, especially pepsin, which requires a pH of 2.0 to 3.5 for its conversion from pepsinogen [15], and enteric enzymes such as trypsin, amylase, maltase, lipase, lactase, and sucrase [16,17]. Overall, acidifiers increase enzymatic activity and improve energy and nutrient utilization [18-20]. This important result favors the use of nutrients by piglets as transition diets in the post-weaning phase generally contain high levels of milk by-products, which increase the gastric and intestinal pH of piglets. As previously verified, most acidifiers decrease the pH in the initial segments of the small intestine and few studies evaluate their action on the pH of the cecum, colon, and rectum of piglets. Based on the low number of observations, it is important to consider the limitations of the results found for pH in these segments of the large intestine for this study. Valid approaches for this context must consider the impact of acidifiers on the modulation of intestinal microflora for each health challenge environment.

A high pH compromises enzymatic activity and can cause disorders in the intestinal microflora, leading to a disturbed environment, reduced digestion and absorption area, and damage to the intestinal mucosal barrier in the gastrointestinal tract [21]. In this study, we identified that the jejunum and colon of piglets fed BLE diets have a pH of 6.0, indicating colonization by unfavorable and pathogenic bacteria. However, dietary blends in nursery challenged piglets do not reduce *Salmonella typhimurium* and *Salmonella enteritidis* colonization [22]. The reduction in pH with acidifiers also helps modulate the bacterial population in the gastrointestinal tract. A low pH helps control pathogenic bacteria, especially gram-negative bacteria, which colonize the gastrointestinal tract at higher pH [23,24]. Associated with a drop in pH, organic acids with lower pKa values allow them to cross the cell barrier of pathogenic bacteria, thereby decreasing the inflammatory response and improving intestinal health [25].

Intestinal morphometry is a good indicator of intestinal health in piglets. In this study, piglets receiving acidifiers (OAC and BLE) were found to have a reduced crypt depth in the jejunum. This may be related to the lower pH in the gastrointestinal tract, which enables greater enzymatic action on nutrients, establishment of a microbiota favorable to the production of short-chain fatty acids, and a less severe inflammatory response in epithelial cells. In this scenario, there are fewer turnover cells in crypts. Acidifiers indirectly contribute to the intestinal health of nursery piglets. Villus height and crypt depth are good indicators of intestinal health. While intestinal villi are responsible for nutrient absorption, crypts are associated with cell proliferation and maturation [26]. Furthermore, reduced enzyme activity in microvilli reduces villus height and increases crypt depth [10]. Additionally, a more significant cellular turnover in epithelial cells results in low absorptive and digestive capacities [10].

In this meta-analysis, 32.5% of the piglets evaluated were subjected to a sanitary challenge and presented lower feed intake and villus height in the ileum. The greater exposure to pathogens associated with the constant activation of the immune system in challenged piglets may explain this result. Piglets challenged by pathogens may consume less feed because they spend less time standing and have food aversion associated with abdominal pain, both of which are caused by intestinal disorders [3,27]. Low feed intake and stress in piglets can lead to reduced gut mucosal integrity, as confirmed by an increase in paracellular transport and a decrease in villus height [27]. In this regard, the lower feed efficiency of challenged piglets can be explained by the increase in competition for nutrients, especially amino acids, between the immune system and tissues for growth. Additionally, under sanitary conditions, the use of acidifiers can minimize the negative impact of pathogen exposure by reducing intestinal pH. These results are more evident in younger piglets housed under poor sanitary conditions [20].

Organic acids in different forms of use (OAC, BLE, or SAL), when associated with diets, are beneficial to nursery piglet performance and intestinal health. This meta-analysis confirmed that adding acidifiers to diets improves performance and favors intestinal health. Research on acidifiers focusing on gut health through microbiome, gene expression, metabolomics, and immune response studies in piglets meets the One Health concept in the pig industry. Integrating information on the impact of acidifiers in their protected/encapsulated form, combined with nutraceuticals and phyto-genic additives, can improve our understanding of their benefits in nursery piglets.

CONCLUSION

Acidifiers in the diet of nursery piglets improve performance by reducing pH in the gastrointestinal tract and indirectly improving intestinal integrity. Organic acids and blends improved the performance of nursery piglets. Additionally, sanitary challenge reduces feed intake and compromises the intestinal morphometry of nursery piglets.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant number 455991/2014-6.

Acknowledgments: We acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Fundação Araucária, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grants awarded.

Conflicts of Interest: The authors declare no conflict of interest.

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