



Evaluation of growth performance and gastro-intestinal parameters on the response of weaned piglets to dietary organic acids

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ABSTRACT

Two experiments (E) were carried out to evaluate the effects of fumaric acid and an acidifier blend [composed by calcium formate, calcium lactate and medium-chain fatty acids (capric and caprylic)] in piglet diets containing colistin (40 ppm) or halquinol (120 ppm) on performance, diarrhea incidence (E1), organs relative weight, pH values, intestinal morphometry and microbiota (E2). In E1, 192 and E2, 24 piglets weaned at 21-day-old were randomly assigned to blocks with 2x2 factorial arrangement of treatments [absence or presence of fumaric acid x absence or presence of acidifier blend], six replicates of eight (E1) and one piglet per pen (E2). For E1, the treatments were control (CD): no acidifier product + 40 ppm of colistin, FA: fumaric acid in absence of acidifier blend, AB: acidifier blend in absence of fumaric acid and, AF+AB: presence of fumaric acid and acidifier blend. For E2, the pre-starter I diet were used and the same treatments as E1 evaluated. No treatment effects ($P>0.05$) were observed on performance, diarrhea incidence (E1), gut pH values and duodenum morphometry of piglets (E2). However, the addition of AB increased ($P<0.05$) large intestine relative weight and, FA addition decreased ($P<0.05$) pancreas relative weight, jejunum villi height and, total coliform and *E. coli* counts in cecum. The inclusion of FA and AB in diets containing colistin or halquinol did not improve performance, although FA exerted an inhibitory effect on cecum microbiota.

Key words: acidifiers, diarrhea, nutrition, pig, weaning.

INTRODUCTION

Early-weaning is one of the most critical management practices in piglet's life, which constitutes a challenge for them and results in

poor growth performance, reduced immunity, and an imbalanced microbiota. The main factors contributing to these results are low feed intake and onset of diarrhea (Barnett et al. 1989). At weaning, the piglets that were physiologically adapted to digest the sow's milk are abruptly submitted to a diet with high amount of vegetable ingredients,

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usually a cereal like corn as main energy source and high in starch (Vente-Spreuwenberg et al. 2003), and another vegetable protein source like soybean meal, for which they are not prepared to digest due to its insufficient production of gastric hydrochloric acid that leads to an increased pH and decreased activity of proteolytic enzymes. Besides that, the lactic acid produced by fermentation of lactose is primarily responsible for acidification of suckling pig stomach and, according to Barrow et al. (1977), this acid acts as HCl-production inhibitor. Also, the presence of large amount of soybean meal in piglets' diet might cause hypersensitivity reactions which result on altered bowel structures (the villi) hindering the digestion of feed and absorption of nutrients (Li et al. 1990). Therefore, the use of enhancing additives can be an alternative to compensate these deficiencies.

Concerning the appearance of resistant bacteria and residues in animal products, the European Union has been prohibited the use of antibiotics as performance-enhancing antimicrobial compounds. Thus, the interest for alternative products, such as organic acids and their salts have increased by the consumer, due to its antimicrobial activity and safety (Blank et al. 2001, Costa et al. 2011). According to Cherrington et al. (1991), these products leave no residues in carcass and do not promote emergence of resistant bacteria, being a natural, safe, and inexpensive alternative to antibiotics in order to improve the health and performance of animals. For pigs, the most commonly used acids are acetic, formic, propionic, lactic, citric and fumaric acid (Partanen 2002), and the efficiency of organic acids in improving piglet's growth performance have been confirmed by other studies (Giesting and Easter 1985, Radecki et al. 1988, Radcliffe et al. 1998).

Therefore, this study aimed to evaluate the effects of dietary supplementation of fumaric acid and an acidifier blend [calcium formate, calcium lactate and medium-chain fatty acids (capric and caprylic)] on growth performance, organs weight,

pH, morphometry and intestinal microbiota of weaned piglets.

MATERIALS AND METHODS

All experimental procedures were previously approved by the Animal Ethics Committee of the Veterinary and Animal Science College from São Paulo State University (UNESP), under protocol number 187/2012-CEUA and, in accordance with directive 2010/63/EU.

EXPERIMENT 1

The experiment was conducted in a nursery facility at a commercial farm in city of Patos de Minas, Minas Gerais State, Brazil. The nursery stall was divided into three rooms, with ceiling height of 2.40 m, and eight pens each, totaling twenty-four pens. Each pen had 4.64 m² with a partially slatted and not suspended flooring, two nipple-type drinkers and a semi-automatic feeder for seven piglets. A hundred forty and four male piglets (castrated) and forty-eight females, totaling 192 animals, from *Agroceres PIC* genetics (Pietrain/Landrace x Duroc/Landrace) were weaned at 21-day-old with average initial body weight of 6.80 ± 0.89 kg. The control temperature within each nursery room was carried out with the management of lateral curtains and heaters.

The experiment duration lasted 41 days and the piglets were fed three types of diets (Table I) according to a nursery feeding system in phases: pre-starter diet I (PI) in the first 14 days after weaning (from 21- to 35-day-old), pre-starter diet II (PII) in the following 14 days (from 36- to 49-day-old) and, starter diet (SI) until the end of the experiment (from 50- to 62-day-old). The formulated diets followed the nutritional requirements proposed by Rostagno et al. (2011), except for calcium. All animals had *ad libitum* access to feed and water.

The experimental design was a randomized block design with four treatments, six replicates and eight animals per experimental unit, in a

factorial arrangement [absence or presence of fumaric acid (FA) x absence or presence of acidifier blend (AB)]. The variables considered for blocks formation were the weight and sex of animals.

The treatments were CD: PI, PII and SI diets without acidifier product; FA: PI, PII, and SI diets containing 0.8%, 0.4% and 0.2% of fumaric acid, respectively; AB: PI, PII, and SI diets containing 0.3%, 0.3% and 0.2% of acidifier blend, respectively; AB + FA: PI, PII, and SI diets containing 0.8%, 0.4% and 0.2% of fumaric acid and 0.3%, 0.3% and 0.2% of acidifier blend respectively. For all treatments, 40 ppm of colistin were added to PI and PII diets and, 120 ppm of halquinol were added to SI diets. The acidifier product used was a mixture of calcium formate, calcium lactate salts and medium-chain fatty acids (capric and caprylic). The inclusions of acidifier, the colistin sulphate and the halquinol in feed were possible by the substitution of equivalent amount of inert material (kaolin).

At beginning of experiment and at each diet switch, the piglet weight was measured individually. At periods 0 to 14, 0 to 28 and 0 to 42 days after weaning, the daily feed intake, daily weight gain and feed:gain ratio were evaluated. During the first 14 days of experiment, the diarrhea incidence was evaluated twice a day by the same observer. Diarrhea status was considered when visually faeces had fluid consistency. The values of diarrhea incidence were calculated as mean percentage of animals with diarrhea in the pen (p), and the data were subjected to angular transformation: $p' = \arcsin[\sqrt{(p/100)}]$ for further analysis of variance. The animals were housed in pens that have not undergone to previous disinfection. The diet pH was evaluated according to the following methodology (AOAC 1990): 5 g of feed sample was weighed into a 100 mL becker, following 25 mL of de-ionized water addition. After mixing the solution and waiting for 30 min, the pH of solution

was registered by a portable Tecnopon pH meter (model mPa-210P, Piracicaba, Brazil).

All data were analyzed using the GLM procedure of SAS 9.0 (SAS 2002). Tukey's multiple range tests were applied to test significant differences between treatments. For all of statistical analysis for this work, the pen served as an experimental unit. Variability in the data was expressed as coefficient of variation (CV) and a probability level of ($P < 0.05$) was considered as statistically significant.

EXPERIMENT 2

The second experiment was conducted in experimental swine production facilities from Faculty of Veterinary Medicine and Animal Science at São Paulo State University (UNESP), Botucatu Campus, Brazil with twelve castrated males and twelve females piglets from commercial genetics (Landrace x Large White) weaned at 21-day-old with an average body weight of 6.46 ± 0.24 kg. The nursery facility had metal suspended pens of 1.75 m² and, a partially slatted flooring with a compact concrete floor under the heater, being equipped with one feeder, one nipple-type drinker and one heater.

The experimental design was a randomized block design with four treatments, six replicates and one animal per experimental unit. The experiment duration lasted 14 days, during which the animals were fed pre-starter I diets (PI) (Table I). All treatments were the same as defined in Experiment I.

At 14th experimental day, all piglets were slaughtered after electrical stunning. A longitudinal incision was made along abdominal cavity for removal of digestive organs. The gastrointestinal tract (with contents) was weighed by full and after removing the stomach, small intestine, cecum and large intestine content, they were weighed again, one by one, to determine its absolute weight. The liver, pancreas, spleen and kidneys were also weighed in order to determine their absolute

TABLE I
Composition of experimental diets provided to piglets
from 21- to 62-day-old.

Ingredients	Diets		
	PI ¹	PII ²	SI ³
Corn	31.70	45.70	62.07
Soybean meal	25.00	27.00	31.00
Cracker/biscuit residue	10.00	7.00	-
Spray-dried blood meal	1.20	0.25	-
Whey	12.00	6.00	-
Energetic concentrate ⁴	3.00	2.00	3.00
Sugarcane yeast	1.00	0.50	-
Pregelatinized corn	3.37	1.94	0.74
Extruded soybean and viscera	8.00	5.00	-
Mineral and vitamin premix ⁵	0.40	0.40	0.50
Dicalcium phosphate (18%)	1.08	1.38	0.97
Ground limestone (37%)	0.25	0.13	0.26
Salt	0.21	0.40	0.50
Sweetener ⁶	0.30	0.30	0.30
Inert (kaolin) ⁷	1.10	0.70	0.40
Fumaric acid	*	*	*
Acidifier blend ⁸	*	*	*
Colistin (4%)	0.10	0.10	-
ZnO (73%)	0.29	0.30	-
CuSO ₄ (25%)	0.07	0.07	-
Halquinol (60%)	-	-	0.02
L-Lys HCl (80%)	0.49	0.45	0.16
DL-Met (99%)	0.22	0.19	0.03
L-Thr (98%)	0.17	0.15	0.03
L-Trp (99%)	0.03	0.02	-
Total (kg)	100.00	100.00	100.00
Calculated values			
Metabolizable energy (kcal kg ⁻¹) ⁹	3,411	3,352	3,284
Crude protein (%)	21.86	20.24	20.18
Ca (%)	0.69	0.63	0.78
Digestible P (%)	0.46	0.45	0.40
Digestible Lys (%)	1.46	1.31	1.09
Digestible Met (%)	0.52	0.48	0.32
Digestible Thr (%)	1.12	0.81	0.68
Digestible Trp (%)	0.24	0.22	0.19
Lactose-equivalent (%)	10.00	5.00	-

¹PI: pre-starter I diet [treatment FA with 0.8% fumaric acid (FA); treatment AB with 0.3% acidifier blend (AB) and treatment FA+AB with 0.8% fumaric acid plus 0.3% acidifier

blend]. ²PII: pre-starter II diet [treatment FA with 0.4% fumaric acid (FA); treatment AB with 0.3% acidifier blend (AB) and treatment FA+AB with 0.4% fumaric acid plus 0.3% acidifier blend]. ³SI: starter I diet [treatment FA with 0.2% fumaric acid (FA); treatment AB with 0.2% acidifier blend (AB) and treatment FA+AB with 0.2% fumaric acid plus 0.2% acidifier blend]. ⁴Energipig[®]: ground corn, extruded soybean, biscuit meal, corn germ meal, vanilla flavor, soy lecithin, enzymatic additive, propionic acid, monosodium glutamate, etoxiquin and, butylated hydroxy toluene. ⁵Agroceres[®] (supplied per kg of diet): Vit. A – 4,000 I.U.; Vit. D₃ – 220 I.U.; Vit. E – 22 mg; Vit. K – 0.5 mg; Vit. B₂ – 3.75 mg; Vit. B₁₂ – 20 µg; Calcium pantothenate – 12 mg; Niacin – 20 mg; Choline – 60 mg; I – 140 µg; Se – 300 µg; Mn – 10 mg; Zn – 100 mg; Cu – 10 mg; Fe – 99 mg. Nutritional values according to Rostagno et al. (2011). ⁶Flavofix[®] (supplied per kg of feed): neohesperidin dihydrochalcone – 1,200 mg/kg; sodium saccharin – 58.20 g/kg; vanilla flavor – 25 g/kg. ⁷The inclusions of acidifier, the colistin sulphate and the halquinol in feed were possible by the substitution of equivalent amount of inert material (kaolin). ⁸AgAcid[®]: calcium formate, calcium lactate, medium-chain fatty acids (capric and caprylic acids). ⁹Calculated values without considering the energetic contribution from FA and AB.

weights. After this procedure, the relative weight of each organ was calculated based on the empty carcass weight (weight with no digesta content). Also pH readings of stomach, jejunum, ileum and colon contents were measured immediately after slaughter by a portable Tecnoport pH meter (model mPa-210P, Piracicaba, Brazil).

The cecum of each animal was removed with its content and stored in styrofoam boxes with ice for further laboratory analysis. The total coliform and *Escherichia coli* counts were measured from 10 g of cecal content, and then added to 90-mL of 0.85% saline and homogenized for 2 min, obtaining a 10:1 dilution. After, a serial decimal dilutions were prepared, transferred to 1-mL of ECTM Petri dishes and incubated at 35°C for 24-hour (AOAC 2000). The *Lactobacillus* spp. count was performed from decimal serial dilutions as described for *Escherichia coli* count, transferring 0.1-mL into Petri dishes containing Man-Rogosa-Sharpe (surface sowing), incubated at 30°C for 5 days in a drying air oven with 5% CO₂ atmosphere

(Downes and Ito 2001). Microflora enumerations were expressed as \log^{10} colony-forming unit (cfu) per gram.

For gut morphometry analysis, samples were collected from 1.5 cm of duodenum and jejunum initial and middle portions which were immersed in 10% formalin solution for 48-hour. Then, samples were immersed in 70% ethanol solution, dehydrated in ethyl alcohol (increasing concentrations). The tissue fragments were cut about 1.0 cm in each segment, diaphanized in benzene and embedded in paraffin. Four semi-serial sections of segments (jejunum and duodenum) with 5 μm thickness were performed for each animal. After processing, the sections were placed on slides, stained by hematoxylin and eosin technique and examined under a light microscope for villi height and width, crypt depth measurements using Leica QWin image processing and analysis workstation software. Thirty readings were performed for each animal and each segment. The final result was the average of 30 measurements.

The scanning electron microscopy technique was used to evaluate the villi density. Duodenum and jejunum samples collected after slaughter were fixed in 3% glutaraldehyde, washed in 0.1 M sodium cacodylate, buffered in pH 7.2, post-fixed in 1% osmium tetroxide. Then, samples were washed in the same buffer and dehydrated in an increasing ethanol concentration. The tissue samples were soaked into isoamyl acetate solution for 3-hour. Drying was performed obtaining the critical point of liquid CO_2 in a dry air oven SEM model 850. A Denton Desk II model vacuum device was used for sputter coated (gold-palladium). The samples were mounted over supports of 1.0 cm in diameter and thickness, using metal tape, observed and registered by a scanning electron microscope JEOL JSM 5410, operated at accelerating voltage of 15 kV. The electron micrographs from four samples were taken counting the villi. Then, to assess the approximate magnitude of the absorptive area per

1 mm^2 of the intestinal mucosa (mm^2/mm^2), the surface of villi was calculated and multiplied by the number of villi, using the following equation: $\pi r^2 + 2\pi rh$, where r is the average radius (villus width/2) and h is the villi height (Skrzypek et al. 2010).

All data were analyzed using the GLM procedure of SAS 9.0 (SAS 2002). Tukey's multiple range tests were applied to test significant differences between treatments. Variability in the data was expressed as coefficient of variation (CV) and a probability level of ($P < 0.05$) was considered as statistically significant.

RESULTS AND DISCUSSION

EXPERIMENT 1

The use of AB decreased ($P < 0.05$) pH values of diets. This reduction was increasingly higher for the acidifier blend, fumaric acid and fumaric acid in combination with the mixture of acids (Table II). No interactions and no effect of main factors ($P > 0.05$) were registered on daily feed intake (DFI), daily weight gain (ADG), feed:gain ratio (F:G) and diarrhea incidence (DI) of piglets (Table III) on studied periods. These results corroborate those reported by Gomes et al. (2011), that the addition of 0.5% of fumaric acid and its combination with 0.5% lactic acid and 0.5% calcium propionate did not improve growth performance of piglets weaned at 15-day-old. Similarly, Zentek et al. (2013) found no effect on growth of weanling piglets fed diets containing the mixture of medium-chain fatty acids (capric and caprylic).

The addition of organic acids and their salts in diet of weanling pigs, such as fumaric acid (Giesting and Easter 1991), lactic acid (Tsiloyiannis et al. 2001, Silva 2002), calcium formate (Partanen and Mroz 1999, Bosi et al. 2005) or the combination as lactic acid, formic acid and phosphoric acid (Freitas et al. 2006) have resulted in benefits on growth performance, while other researches have shown

negative responses on piglets' growth performance due to formic acid usage (Manzanilla et al. 2004) and mixture of fumaric and formic acids (Gomes et al. 2007).

The divergence in results due to usage of acidifiers in weaned piglets diets might be related to differences on raw material composition of experimental diets. Throughout this experiment, the diets had high inclusion of animal protein and lactose, which might be a substrate for microorganisms present in digestive tract, such as *Lactobacillus* spp., which produces lactic acid and decreases the pH. Owsley et al. (1988) observed that the use of simpler diets based on corn and soybean meal and low inclusion of dairy products have resulted on positive effects after acidifier inclusion. Other reasons for the divergence of results observed in researches performed with acidifiers are the palatability of diets that can inhibit or stimulate consumption, the buffering capacity of diets, the level of acidifier inclusion in diets and the animal's age (Ravindran and Kornegay 1993). In this study, the addition of organic acids in diets had no effect on palatability, since the feed intake was not different among treatments.

The addition of antibiotics and antimicrobial agents as copper sulfate and zinc oxide has also influenced the absence of responses from acidifiers (they might have masked the positive effects of organic acids), since they act similarly to those products (Katouli et al. 1999). Another important factor was high weight at weaning that might have contributed to reduce the organic acids responses, because piglets were healthier and mature (Radecki et al. 1988).

The feed provided to piglets in farrowing stall benefits the development of digestive enzymes activity, improving diet utilization by weaned piglets (Lindemann et al. 1986). The animals of this experiment received pre-starter diets in farrowing rooms, which might have contributed to the absence of acidifiers' responses due to, according

to Radecki et al. (1988), this management practice can affect the efficiency of organic acids, once feed consumption in the farrowing house accelerates the maturation of piglets' digestive system at weaning, promoting their greater adaptation.

The diarrhea, the most serious problems of weaned piglets, is characterized by loss of solutes and water, leading to depletion of electrolytes, acid-base imbalance and dehydration, which can be fatal if not treated properly (Zlotowski et al. 2008). The use of additives, such as organic acids and their salts reduces or even eliminates the post-weaning diarrhea problems, acting in pathogenic bacteria control (Tsiloyiannis et al. 2001, Mazzoni et al. 2004, Bosi et al. 2005, Roselli et al. 2005, Freitas et al. 2006). No differences ($P>0.05$) were observed on diarrhea incidence among animals (Table III). Similar results reported by Risley et al. (1993) and Braz et al. (2011) found no differences on diarrhea occurrence when diets were evaluated with combinations of organic acids for piglets. The fact that animals had been housed in pens subjected to a long empty period before the beginning of the experiment, although not disinfected, have contributed to a low health challenge what might explain the ineffectiveness of acidifier blend or fumaric acid on diarrhea reduction in piglets kept on that health challenge status.

EXPERIMENT 2

No FA x AB interaction effects neither differences on pH values of stomach, jejunum, ileum, colon and rectum ($P>0.05$) were registered (Table II). The stomach pH ranged from 3.10 to 3.68, which are within the range of pepsin action, that is 1 to 4 (Yamamoto 1975). Gomes et al. (2011) found no statistical differences when added 0.5% fumaric acid and 0.5% fumaric acid plus 1% lactic acid in piglet diets and, reported gastric pH values of 4.13 and 4.23, respectively, for piglets at 38-day-old.

TABLE II
Mean pH values of diets and piglets' digestive tract.

		Diet-pH values*				
Fumaric acid	Acidifier blend	PI	PII	SI		
Absent	Absent	6.29	6.27	6.17		
	Present	6.08	6.16	6.14		
Present	Absent	5.32	5.32	5.83		
	Present	5.26	5.24	5.75		
		Segment-pH values**				
		S	J	I	C	R
Absent	Absent	3.42	6.02	6.80	6.15	6.25
	Present	3.82	5.58	6.78	6.12	6.48
Present	Absent	3.10	5.62	6.88	6.02	6.43
	Present	3.47	5.72	6.77	6.03	6.27
<i>Main factors</i>						
Fumaric acid (FA)						
	Absent	3.62	5.80	6.79	6.13	6.37
	Present	3.28	5.67	6.83	6.03	6.35
Acidifier blend (AB)						
	Absent	3.26	5.82	6.84	6.08	6.34
	Present	3.64	5.65	6.78	6.08	6.38
FA x AB		NS ¹	NS ¹	NS ¹	NS ¹	NS ¹
CV (%) ²		21.48	7.55	3.47	5.53	3.76

*Values of diet-pH from experiment 1. PI = pre-starter I diet; PII = pre-starter II diet; SI = starter I diet; S = stomach; J = jejunum; I = ileum; C = colon; R = rectum. ** Values of segment-pH from experiment 2. ¹NS: Non-significant (P>0.05). ²Coefficient of variation.

The absence of effects of AB on pH of stomach digesta (Risley et al. 1992, Freitas et al. 2006, Bosi et al. 2007, Braz et al. 2011), duodenum pH (Bosi et al. 2007), jejunum pH (Risley et al. 1992) and ileum pH (Gabert and Sauer 1995, Bosi et al. 2007) was similar to the observed in this experiment, suggesting that this is not the most efficient mode of action for acidifiers' usage. The differences on pH values found in segments of digestive tract might occur depending on the location where measurements were taken, the methodology used and the time after eating (Ferreira 1986). In

the present study, sampling was performed after the slaughter of animals, which did not undergo fasting. Another factor that might have influenced the response of acidifier was the lactose inclusion in diets, which is used as substrate for *Lactobacillus* spp. to produce lactic acid, which in turn reduces the pH.

However, some researches have shown acidifiers efficiency in reducing pH of stomach digesta (Thomlinson and Lawrence 1981, Bolduan et al. 1988, Maribo et al. 2000), cecum pH (Maribo et al. 2000) and colon pH (Maribo et

TABLE III
Means of performance variables at periods 21 to 35, 21 to 49, and 21 to 62 of piglets' age.

Fumaric acid	Acidifier blend	Growth performance variables										
		DFI (g)			DI (%)		ADG (g)			F:G		
		21 to 35	21 to 49	21 to 62	21 to 35	21 to 35	21 to 49	21 to 62	21 to 35	21 to 49	21 to 62	
Absent	Absent	270	555	770	13.46	228	383	457	1.19	1.45	1.69	
	Present	252	530	725	12.02	206	388	440	1.23	1.37	1.65	
Present	Absent	250	526	742	12.50	200	361	437	1.25	1.46	1.70	
	Present	263	542	768	13.94	215	375	458	1.23	1.44	1.68	
<i>Main factors</i>												
Fumaric acid (FA)												
	Absent	261	542	748	12.74	217	385	448	1.20	1.41	1.68	
	Present	256	534	756	13.22	208	368	448	1.24	1.45	1.69	
Acidifier blend (AB)												
	Absent	260	540	748	12.98	214	372	447	1.22	1.45	1.69	
	Present	258	536	756	12.98	211	381	449	1.23	1.41	1.66	
FA x AB		NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	
CV (%) ²		13.5	10.7	8.71	24.66	16.2	9.95	8.52	5.70	4.61	3.45	

DFI = daily feed intake; DI = diarrhea incidence; ADG = average daily gain; F: G = feed:gain ratio. ¹NS: Non-significant (P>0.05). ²Coefficient of variation.

al. 2000, Namkung et al. 2004). The reduction on digesta pH limits the development of pathogenic microorganisms, such as *Escherichia coli*, which does not survive in acidic conditions (Barcellos et al. 2007), but usually grows in a pH range from 5 up to 9 (Zilberstein et al. 1984).

No FA x AB interaction or main factors effects were observed (P>0.05) on relative weights of stomach, small intestine, cecum, liver, spleen and kidneys. However, the addition of FA in diet determined lower relative weight of pancreas (P = 0.009) and, the addition of AB resulted on higher relative weight of large intestine (P = 0.009) (Table IV). Similar results were reported by Bhandari et al. (2008), which added the mixture of organic acids in piglet diets and did not observe effect on spleen relative weight, because this organ weight

varies depending on the energy and/or protein intake (Rao and McCracken 1992). The diets used in this experiment were isoproteic and had similar levels of metabolizable energy, which could justify the fact that no differences were found on relative weights of stomach, small intestine, cecum, liver, spleen and kidneys.

No FA x AB interaction effects and no effects of main factors (P>0.05) were observed on villi height (VH) of duodenum, villi width (VW), crypt depth (CD), villi height: crypt depth ratio (VH:CD), and absorption areas of duodenum and jejunum (Table V). The jejunum villi height of piglet fed diet containing fumaric acid was lower (P = 0.009) compared to animals fed diet without FA; however, the jejunal absorption area (Table V) was similar,

TABLE IV
Mean of organ's relative weights calculated based on empty weight of piglet's carcass.

Fumaric acid	Acidifier blend	Relative weights							
		S	SG	LG	CC	L	P	SP	KD
Absent	Absent	0.98	5.26	1.65	0.27	2.67	0.26	0.20	0.64
	Present	0.98	5.64	1.77	0.32	2.72	0.26	0.19	0.63
Present	Absent	0.91	5.37	1.64	0.29	2.65	0.25	0.22	0.61
	Present	0.90	5.51	1.77	0.28	2.68	0.23	0.20	0.58
<i>Main factors</i>									
Fumaric acid (FA)									
	Absent	0.98	5.45	1.71	0.29	2.69	0.26 ^a	0.19	0.63
	Present	0.91	5.44	1.70	0.29	2.66	0.24 ^b	0.21	0.60
Acidifier blend (AB)									
	Absent	0.95	5.31	1.64 ^b	0.28	2.66	0.26	0.21	0.62
	Present	0.94	5.58	1.77 ^a	0.30	2.70	0.25	0.19	0.60
FA x AB		NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹	NS ¹
CV (%) ²		14.20	7.48	5.64	16.72	12.60	9.91	14.21	11.31

S = stomach; SG = small intestine; LG = large intestine; CC = cecum; L = liver; P = pancreas; SP = spleen; KD = kidneys. ^{a,b}Values followed by different letters in the column are statistically different (P = 0.009). ¹NS: Non-significant (P>0.05). ²Coefficient of variation.

indicating that presence of FA in diet resulted in an increase on villi density.

After evaluate FA inclusion of 0.5 to 1% in diets, Gomes et al. (2007) found no effects on small intestine morphometric characteristics. In contrast, Bosi et al. (2007) reported greater villi height values in small intestine of piglets weaned at 21-day-old when calcium formate were provided in diets.

The reduction on intestinal villi height of weaned piglets occur at 3rd to 7th days after weaning (Cera et al. 1988) and, the intensity of intestinal disorders are more associated with ingredients used in diet formulation (Li et al. 1990) and the quantity of ingested feed (Van Beers-Schreurs et al. 1998). The decrease on villi height are mainly due to the increase in cell desquamation rate (Thomaz et al. 2002) which predisposes animals to

nutrient malabsorption, diarrhea and dehydration (Scandolera et al. 2005). Higher VH:CD ratios indicate the presence of increased amount of mature and functional enterocytes, which results in better digestion and absorption of nutrients and also, minor injuries in intestinal mucosa (Tucci et al. 2011).

The animals were kept during the experimental period in a controlled environment, under good conditions of temperature and hygiene, as well as receiving pre-starter diets with high inclusion of lactose, which help to reduce the stress of weaning, contributing to minor changes of intestinal mucosa (Figueiredo et al. 2003, Thomaz et al. 2011).

The nutrient absorption capacity is proportional to density and size of the villi (Macari 1995). Skrzypek et al. (2010) used piglets at 14-day-old

TABLE V
Means of morphometric variables of piglet's duodenum and jejunum.

Morphometric variables of duodenum						
Fumaric acid	Acidifier blend	VH (µm)	VW (µm)	CD (µm)	VH:CD	AA (mm²/mm²)
Absent	Absent	336	161	196	1.77	10.35
	Present	374	154	216	1.80	9.06
Present	Absent	345	148	194	1.84	8.00
	Present	318	154	179	1.85	7.26
<i>Main factors</i>						
Fumaric acid (FA)						
	Absent	355	157	206	1.78	9.71
	Present	331	151	187	1.85	7.63
Acidifier blend (AB)						
	Absent	340	155	195	1.81	9.18
	Present	346	154	197	1.82	8.16
FA x AB		NS ¹	NS ¹	NS ¹	NS ¹	NS ¹
CV (%) ²		16.41	13.64	13.24	14.36	43.40
Morphometric variables of jejunum						
Absent	Absent	337	125	182	1.89	4.89
	Present	374	115	190	2.03	6.10
Present	Absent	302	120	162	1.90	6.29
	Present	295	121	176	1.75	6.68
<i>Main factors</i>						
Fumaric acid (AF)						
	Absent	356 ^a	120	186	1.96	5.50
	Present	298 ^b	121	169	1.82	6.48
Acidifier blend (AB)						
	Absent	319	122	172	1.90	5.59
	Present	335	118	183	1.89	6.39
FA x AB		NS ¹	NS ¹	NS ¹	NS ¹	NS ¹
CV (%) ²		19.84	11.04	11.16	13.70	38.54

VH = villi height; VW = villi width; CD = crypt depth; VH:CD = villi height: crypt depth ratio; AA = absorptive area. ^{a, b}Values followed by different letters in the column are statistically different (P = 0.009). ¹NS: Non-significant (P>0.05). ²Coefficient of variation.

and the mean values obtained from duodenum and jejunum absorption area of 9.3 mm²/mm² and 14.4 mm²/mm², respectively were higher than those found in this study for piglets of 35-day-old; although they reported that gut absorption area increased rapidly until 7th day of age, and reduced on 14th day of piglets' age.

The use of ingredients with anti-nutritional factors and/or antigenic protein in diet causes changes on intestinal mucosa, including damage to villi and impair absorption of nutrients (Chamone et al. 2010). The diets used in this experiment were formulated, mostly using highly digestible ingredients, which may have contributed to absence of acidifier effects for most of the variables.

No FA x AB interaction effects were observed on total coliform counts (TC) and *Lactobacillus* counts (LC) (Table VI). However, animals fed diet containing FA showed lower (P<0.05) total coliform and *Escherichia coli* (EC) counts than piglets fed diet without FA, proving the antimicrobial capacity of the acid. The basic principle of FA action is related to its undissociated forms (non-ionized, lipophilic form) that diffuse across cell membranes of bacteria, inhibiting their growth through inactivation of bacterial decarboxylases and catalases or even though, over long periods of exposure to FA, disrupting their cell wall (due to an excess of intracellular H⁺ ions) and/or also to the intestinal lumen dissociation that liberates H⁺ ions serving as a pH barrier against bacteria colonization on brush border (Suiryanrayna and Ramana 2015). Although, after a reduction on total coliform counts, we also expected a reduction on *Escherichia coli* counts, once these microorganisms constitute the major part of enterobacteria population, the results obtained in this study were contrary to those reported by Risley et al. (1992), which found no reduction on *Escherichia coli* counts in cecum of weaned piglets fed diet containing 1.5% FA. These results agreed with results of studies in which the presence of acidifier has determined a reduction on

TABLE VI
Mean cfu-values of piglet's cecum microbiota.

Fumaric acid	Acidifier blend	Colony-forming unit (cfu) (log ¹⁰ /g)		
		TC	EC	LC
Absent	Absent	7.15	7.02	8.78
	Present	6.91	6.50	8.51
Present	Absent	5.98	5.27	8.56
	Present	5.71	5.80	8.30
<i>Main factors</i>				
Fumaric acid (FA)				
	Absent	7.04 ^a	6.76 ^a	8.64
	Present	5.84 ^b	5.53 ^b	8.43
Acidifier blend (AB)				
	Absent	6.57	6.15	8.67
	Present	6.31	6.15	8.41
FA x AB		NS ¹	NS ¹	NS ¹
CV (%) ²		19.37	22.87	6.72

TC = total coliform counts; EC = *Escherichia coli* counts; LC = *Lactobacillus* spp. counts. ^{a, b}Values followed by different letters in the column are statistically different (P = 0.009). ¹NS: Non-significant (P>0.05). ²Coefficient of variation.

Escherichia coli counts in faeces (Tsiloyiannis et al. 2001, Knarreborg et al. 2002).

The lack of responses regarding the acidifier blend usage confirms the results reported by Walsh et al. (2007), that evaluated a combination of organic acids and did not observe reduction on *Escherichia coli* counts in weaned piglets fecal content, as well as those described by Torrallardona et al. (2007), that analyzed inclusion of 1.8% of calcium formate in diets and found no difference on *Escherichia coli* counts in cecum and ileum of weaned piglets compared to other treatments. Bhandari et al. (2008), working with piglets weaned at 17-day-old and evaluating combinations of organic acids in diets, found no difference on *Escherichia coli* counts in ileum and cecum among treatments, highlighting that low number of replicates and inherent variability at post-weaning might have

caused the lack of answers. Moreover, lack of responses on the reduction of total coliform and *Escherichia coli* counts might also be explained by the low AB inclusion (0.3%) in experimental diets.

CONCLUSIONS

The inclusion of fumaric acid and the acidifier blend in diets containing colistin or halquinol is not indicated for weaned piglets from 21- to 62-day-old, regarding the growth performance results, pH values of digesta, relative weight of organs and intestinal morphometry. Also, the fumaric acid exerted inhibitory action on growth of total coliform and *Escherichia coli* population in piglets' cecum.

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