http://dx.doi.org/10.1590/1678-4162-12464

Arq. Bras. Med. Vet. Zootec., v.74, n.1, p.185-194, 2022

Acidifiers blend on digestibility, blood metabolites and performance of nursery piglets

[*Acidificantes na digestibilidade, nos parâmetros sanguíneos e no desempenho de leitões na fase de creche*]

J.M. *Oliveira Júnior*[1], A.R. *Mendes*[2], J.H. *Montes*[3], B.R. *Alves*[3], F. *Moreira*[1], I. *Bianchi*[1], E. *Schwegler*[1], V. *Peripolli*[1,*]

[1]Instituto Federal Catarinense, *Campus* Araquari, Araquari, SC, Brasil
[2] Graduate, Universidade Federal do Paraná, Curitiba, PR, Brasil
[3] Graduate, Instituto Federal Catarinense, *Campus* Araquari, Araquari, SC, Brasil

ABSTRACT

The aim of this study was to evaluate the effect of inclusion of two acidifiers blend (with different protection levels) on the acidification potential in vitro, apparent total tract digestibility of diet nutrients, blood metabolites, and average daily gain of nursery piglets. Two commercial acidifiers blend additives (AC1 and AC2) were evaluated. The acidification potential of the two acidifiers blend added to the diet were tested by buffering capacity and initial pH in vitro. For the evaluation of apparent digestibility of diet nutrients, blood metabolites and average daily gain, two experiments were carried out. The AC2 acidifier had a greater effect on the initial reduction in pH and a lower linear buffering rate of the diet. The use of acidifiers did not improve the nutrient digestibility of the diets and had no effect on the blood metabolite concentrations of urea, total protein, albumin, globulin, and lactate. Although the inclusion of acidifiers in the diets did not affect the apparent total tract digestibility of the diet nutrients the inclusion of the AC2 acidifier at different levels showed a negative quadratic effect on the average daily gain, with the best inclusion level being 0.26%.

Keywords: acidification potential; buffering capacity; organic acid; swine

RESUMO

O objetivo deste estudo foi avaliar o efeito da inclusão de dois acidificantes (com diferentes níveis de proteção) sobre o potencial de acidificação in vitro, *a digestibilidade aparente do trato total dos nutrientes da dieta, os metabólitos sanguíneos e o ganho médio diário de leitões. Foram avaliados dois acidificantes comerciais (AC1 e AC2). O potencial de acidificação dos acidificantes adicionados às dietas foi testado quanto à capacidade tamponante e ao pH inicial* in vitro. *Para avaliação da digestibilidade aparente dos nutrientes da dieta, dos metabólitos sanguíneos e do ganho médio diário, foram realizados dois experimentos. O acidificante AC2 teve maior efeito na redução inicial do pH e menor taxa linear de tamponamento linear da dieta. O uso de acidificantes não melhorou a digestibilidade dos nutrientes das dietas e não teve efeito sobre as concentrações dos metabólitos sanguíneos de ureia, proteína total, albumina, globulina e lactato. Embora a inclusão de acidificantes nas dietas não tenha afetado a digestibilidade aparente do trato total dos nutrientes da dieta, a inclusão do acidificante AC2 em diferentes níveis apresentou efeito quadrático negativo no ganho médio diário, com o melhor nível de inclusão sendo 0,26%.*

Palavras-chave: suínos, ácidos orgânicos, capacidade tamponante, potencial de acidificação

INTRODUTION

The transition from sow milk to a high-protein diet, combined with the inability of piglets to secrete enough hydrochloric acid (HCl) into the immature gastrointestinal tract, can lead to a digestive disturbance and the invasion of acid-intolerant bacteria such as *Escherichia coli* and *Salmonella sp*. (Ahmed *et al*., 2014). To overcome these problems, the use of antimicrobial

*Corresponding author: vanessa.peripolli@hotmail.com
Submitted: June 22, 2021. Accepted: November 25, 2021.

additives in animal feed became common, since its use can provide several beneficial effects, such as improving intestinal health and performance. However, this practice is questionable due to the possibility of cross-resistance development in bacteria, that results from co-resistance, as well as multiple resistance mechanisms in a single host (Miguel *et al*., 2011) that can affect systemic metabolism (Mu *et al*., 2017).

As an alternative, studies have been carried out using acidifiers blend as feed additive to reduce gastric pH, altering the action of digestive enzymes and reducing the proliferation of pathogenic microorganisms, with the aim of improving the apparent total tract digestibility, feed conversion and performance of the animals as a whole (Diao *et al*., 2014). The main action mechanisms of acidifiers are the reduction of gastric pH, the antimicrobial effect and pathogen inhibition and the stimulation of pancreatic enzyme secretion (Papatsiros *et al*., 2012). Additionally, acidifiers serve as an energy source during the intermediate metabolism of the gastrointestinal tract and can improve the mineral utilization by the process of chelation (Suryanarayana *et al*., 2012). Therefore, the use of acidifiers in combination or as a substitute of antibiotics could be a potential alternative. The use of organic acids blends as antibiotic substitute improved performance, serum immunity, intestinal morphology, microbiota (Long *et al*., 2018) and reduced the diarrhea in weaned piglets (Lei *et al*., 2017). However, the action potential of acidifiers in diets depends on its composition, pKa and level of inclusion in the piglets diets.

Results from studies using acidifiers blend as feed additive in piglets' diets are still quite controversial, where inclusion levels depend on both the chemical composition and the physicochemical properties of the diet as well as on the acidifying and buffering power of the acidifier used (Oliveira Jr. *et al*., 2017). Additionally, few studies have evaluated the impact of acidifiers on pH and buffer capacity in order to determine the appropriate inclusion level in diets prior to *in vivo* experimentation (Bockor *et al*., 2017).

Due to the properties of acidifiers, positive effects are expected on the feed palatability and digestibility, reducing buffering capacity of feed, improving the digestive system, health, and animal performance (Nowak *et al.*, 2021). However, this expected effect depends on the quantity and quality of the acidifier, also considering the composition of the diet. Furthermore, unprotected acidifiers can be easily buffered by the effect of certain ingredients or absorbed prior to the effect on the intestinal environment (Canibe *et al.*, 2001).

Through advances in technology, the incorporation of acidifiers has improved the palatability aspects and potential of dissociation through the process of micro-encapsulation (Grilli *et al*., 2010). This process involves molecules of organic acid or a blend, with a thin layer of polymers or lipids, which when ingested by the animal slowly dissociate the acids in the small intestine, showing better results compared to unprotected acidifiers (Piva *et al*., 2007; Grilli *et al*., 2010). Furthermore, encapsulated compound acidifiers improve the intestinal morphology and function by reducing the gastrointestinal pH, so as enhance the intestinal adaptation and immunity, the growth performance improvement in weaning piglets occurs (Jia *et al.*, 2010).

Thus, the aim of this study was to evaluate i) the effect of inclusion of two acidifiers blend (with different protection levels) on the acidification potential in vitro, apparent total tract digestibility of diet nutrients and blood metabolites of nursery piglets; ii) the effect of different inclusion levels of the acidifier blend with a higher protection level on the average daily gain of the nursery piglets.

MATERIAL AND METHODS

Animal care procedures throughout the study followed the protocols approved by the Ethics Committee for Animal Use (ECAU) at the Federal Catarinense Institute (IFC), numbers 105/2015 (experiment 1) and 152/2016 (experiment 2).

Two commercial acidifiers blend additives (AC1 and AC2, Sanex Comércio e Indústria Veterinária Ltda, Curitiba, Paraná, BR) were evaluated in vitro. The AC1 acidifier blend was a compound of five organic acids including benzoic, lactic, formic, fumaric and citric acids, with a concentration of 7286 mEq H^+/kg, 23.5% in a protected form. The AC2 acidifier blend was composed of benzoic, sorbic, formic, lactic and phosphoric acids, with a concentration of

7302mEq of H^+/kg, 53.8% in a protected form. Both products were subjected to the same lipid protection process, patented by the company.

To evaluate the buffering capacity, nine inclusion levels (0.25, 0.50, 0.75, 1, 2, 5, 9, 12 and 25%) of the AC1 and AC2 acidifiers blend were added to the diet and evaluated for buffering capacity by the linear buffering rate according to Bockor *et al*. (2017). To evaluate the initial pH of the diet, five inclusion levels (0.25, 0.50, 0.75, 1 and 2%) of the AC1 and AC2 acidifiers blend were added to the diet.

The pH was measured with the pH meter (pH 330, SET, WTW) using a 0.5 g sample in a solution with distilled and deionized water under constant agitation. Buffering capacity was measured using titration curves. The initial pH was measured, then NaOH (0.05 mol/l) was used to raise the pH to 8.0, and HCl (0.05 mol/l and 0.1 mol/l, respectively) was used to lower the pH to 2.0, with constant reductions around 0.200 pH points. From the titration curves pH values were transformed by exponential function (1/ pH) and the linear buffer rate (LBR) was obtained as the inverse of the angular coefficient between the transformed pH measurement as the dependent variable and the amount of acid added as independent variable.

The diet used for both the initial pH and the LBR evaluations was an isoenergetic and isoproteic diet formulated to attend to the nutritional requirements of piglets during the nursery phase, according to Rostagno *et al*. (2011) (Table 1).

Table 1. Ingredients and chemical composition calculated of experimental diet for nursery piglets

Feed ingredients, %		Chemical composition, %	
Corn 7.8%	42.95	Dry matter	91.67
Soybean meal 44,5%	25.62	Ash	6.40
Meat and bone meal 45%	3.29	Crude protein	21.03
Milk whey	20.24	Crude fiber	4.86
Soybean oil	3.9	EEAH	7.22
Butylated hydroxytoluene (BHT)	0.02	GE, kcal/kg	4130
DL-Methionine	0.21	EM, kcal/kg	3304
L-Lysine	0.67	NDF	12.8
L-Threonine	0.29	ADF	3.85
L-Thryptofane	0.08	Lignin	0.20
Bicalcium phosphate 15%	0.71	Calcium	5.94
Choline chloride 60%	1.39	Phosphorous	5.90
Mineral premix[1]	0.05	Lysine	1.16
Vitamin premix[2]	0.02	Methionine	0.48
		Threonine	0.76
		Thryptofane	0.22

EEAH: ether extract after acid hydrolysis; GE: gross energy; ME: metabolizable energy; NDF: neutral detergent fiber; ADF: acid detergent fiber. [1]240 mg/kg Co; 46.7 g/kg Fe; 15.6 g/kg Mn; 50 g/kg Cu; 52.9 g/kg Zn; 620 mg/kg I; 180 mg/kg Se; [2]5,000.000 UI vitamin A; 495 mg/kg vitamin B1; 1,520 mg/kg vitamin B2; 5,000 mcg vitamin B12; 790 mg/kg vitamin B6; 1,000.000 UI vitamin D3; 12,500 UI vitamin E; 1,000 mg vitamin K3; 95 mg/kg folic acid; 10 g/kg nicotinic acid; 4,500 mg/kg pantothenic acid; 24 mg/kg biotin; 50 g/kg choline; 1,320 mg/kg ethoxyquin.

Statistical analysis was performed using SAS version 9.3 (Statistical Analysis Institute, Cary, NC, USA). The results obtained from the initial pH and LBR (dependent variables) were adjusted for linear regressions and the level of inclusion of the acidifiers blend was considered as an independent variable. The regression lines were compared with a significance level of 5%. Differences between the intercept and the slope of the data adjustment for the linear regressions of the different acidifiers blend were evaluated.

To evaluate the apparent total tract digestibility of diet nutrients, blood metabolites, and average

daily gain of nursery piglets two experiments were carried out in at a Pig Experimental Farm located in Santa Catarina State in southern Brazil (26°22'12"S and 48°43'20"W), with an altitude of 9 m. The climate is Cfa (moist mesothermal with hot summer), according to the Koppen classification system.

Experiment 1 - To evaluate the apparent total tract digestibility three treatments (control, AC1 and AC2) were used, with six animals per replication, allocated in 18 cages. The experiment was repeated twice in time, totalizing 12 animals per treatment. After weaning at 28 days of age, the animals (Large White x Landrace; 50% males and 50% females) were identified with ear tags, individually weighed (6.78 ± 1.01 kg), and placed into adjustable individual cages with feces and urine collection systems and pacifiers installed. The animals underwent a five-day diet adaptation period followed by a five-day collection period, totaling an experimental period of 10 days. The room temperature was 28ºC.

All animals received an isoenergetic and isoproteic diet formulated to attend to the nutritional requirements of piglets during the nursery phase, according to Rostagno *et al*. (2011) (Table 1) used also in the in vitro test. However, the treatments differed in the acidifier blend used as feed additive (AC1 and AC2). Thus, the three experimental treatments were: control group, which received the basal diet and 0.3% addition of refined sugar cane (CONT); AC1 group, which received the basal diet and the addition of the AC1 acidifier blend (AC1); and the AC2 group, which received the basal diet and the addition of the AC2 acidifier blend (AC2), both acidifier blend with a 3% inclusion level in the basal diet. Refined sugar cane was added in the control group to equalize the energy provided by the acidifier blend. The animals received diets in two daily meals (6:00 am and 6:00 pm), with free access to water. After the adaptation period, the amount of feed provided daily for each animal was calculated based on the metabolic weight ($LW^{0.60}$) and adjusted to the level of metabolizable energy of diet according to Noblet and Perez (1993).

The feces (total fecal collection method) were collected twice a day for a five-days period, weighed, packed in plastic bags individually identified, pre-dried at 65°C, milled (with a 1 mm mesh) and homogenized. The pre-dried feces were analyzed for dry matter (DM), organic matter (OM), crude protein (CP) and ash (A) according to AOAC (Official…, 1990). These results were used to calculate the apparent total tract digestibility of the diet nutrients ($ATTD_{nutrient}$), as follow: (((Nutrient diet-Nutrient feces) / Nutrient diet) x100).

To evaluate blood metabolites, 12 nursery piglets (Large White x Landrace; 50% males and 50% females) were identified with ear tags, individually weighed (6.85 ± 0.85 kg), standardized according to sex and live weight and allocated into three pens with a total area of 2.68 m^2 with a fenestrated plastic floor suspended 0.90 m from the ground and with pacifiers installed, and received one of the experimental diets (CONT, AC1 or AC2) ad libitum. The initial room temperature was 28ºC, being reduced by 2 degrees per week. The number of animals per treatment (4) was determined using the minimum number of replications formula (Kaps and Lamberson, 2009) to detect differences between treatments at a level of 5% and 80% power of the test being in accordance with ECAU regulations.

Animal blood collections for blood metabolites analyzes were performed by venipuncture of the jugular using 10 ml vacutainer tubes at weaning (beginning of the experiment) and again 12 days later. To obtain the serum, the samples were centrifuged at 7,000G for 5 min. The serum was then stored in Eppendorf tubes and frozen at -20°C for further analysis of urea (1,013-4/50, Labtest, Lagoa Santa, MG, Brazil; sensitivity 0.94mg/dL), albumin (1,007-250, Labtest, Lagoa Santa, MG, Brazil; sensitivity 0.015g/dL), lactate (138-1/50, Labtest, Lagoa Santa, MG, Brazil; sensitivity 0.4 mg/dL), and total protein (99-1/250, Labtest, Lagoa Santa, MG, Brazil; sensitivity 0.0168g/dL), that were colorimetrically measured in duplicate using commercial kits developed for veterinary or human serum validated for multi-species (all coefficients of variation below 10%). All analyzes were performed in a commercial laboratory. Globulin was calculated as follows: globulin=total protein-albumin.

Statistical analysis was performed using SAS version 9.3 (Statistical Analysis Institute, Cary, NC, USA). For the digestibility analyses a completely randomized design with three treatments and twelve replications (repeated twice

in time) was used. The GLM procedure was used to test the effects of treatment (CONT, AC1 and AC2) on apparent total tract digestibility of diet nutrients. Sex was included in the model as a random effect. The effect of the experiment repetition over time was included in the model. The animal was considered the experimental unit. The model used was $Yijl= \mu+ \alpha i+ \beta j + \gamma l + \varepsilon ijl$, where Yijl is the observation given acidifiers i, time j and sex l; μ is the overall mean; αi is the effect of acidifier i; βj is the effect of time j; γl is the effect of sex l and εijl is the random error associated with each observation, being $\mu= 0$ e variance= 1. Means were compared using the Tukey test with a significance level of 5%. There were no significant interaction effects, therefore they were removed of the final model.

For the blood metabolites analyses a completely randomized design with three treatments and four replications was used. The GLM procedure was used to test the effects of treatment (CONT, AC1 and AC2) on blood metabolites. Sex was included in the model as a random effect. Blood metabolite data was analyzed as a repeated measure over time. The animal was considered the experimental unit. The model was the same used for digestibility analyses, except that instead of considering the effect of the experiment repetition over time, the effect of the blood sample collection was considered. Means were compared using the Tukey test with a significance level of 5%.

Experiment 2 – To evaluate the average daily gain (ADG) of nursery piglets an experiment was carried out with 24 nursery piglets (Large White x Landrace; 50% males and 50% females) at 28 days of age, allocated in four treatments with six animals per replication. After weaning, the animals were identified with ear tags, individually weighed (8.59 ± 1.19kg), standardized according to sex and live weight and allocated into groups of three animals in pens with a total area of 2.68 m^2, with a fenestrated plastic floor suspended 0.90 m from the ground and with pacifiers installed. The initial room temperature was 28ºC, being reduced by 2 degrees per week.

All animals received the same basal diet used in Experiment 1, however, the treatments differed in the inclusion level (0, 0.2, 0.4 and 0.6%) of the AC2 acidifier blend. The concentration of the acidifier blend in each diet was calculated according to the predicted buffering capacity. The number of animals per treatment (6) was determined using the minimum number of replications formula (Kaps and Lamberson, 2009) to detect differences between treatments at a level of 5% and 80% power of the test being in accordance with ECAU regulations. Also, the animal was considered the experimental unit.

During the 21-day experimental period, the animals were weighed individually every 7 days to evaluate the average daily gain (ADG) of each animal.

Statistical analysis was performed using SAS version 9.3 (Statistical Analysis Institute, Cary, NC, USA). The experimental design was a completely randomized with four treatments and six replications. To evaluate the effect of the treatment (0, 0.2, 0.4 and 0.6% of AC2 in the diet) on the piglets ADG the REG procedure was used to determine the optimal level of acidifier blend in the diet to obtain the higher ADG.

RESULTS

The initial drop in pH differed according to the acidifier blend used (Figure 1, Table 2), with the addition of AC2 showing the greatest decrease in pH ($P < 0.001$), despite the concentrations of AC1 and AC2 being similar (7289 and 7302 mEq H^+/kg, respectively).

Linear regression analysis demonstrated that the addition of the acidifier blend to the diet presented different linear buffering rates (LBR) (Figure 2, $P < 0.001$). The diet using AC2 as feed additive had a lower LBR than the diet with AC1 (Table 2, $P < 0.001$).

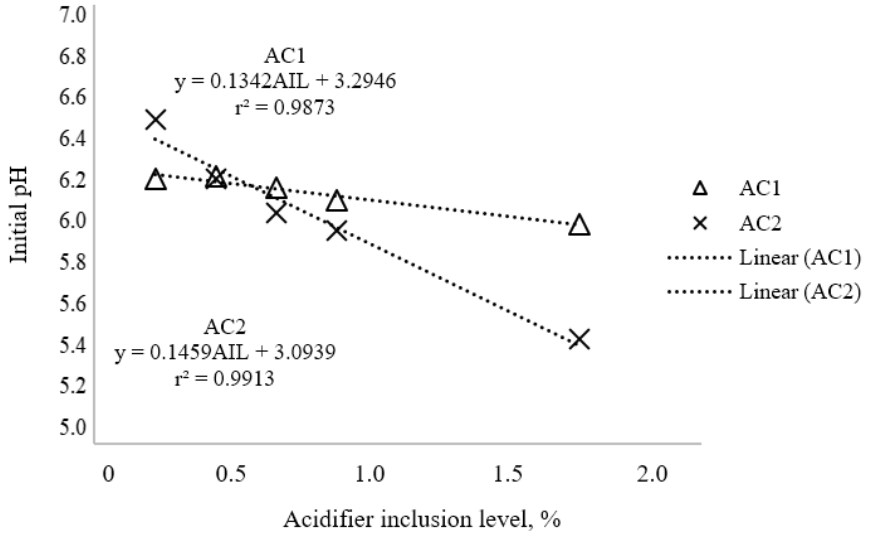
There was no effect of treatment on dry matter, organic matter, ash, and crude protein digestibility ($P > 0.05$), with average values of 86.93, 86.93, 68.26 and 85.46%, respectively (Table 3).

Regardless of the collection time (at weaning and 12 days later), there was no effect of treatment ($P > 0.05$) on the concentrations of urea, total protein, albumin, globulin, and lactate, with mean values of 17.36 mg/ dL, 4.88 g/dL, 3.32 g/dL, 1.56 mg/dL and 9.23 mmol/L, respectively (Table 4).

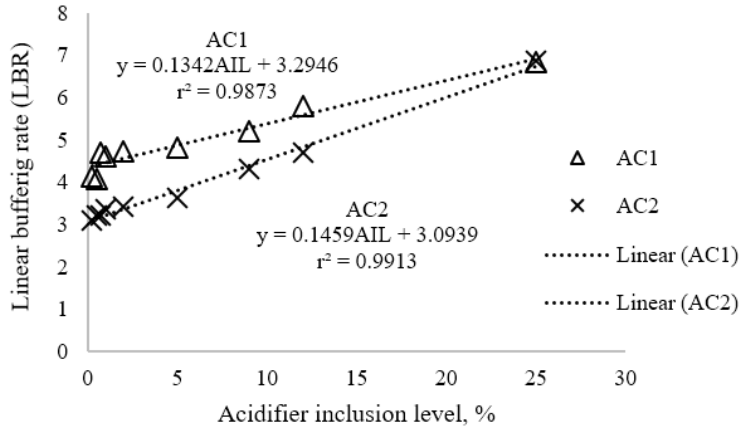
Table 2. Comparison of different acidifiers (AC1 and AC2) added to diets in relation to the constant (a) and angular coefficient (b) of the adjustment of data to linear regressions of initial pH and buffering capacity expressed as linear buffering rate (LBR)

	Constant (a)	Angular coefficient (b)	Coefficient of determination (r^2)
Initial pH			
AC1	6.2599[b]	-0.1310[b]	0.9577
AC2	6.5205[a]	-0.5362[a]	0.9696
LBR			
AC1	3.2946[a]	0.1342[b]	0.9873
AC2	3.0939[b]	0.1459[a]	0.9911

Means followed by different lower-case letters in the column differ at 5% by the Tukey test ($P < 0.05$).



AIL: acidifier blend inclusion level; r^2: coefficient of determination.
Figure 1. Effect of the inclusion level of AC1 and AC2 acidifiers blend on initial pH of the diet.



AIL: acidifier blend inclusion level; r^2: coefficient of determination.
Figure 2. Effect of the inclusion level of AC1 and AC2 acidifiers blend on buffering capacity expressed as linear buffering rate (LBR) of the nursery piglets' diet.

Table 3. Apparent total tract digestibility of dry matter, organic matter, ash and crude protein of the nursery piglets' diet

Nutrient digestibility, %	Treatment			Pr > F
	CONT	AC1	AC2	
Dry matter	87.0±0.97	86.3±0.92	87.5±0.84	0.65
Organic matter	87.0±0.97	86.3±0.92	87.5±0.84	0.65
Ash	69.2±1.90	67.0±1.81	68.6±1.65	0.70
Crude protein	85.1±1.35	84.9±1.28	86.4±1.17	0.63

CONT: group which received only basal diet; AC1: group which received basal diet and addition of acidifier AC1; AC2: group which received basal diet and addition of acidifier AC2, both with inclusion level of 0.3% in the basal diet; Pr>F: probability; means followed by standard error of the mean.

Table 4. Effect of treatment and blood collection time on urea, total protein, albumin, globulin, and lactate concentrations of nursery piglets

Blood metabolites	Treatment						Probability			
	CONT		AC1		AC2		Pr>F[1] (Col)			Pr>F[2]
	Col 1	Col 2	Col 1	Col 2	Col 1	Col 2	CONT	AC1	AC2	Treatment
Urea, mg/dL	15.96± 0.05	23.55± 2.35	19.45± 5.35	13.25± 0.15	17.50± 0.70	4.50± 3.5	0.28	0.55	0.71	0.10
Total protein, g/dL	5.40± 0.20	5.05± 0.05	5.10± 0.10	4.35± 0.95	4.45± 0.15	4.95± 0.05	0.32	0.54	0.36	0.66
Albumin, g/dL	3.55± 0.05	3.60± 0.10	3.55± 0.15	2.75± 0.75	3.15± 0.15	3.30± 0.10	0.29	0.66	0.17	0.47
Globulin, mg/dL	1.85± 0.25	1.45± 0.05	1.55± 0.25	1.60± 0.20	1.30± 0.05	1.65± 0.15	0.36	0.10	0.36	0.65
Lactate, mmol/L	8.55± 0.55	10.65± 0.65	9.10± 1.30	11.00± 2.60	6.10± 0.10	10.00± 3.7	0.48	0.86	0.54	0.96

CONT: group which received only basal diet; AC1: group which received basal diet and addition of acidifier blend AC1; AC2: group which received basal diet and addition of acidifier blend AC2, both with inclusion level of 0.3% in the basal diet; Pr>F: probability; means followed by standard error of the mean; col 1: blood sample collected at weaning; col 2: blood sample collected 12 days after weaning. [1]Variance analysis between collection time; [2]Variance analysis between treatments of blood sample collected 12 days after weaning.

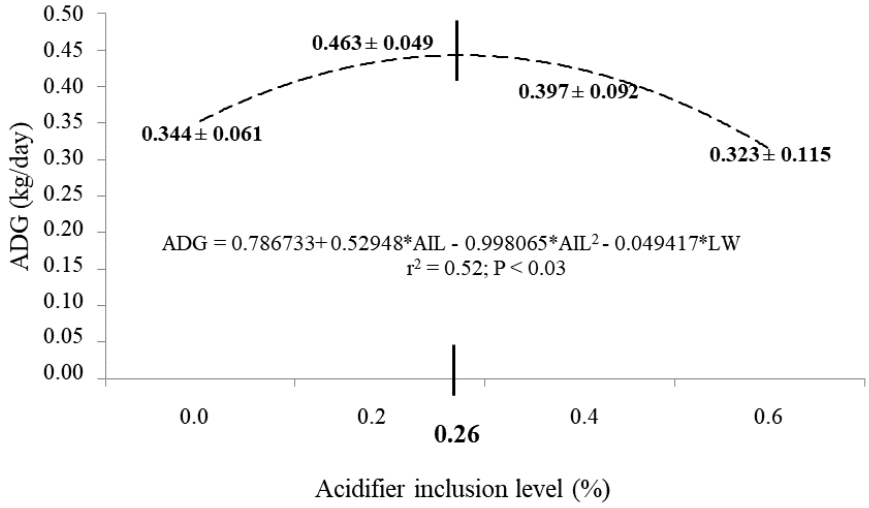
A negative quadratic effect on the ADG of piglets was observed with the addition of the AC2 acidifier blend to the diet ($P < 0.05$), indicating 0.26% as the best level of AC2 inclusion, in terms of the higher ADG (Figure 3).

DISCUSSION

The efficiency of acidifiers additives in weaned piglet diets are controversial due to several factors, such as the growth stage, the diet complexity, the type of acid used and level of inclusion (Suryanarayana *et al*., 2012), as well as the palatability of the diet, which can stimulate or inhibit consumption (Kil *et al*., 2011). These inconsistencies are also related to the non-evaluation of the diet buffer capacity with the inclusion of acidifiers at the formulation time (Corassa *et al*., 2006).

Increasing the inclusion of the acidifiers blend AC1 and AC2 as feed additive differently effected the initial dietary pH. The use of AC2 demonstrated a greater efficiency in reducing the initial pH, especially when the inclusion level was greater than 0.75%. Additionally, the buffering capacity of the diets was also lower when AC2 was added at different inclusion levels. This was expected, since in the formulation of this compound, the buffering capacity of each acid was taken into consideration, aiming to minimize the effects on the buffering capacity of the diet. The presence of phosphoric acid in the AC2 acidifier blend, which had a higher water solubility, may have promoted the greater initial

pH decrease and influenced the buffering capacity, besides being the only inorganic acid present in the compounds. Another factor that can influence the buffering capacity of acidifiers is related to the level of protection used. The reason is that unprotected acidifiers can be easily buffered by components present in food ingredients or absorbed prior to their effect on the intestinal lumen. (Nowak *et al.*, 2021).



Means followed by standard deviation of the mean; ADG: average daily gain, AIL: acidifier blend inclusion level; LW: live weight; r^2: coefficient of determination.

Figure 3. Quadratic effect of the inclusion level of the AC2 acidifier blend on average daily gain (ADG) of piglets.

The main action mechanisms of acidifiers additives are the antimicrobial effect and the inhibition of pathogens, and may stimulate the secretion of pancreatic enzymes, reduce gastric pH, act as an energy source during the intermediate metabolism of the gastrointestinal tract and improve the use of minerals by the chelation process (Suryanarayana and Ramana, 2015), aiming to improve total apparent digestibility, feed conversion and performance of the animals (Diao *et al*., 2014). Protected acidifiers can improve intestinal morphology and function, reduce gastrointestinal pH, and improve intestinal adaptation (Jia *et al.*, 2010), expecting positive effects on digestibility. However, in this study there was no effect of treatment on nutrient digestibility, showing that neither the addition of AC1 and AC2 acidifiers blend improved the overall nutrient digestibility of nursery piglet diets. Likewise, in a study with complex diets, the addition of acidifiers (blend and sodium butyrate) as growth promoter had no effect on increasing the nutrients digestibility of the diet (Boas *et al*., 2016).

Serum protein was associated with the diet provided at each stage of development and may vary according to the age and genotype of the animals (Stukelj *et al*., 2010). In this study, regardless of the collection time, the use of AC1 and AC2 acidifiers blend as feed additive in the diet did not influence the concentrations of urea, total protein, albumin, globulin, and lactate, with values within the reference range for the age and species evaluated, corroborating with previous studies (Stukelj *et al*., 2010; Mu *et al*., 2017).

A high level of lactate in the stomach tends to inhibit the secretion of hydrochloric acid and therefore, generates insufficient enzyme secretion (Suryanarayana and Ramana, 2015). Therefore, as presented in this study, blood lactate levels were not influenced by the addition of AC1 and AC2 acidifiers blend, regardless of the collection time. This can be considered a positive point for additive supplementation, as it did not interfere in the stomach acidity process of piglets.

Moreover, the lack of effect of AC1 and AC2 acidifiers blend on blood metabolites may be attributed to inherent piglet factors, such as greater weight at weaning and a consequently better immune response (Denck *et al*., 2017), which could contribute to the low response of acidifiers during the supplementation period.

In terms of the higher ADG, the best inclusion level of the AC2 acidifier blend to the diet was found to be 0.26%, agreeing with previous studies that observed that dietary supplementation with acidifiers improved growth performance in weanling piglets (Miguel *et al*., 2011; Lei *et al*., 2017; Long *et al*., 2018).

The improvement in the ADG of piglets supplemented with acidifier blend in the diet can be due to the modulation of pH, improving the action of digestive enzymes with a probable improvement in the nutrient utilization of the diet and in the animal performance (Diao *et al*., 2014).

CONCLUSION

The use of an acidifier composed of benzoic, sorbic, formic, lactic and phosphoric acids in a protected form (AC2) as feed additive had a greater effect on the initial reduction in pH and a lower linear buffering rate of the diet than those composed of five organic acids including benzoic, lactic, formic, fumaric and citric acids (AC1). The use of acidifiers blend with inclusion of 0.3% did not improve the nutrient digestibility of nursery piglets and had no effect on blood metabolites. Considering the average daily gain of the animals, the best inclusion level of the AC2 acidifier blend in the diet was 0.26%.

REFERENCES

AHMED, S.T.; HWANG, J.A.; HOON, J. *et al.* Comparison of single and blend acidifiers as alternative to antibiotics on growth performance, fecal microflora, and humoral immunity in weaned piglets. *Asian-Australas. J. Anim. Sci*., v.27, p.93-100, 2014.

BOAS, A.D.C.V.; BUDIÑO, F.E.L.; TRINDADE NETO, M.A. *et al*. Organic acids in diets of weaned piglets: performance, digestibility and economical viability. *Arq. Bras. Med. Vet. Zootec.*, v.68, p.1015-1022, 2016.

BOCKOR, L.; OLIVEIRA JR, J.M.; MENDES, A.R. *et al*. Método de linearização de curvas de titulação para determinação da capacidade tamponante de alimentos para animais. *Arch. Vet. Sci*., v.22, p.66-74, 2017.

CANIBE, N.; STEIEN, S.H.; OVERLAND, M. *et al*. Effect of K-diformate in starter diets on acidity, microbiota, and the amount of organic acids in the digestive tract of piglets, and on gastric alterations. *J. Anim. Sci.*, v.79, p.2123-2133, 2001.

CORASSA, A.; LOPES, D.C.; OSTERMANN, J.D. *et al*. Níveis de ácido fólico em dietas contendo ácido fórmico para leitões de 21 a 49 dias de idade. *Rev. Bras. Zootec*., v.35, p.462-470, 2006.

DENCK, F.M.; HILGEMBERG, J.O.; LEHNEN, C.R. Uso de acidificantes em dietas para leitões, em desmame e creche. *Arch. Zootec.*, v.66, p.629-638, 2017.

DIAO, H.; ZHENG, P.; YU, B. *et al.* Effects of dietary supplementation with benzoic acid on intestinal morphological structure and microflora in weaned piglets. *Livest. Sci*., v.167, p.249-256, 2014.

GRILLI, E.; MESSINA, M.R.; TEDESCHI, M. *et al*. Feeding a microencapsulated blend of organic acids and nature identical compounds to weaning pigs improved growth performance and intestinal metabolism. *Livest. Sci*., v.133, p.173-175, 2010.

JIA, G.; YAN, J.Y.; CAI, J.Y. *et al*. Effects of encapsulated and non-encapsulated compound acidifiers on gastrointestinal pH and intestinal morphology and function in weaning piglets. *J. Anim. Feed Sci.*, v.19, p.81-92, 2010.

KAPS, M.; LAMBERSON, W.R. *Biostatistics for animal science*: an introductory text. Cambridge: CABI, 2009.

KIL, D.Y.; KWON, W.B.; KIM, B.G. Dietary acidifiers in weanling pig diets: a review. *Rev. Colom. Cienc. Pecu.* v.24, p.231-247, 2011.

LEI, X.J.; PARK, J.W.; BAEK, D.H. *et al*. Feeding the blend of organic acids and medium chain fatty acids reduces the diarrhea in piglets orally challenged with enterotoxigenic Escherichia coli K88. *Anim. Feed Sci. Technol*., v.224, p.46-51, 2017.

LONG, S.F.; XU, Y.T.; PAN, L. *et al*. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Anim. Feed Sci. Technol*., v.235, p.23-32, 2018.

MIGUEL, W.C.; TRINDADE NETO, M.A.; BERTO, D.A. *et al*. Suplementação de acidificantes em rações de leitões desmamados: desempenho e digestibilidade. *Braz. J. Vet. Res. Anim. Sci.*, v.48, p.141-146, 2011.

MU, C.; YANG, Y.; YU, K. *et al*. Alteration of metabolomic markers of amino acid metabolism in piglets with in feed antibiotics. *Amino Acids*, v.49, p.771-781, 2017.

NOBLET, J.; PEREZ, J.M. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. *J. Anim. Sci.*, v.71, p.3389-3398, 1993.

NOWAK, P.; ZAWORSKA-ZAKRZEWSKA, A.; FRANKIEWICZ, A. *et al*. The effects and mechanisms of acids on the health of piglets and weaners–a review. *Ann. Anim. Sci.*, v.21, p.433-455, 2021.

OFFICIAL methods of analysis of AOAC international. 15.ed. Gaithersburg: AOAC International, 1990.

OLIVEIRA JR, J.M.; NARDI, V.K.; SCANDOLERA, A.J. *et al*. Acidifier and nutrients digestibility in growing pigs. *Cienc. Anim. Bras*., v.18, p.1-10, 2017.

PAPATSIROS, V.G.; CHRISTODOULOPOULOS, G.; FILIPPOPOULOS, L.C. The use of organic acids in monogastric animals (swine and rabbits). *J. Anim. Sci. Biotechnol*., v.6, p.154-159, 2012.

PIVA, A.; GRILLI, E.; FABBRI, L. *et al*. Free versus microencapsulated organic acids in medicated or not medicated diet for piglets. *Livest. Sci*., v.108, p.214-217, 2007.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L. *et al. Tabelas brasileiras para aves e suínos*: composição de alimentos e exigências nutricionais. 3.ed. Viçosa: Universidade Federal de Viçosa, 2011. 252p.

STUKELJ, M.; VALENCAK, Z.; KRSNIK, M. *et al.* The effect of the combination of acids and tannin in diet on the performance and selected biochemical, haematological and antioxidant enzyme parameters in grower pigs. *Acta Vet. Scand*., v.52, p.1-19, 2010.

SUIRYANRAYNA, M.V.A.N.; RAMANA, J.V. A review of the effects of dietary organic acids fed to swine. *J. Anim. Sci. Biotechnol*., v.6, p.45, 2015.

SURYANARAYANA, M.V.A.N.; SURESH, J.; RAJASEKHAR, M.V. Organic acids in swine feeding: a review. *Agric. Sci. Res. J*., v.2, p.523-533, 2012.