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ANIMAL PRODUCTION

Meta-analytical approaches to assess citric and ascorbic acid use in nursery piglets

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ABSTRACT: This study evaluated different meta-analytical approaches tousing citric and ascorbic acids in the performance of nursery piglets. The study included 24 publications between 1985 and 2018 (mode 1998), totaling 4215 nursery piglets. The Higgins index indicated high heterogeneity (98.5%) among the studies concerning citric acid (96.9%) and ascorbic acid (91.4%). In variances and forest plot analysis, using citric and ascorbic acids and their analogs was no significant effect ($P > 0.05$) on piglet weight gain. In the meta-analysis approach proposed by LOVATTO et al. (2007) and SAUVANT et al. (2008), there was no significant effect ($P > 0.05$) of the addition of citric and ascorbic acids on piglet performance. Numerically, was observed an increased ADG ($\Delta > 0$) related to acids (supplemented over control treatments) in 59.7% (ascorbic acid) and 46.6% (citric acid) of the ADG comparisons. The effect of ascorbic supplementation on the respective control treatment of the overall nursery period was on average 0.98%, 1.07%, and -1.3% for ADFI, ADG, and FCR, respectively. Citric acid supplementation to the respective control treatment of the overall nursery period was on average 1.67%, 4.40%, and -2.29% for ADFI, ADG, and FCR. Adding citric and ascorbic acids in diets for nursery piglets does not alter performance regardless of the meta-analysis method used.
Key words: forest plot, performance, systematic review, weaning, vitamin C.

Abordagens meta-analíticas para avaliar o uso do ácido cítrico e ascórbico em leitões em creche

RESUMO: Neste estudo, o objetivo foi avaliar diferentes abordagens de sistematização e meta-análise sobre o uso de ácidos cítrico e ascórbico no desempenho de leitões de creche. O estudo incluiu 24 publicações entre 1985 e 2018 (moda 1998), totalizando 4215 leitões de creche. O índice de Higgins indicou alta heterogeneidade entre os estudos envolvendo ácido cítrico (96,9%) e ácido ascórbico (91,4%). Na análise por forest plot a adição de ácido ascórbico em dietas de leitões não melhora ($P > 0.05$) o ganho de peso de leitões em creche. Na abordagem de meta-análise proposta por LOVATTO et al. (2007) e SAUVANT et al. (2008) não houve efeito significativo ($P > 0.05$) da adição de ácidos cítrico e de ascórbico no desempenho dos leitões. Numericamente, observou-se aumento do ganho de peso ($\Delta > 0$) relacionado aos ácidos (suplementado em relação aos tratamentos controle) observado em 59.7% (acido ascórbico) e 46.6% (ácido cítrico) das comparações do ganho de peso diário. A suplementação de ácido ascórbico em relação ao tratamento controle no período total de creche teve variação de 0,98%, 1,07% e -1,3% para ADFI, ADG e FCR, respectivamente. A adição de ácido cítrico em relação ao tratamento controle teve variação de 1,67%, 4,40% e -2,29 para ADFI, ADG e FCR. A adição de ácidos cítrico e ascórbico em dietas para leitões de creche não altera o desempenho, independentemente da abordagem de meta-análise utilizada.
Palavras-chave: desempenho, desmame, gráfico de floresta, revisão sistemática, vitamina C.

INTRODUCTION

Early weaning of piglets can increase oxidative stress and compromise the performance and health of the animal during the nursery phase. Stressful conditions such as nutritional, environmental, and social alterations in this phase lead to performance impairments due to changes in intestinal morphology, and immune and physiological response (ZENG et al., 2015), triggering an increase in the production of reactive oxygen species. For many decades citric and ascorbic acids have been explored in performance studies to minimize free radical production and improve the immune response of piglets. However, many studies indicate low expression of these acids on the performance of nursery piglets (BEZERRA et al., 2015; REY et al., 2017).

Ascorbic acid, known as vitamin C, has several functions in the living body, including removing free radicals acting as an enzymatic cofactor improving the body's immune response (SILVA, et al., 2015). Citric acid is an acidifier that can change

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the pH of the stomach and the gastrointestinal tract increasing the action of digestive enzymes and having significant effects on improving nutrient digestibility (KO et al., 2018). Therefore, the elements are often used in younger animals, especially for maintaining piglets' intestinal and metabolic integrity in the nursery phase (RODAS, et al., 1998). However, the significant variability of results in published studies causes inconsistencies in interpreting responses on zootechnical variables.

Meta-analysis allows obtaining new results through statistical analysis on a larger sample population. This approach allows an unbiased estimate and expresses with greater accuracy (ST-PIERRE, 2007). The interpretation of the results of a forest plot meta-analysis allows quick visualization of the result. In this approach, a dependent variable is considered the basis for interpreting the study results and then statistical analyses are applied (NEYELOFF et al., 2012). By integrating several factors or effects through predictive equations, the quantitative approach can predict phenomena or behaviors that could not be determined in a single study (LOVATTO et al., 2007; SAUVANT et al., 2008). However, each meta-analytic approach has its particularities and limitations. In this sense, this study evaluated different meta-analytic methods on the use of citric and ascorbic acids in the performance of nursery piglets.

MATERIALS AND METHODS

Systematic review

Indexed publications based on in vivo experiments of post-weaning piglets fed diets supplemented with ascorbic acid or citric acid were selected from Elsevier, Science Direct, Scopus, Scielo, PubMed, and Periodic CAPES. Only papers reporting the performance of post-weaning piglets and experiments applying supplementation levels ascorbic acid and citric acid were retained. From a total of 47 publications, only 24 were included in the database. The selected papers were critically evaluated for their relevance and quality in relation to the objectives of the meta-analysis regarding the experimental design, treatments, variables, and data analysis. Each study was evaluated independently by two evaluators. The outcome of a single study (i.e., whether ascorbic acid and citric acid were beneficial or not) was not considered as criteria for inclusion in this database. The reasons for the exclusion of the publications were: shown results as graphics or images; is outside the objective of this meta-analysis, like a pig in growth or finish phases; association of vitamin C/ascorbic acid with other additives, example: vitamin E, phytogenic, acidifiers; association of citric acids with other organic acids like blends or with other additives or being published without any evaluation criteria. To assess the effect of ascorbic and citric acids on piglet performance, two meta-analytical approaches were applied to selected papers.

A) Spreadsheet and Forest plot (NEYELOFF et al., 2012)

A Microsoft Excel spreadsheet was used to compilation data of 24 articles. This approach follows the propositions of Neyeloff et al. (2012) for the compilation of information for each paper, data analysis and elaboration of a forest plot. Briefly, a) outcome (effect size) was calculated by average daily weight gain of piglets and was considered as the main event (absence or presence of effect) for post weaned piglets fed diets containing citric acid (Figure 1) and ascorbic acid (Figure 2). Each article, the event ratio about sample size was relativized; b) standard error (SE); c) variance (SE^2); d) individual study weights; e) weighted effect size, multiplying each effect size by the study weight; f) Q test, measures heterogeneity among studies. It was calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method. Q is distributed as a chi-square statistic with k (number of studies) minus 1 degrees of freedom; f) Higgins's index (I^2), quantify heterogeneity and it is expressed in percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, to between-studies variability; g) deciding on effect summary, based on I^2 if the heterogeneity is low, a fixed-effects model is appropriate. However, in this study, the I^2 was upper 90%. In this case, the random-effects model is the most suitable for analyzing variance.

In the forest plot (Figure 1; Figure 2), each square represents a study with its respective confidence interval (IC 95%), represented by horizontal lines. The graph has a vertical trend line (Central Tendency), indicating no significance ($P > 0.05$) on the responses for each study when it intersects with the confidence interval lines. A summary value in the last line of the graph is indicated with a diamond (meta-analysis).

B) Meta-analytical approach proposed by LOVATTO et al. (2007) and SAUVANT et al. (2008)

After the final selection, a database with information specific to each selected paper was created in Microsoft Excel (2016) spreadsheet. Each column represented a study variable and each row

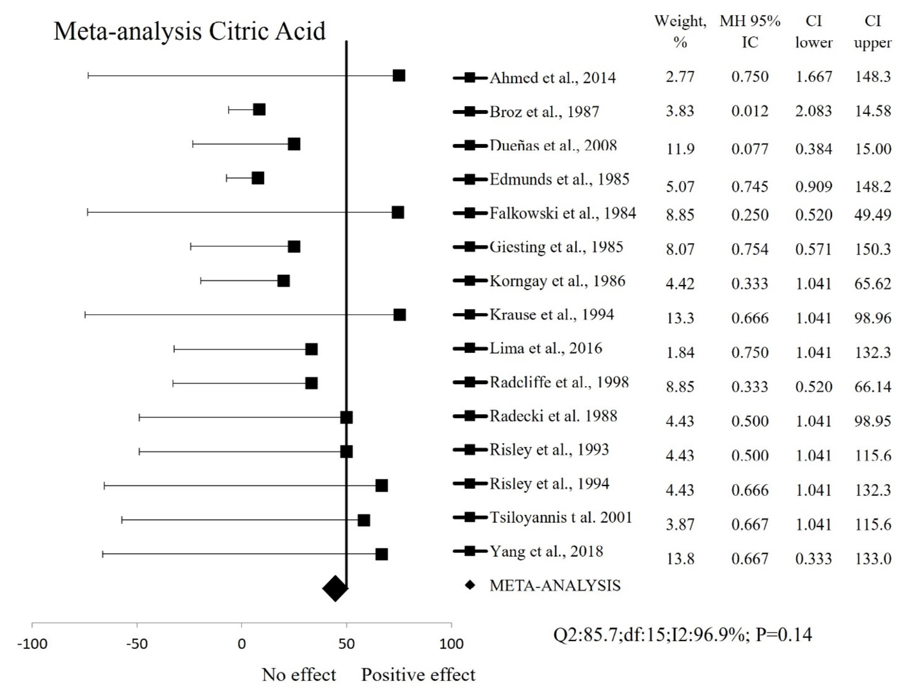

Figure 1 - Effect of citric acid on average daily gain of post weaning piglets.

represented a treatment. The tabulated data referred to bibliographic aspects (authors, year, country), experimental characteristics (experimental design, ascorbic acid and citric acid level in the diet, nutritional composition, ambient temperature, age and weight of piglets, and the variables tested (growth performance in terms of average daily feed intake, average daily weight gain, and feed conversion ratio, as well as).

A 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 (LOVATTO et al., 2007). The definition of the dependent and independent variables and the codification of the data for the analysis of inter-and intra-experimental effects were done according to LOVATTO et al. (2007), SAUVANT et al. (2008), ST-PIERRE (2007), and REMUS et al. (2014). Briefly, sequential numbers were used to encode every single study (general encoding), encode every single treatment within an experiment (inter encoding, i.e., each treatment received a sequential number and concatenated to the previously given study code) or when a study had more than one experiment in the same study, and encode repeated measures for different time intervals or dose when available (intra encoding). Additional encodings were made to facilitate the graphical and statistical analysis of the database.

The relationship between average daily feed intake (ADFI), daily average weight gain (ADG) and feed conversion ratio (FCR) was determined by expressing performance response to the control (set to zero), thus, expressing values as a percentage change (Δ, %) as described by ANDRETTA et al. (2012). This procedure was adopted because it considerably reduced the effect of variation among experiments in the database (PASTORELLI et al., 2012). Analysis of variance was conducted by applying a generalized linear model with covariate adjustment (LS-means). Basically, the analytical model considered the effects of the citric and ascorbic acids (Additive), study (fixed effect), and aleatory error. Additionally was included in the model the year of publication, age (initial and final age of each evaluation) or body weight (at average between initial and final body weight) as random effects. Sex effect (males/females or males) was not significant being removed from the

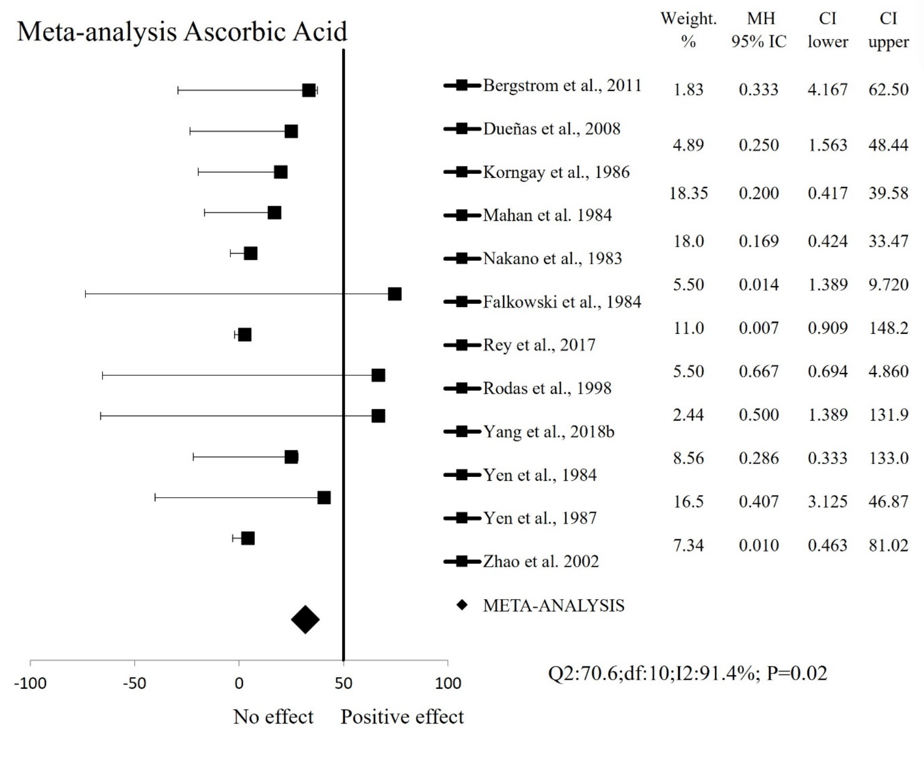

Figure 2 - Effect of ascorbic acid on average daily gain of post weaning piglets.

model. 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. All analyses were performed using MINITAB 19 (Minitab Inc., State College, USA).

RESULTS AND DISCUSSION

The database was composed of papers published between 1985 to 2018 (mode year:1998). The studies comprised a total of 4215 piglets, with an average of 120 animals per treatment. Average initial age was 22 days (ranging between 21 and 30 days), divided into 285 different treatments. Of the 285 treatments, 72 (25.2%) treatments with ascorbic acid and 75 (26.3%) with citric acid, and the other 138 (48.4%) were without additives or control group. 60.72% of experiments were conducted with males and females, 32.14% only male piglets, and in 7.14%,there was no information regarding the sex of the animals.

The level of ascorbic acid in the diets was 376 mg/kg (mean) and ranged from 50 to 900 mg/kg, and citric acid in the diets was 13720 mg/kg and ranged from 500 to 30000 mg/kg (0.5 to 3%). Most studies were carried out in American institutions (54.8%, (n=17 articles), English institutions (9.67% (n=3 articles), or Canadian, Chinese, and Brazilian institutions. 57.14% were published in the Journal of Animal Science, 17.85% in Animal Feed Science and Technology, and 7.14% in Animal Production (information presented in the supplementary material). The nutritional composition of diets was presented in 75% of the studies (21 articles), presenting in mean 3240 kcal/kg ME, 19.6% CP, 1.14% digestible lysine, 0.4% methionine, 0.51% threonine, 0.76% calcium and 0.79% phosphorus.

In the forest plot analysis of citric acid for weaning piglets, 15 articles were evaluated, 6 of which presented positive results for adding citric acid in diets, and another 7 studies showed adverse or null effects (Figure 1). The graph indicates the heterogeneity between studies when the Higgins'index (I2) observed is greater than 50% (NEYELOFF et al., 2012). I2 found was 96.9%, which indicates that there is high heterogeneity between the articles. For the forest plot ascorbic acid on daily gain of weaning piglets, 12 articles were evaluated, 3 of which presented positive results for adding ascorbic acid in diets, and another 9 studies showed adverse or null effects. Higgins' index observed was 91.4%. The diamond in the lower region of the graph represents the effect resulting from the combination of the different studies, that is, the result of the meta-analysis. When the diamond is over the central trend line, it indicates no significance ($P > 0.05$). This is the case for citric acid; however, in the forest plot ascorbic acid (Figure 2) was significant ($P < 0.02$). In this study, there was none or positive impact on citric acids on the weight gain of nursery piglets. Heterogeneity indicates the proportion of variability due to inter-study variance. This result is expected due to discrepancies between studies are probably a result of multiple variations such as experimental design, experimental diets, differences between dosages, sample size, age and weight of the animals, and sex category of the piglets, analytical and statistical methodologies (UPADHAYA & KIM, 2021).

Following the propositions of this methodology, the recommendation is to nest the studies in subgroups and then apply the meta-analysis to each subgroup (LOVATTO et al., 2007). However, the statistical results can be doubtful in this format due to publication bias since studies are separated by dosage, the form of administration, or sample size (ST-PIERRE, 2007). As verified in the forest plot graphic for average daily gain there were no significant differences in the performance of piglets fed diets containing ascorbic or citric acids (Table 1). This analysis proposed by REMUS et al. (2014) and SAUVANT et al. (2008) were considered, in addition to the treatment effect, the inter-study as fixed-effect class and nursery days as a random effect, allowing a better prediction of the model.

Table 1 - Performance of nursery piglets feeding with diets containing ascorbic or citric acid in post-weaning (0-14 d) and nursery period.

--------Additive--------									
	Control	----Ascorbic acid----		----Citric acid----		SEM	------P-value[4,5]------		
N [1]	138	72		75					
--------BW, kg--------							Additive	Age	BW
Initial	6.98	6.93		6.82		1.24	0.978	0.232	0.001
Final	18.6	20.2		17.4		1.24	0.978	0.232	0.001
ADFI, kg/d	LSM[2]	LSM	Δ, %[3]	LSM	Δ, %				
0-14d	0.355	0.335	0.76	0.381	2.82	0.07	0.928	0.009	0.001
> 15d	0.616	0.627	1.56	0.652	0.87	0.09	0.738	0.868	0.001
Overall	0.489	0.478	0.98	0.550	1.67	0.09	0.922	0.001	0.001
--------ADG, kg/d--------									
0-14d	0.235	0.230	0.68	0.240	0.68	0.05	0.683	0.800	0.001
>15 d	0.389	0.420	2.60	0.412	2.31	0.04	0.846	0.099	0.001
Overall	0.319	0.326	1.07	0.331	4.40	0.06	0.809	0.062	0.001
--------FCR--------									
0-14d	1.68	1.69	-2.3	1.66	-3.7	0.32	0.569	0.614	0.072
>15 d	1.74	1.68	0.31	1.76	3.00	0.12	0.881	0.002	0.980
Overall	1.72	1.65	-1.3	1.72	-2.29	0.28	0.393	0.085	0.420

[1]N, number of observations; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; SEM, standard error of mean.

[2]LSM (least square means) of inter experimental groups followed by distinct letters differ by the Tukey test ($P < 0.05$).

[3]Δ, obtained by the difference between the treatments (intra-experimental) with ascorbic acid or citric acid compared to the control (set to zero), expressed in percentage.

[4]Additive and studies (experiments) entered in the model as a fixed-effect class variable, age (average between the initial and final age of each evaluation, expressed in d), and BW (body weight at average between initial and final body weight of each evaluation. expressed in kg) as random effects.

[5]Probability at 5%.

As shown in figure 3, the variation of ascorbic and citric acid (Δ) on performance was evaluated in each study. The grouped information indicated large variability in treatments supplemented with ascorbic or citric acid of post-weaning piglets. Disregarding the statistical significance in the original analysis, increased ADG (Δ > 0) related to acids (supplemented over control treatments) was observed in 59.7% (ascorbic acid) and 46.6% (citric acid) of the ADG comparisons. Numerically, the effect of ascorbic supplementation to the respective control treatment of overall nursery period was on average 0.98%, 1.07%, and -1.3% for ADFI, ADG, and FCR, respectively (Table 1). Citric acid supplementation to the respective control treatment of overall nursery period was on average 1.67%, 4.40%, and -2.29% for ADFI, ADG, and FCR, respectively.

Although, this study identified inter-study heterogeneity both methods' fixed and random effects allowed for adequate statistical analysis. Thus, this meta-analysis showed that the addition of ascorbic and citric acid does not alter the performance of nursery piglets. Results obtained by REY et al. (2017) and FERNÁNDEZ-DUEÑAS et al., (2008), corroborate those results in this meta-analysis. Even when ascorbic acid is provided in a protected form, its effectiveness can be quickly reduced due to high instability (NRC, 1998). This can explain the behavior of the data in figure 3, where the effects between studies were very dispersed and showed significant variations, both negative and positive.

Ascorbic acid or citric acid in diets for piglets after weaning (0-14 days), and nursery period (overall) did not differ performance (P > 0.05) (Table 1). In the variance-covariance study, we identified that the age and weight of the piglets strongly influenced the performance in this phase. The first 14 days after weaning are associated with greater physiological stress, less use of nutrients, and favorable conditions for the emergence of diarrhea (UPADHAYA & KIM, 2021). Although, the variation in relation to the control treatment (Δ, %) is numerical, piglets fed diets containing citric acid present better post-weaning performance. Citric acid improves post-weaning performance by reducing stomach pH by stimulating the piglet's enzymatic action on the feed intake (AHMED et al., 2014). The abrupt changes in digestive physiology, that occur during the transition phase, can probably benefit the use of organic acids in the first weeks after weaning pigs (DENCK et al.

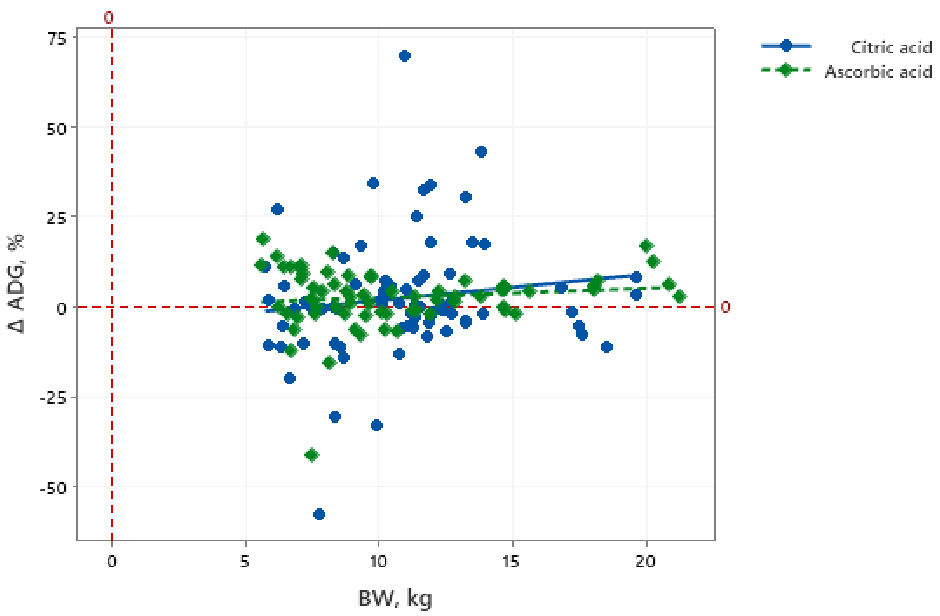

Figure 3 - Average daily gain (ADG) variation (Δ) between treatments supplemented with ascorbic acid or citric acid relativized to the respective control treatment(zero) in the database. Each symbol in the figure represents an individual comparison available in the original publication. BW, kg average body weight.

2017). However, this meta-analysis of two different approaches indicates that citric acid alone does not improve statistically the performance of nursery piglets. One of the causes may be associated with the negative influence of citric acid on the palatability of diets, decreasing the feed intake of piglets (DENG et al., 2021) and very low doses added to the diets, decreasing its action on gastric pH (DENG et al., 2021; FERREIRA et al., 2020).

Ascorbic acid is unstable when exposed to oxygen, and when associated with some minerals, it can undergo irreversible oxidation processes, transforming into diketogluconic acid and L-thronic acid, which are elements not absorbed by the animal body and excreted via feces and urine (LI et al., 2020). In most studies, ascorbic acid or analogs were added in their pure or natural form, as seen in the supplementary table, which may partly explain the absence of positive or negative effects on piglet performance (NJUS et al., 2020). Recent studies indicated that the addition of ascorbic acid in diets for nursery piglets in the stabilized forms of magnesium-l-ascorbyl-2-phosphate (MAHAN et al., 1994; SVINJA et al., 2005) and L-ascorbyl-2-polyphosphate improve piglet performance (REY et al., 2017; TYMCZYNA et al., 2020). In last years, natural citric acid and ascorbic acid have been associated with essential oils and polyphenols of citrus fruits (ascorbic acid, quercetin, rutin and naringenin), which due to the extraction and encapsulation process show promising results in newly and weaned piglets (PEREIRA et al., 2019; LEHNEN et al., 2010). Synergistic action between vitamin C and flavonoids improves the palatability of diets and increases feed intake (NEPOMUCENO et al., 2018), as well as in growing pigs enhances the utilization of nutrients (LANFERDINI et al., 2013).

In the compilation of studies with ascorbic and citric acid for piglets, it was observed that a relevant part of the studies was carried out in the 1980s, 1990s and 2000s, and many of these studies did not present information on intestinal morphometry or biochemical parameters in piglets. Conversely, studies after 2015 explore the action of these acids on oxidative stress in post-weaning piglets or immune response. In the statistical analysis of this study, we identified that in response variables such as weight gain, it is necessary to include the various factors (age of the piglets, source, and acid levels) that act on animal performance, which are better explained by the methodology proposed by LOVATTO et al. (2007) and SAUVANT et al.(2008). However, in Neyloff's approach, we can separate these factors into categories and separately analyze the effects on weight gain. In general, the results of studies that involve categorical variables in the meta-analysis are better expressed in forest plots.

CONCLUSION

The addition of citric or ascorbic acids in diets for nursery piglets does not alter performance regardless of the meta-analysis method used.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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