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Mannan-oligosaccharide and organic acids for weaned piglets

Mananoligossacarídeo e ácidos orgânicos para leitões desmamados

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Abstract

This study aimed to evaluate the effect of acetic, propionic, and formic (50%) organic acids and mannan-oligosaccharide (50%) on growth performance, digestibility, and faecal score in challenged weaned piglets. Twenty male piglets (5.57 ± 0.32 kg of BW; 21-24 days of age) were housed individually in metabolic cages for 28 days in an acclimatised room. The treatments were composed of the inclusion (0.1%; n = 10) or not (n = 10) of additive in the diet. The experimental design was completely randomised with two treatments, 10 replicates, and one piglet per replicate. The nutritional matrix was supplemented with 10% of barley and 35.9 to 34.0% of soybean meal in the pre-starter diet (3-14 days post-weaning) and the starter diet (15-28 days post-weaning), respectively, to cause an intestinal challenge. Diets did not include any antimicrobial or growth promoters. Weekly, the animal and the leftover diet were weighed to evaluate growth performance. Digestibility was evaluated through total faeces and urine collection. Piglets fed diets with additive had 8.7% greater weight gain ($P < 0.05$) compared to those piglets in the control treatment in the starter phase. For other growth performance responses there was no treatment effect. Similarly, the inclusion of additive in the piglet diets did not affect the faecal score or the energy and nutrient digestibility. In the starter phase and throughout the experimental period, piglets fed diets with additive had 18.37% and 15.07% greater nitrogen (N) intake and 19.53% and 16.05% greater N retention, respectively, compared to piglets in the control treatment ($P < 0.05$). In conclusion, the addition of additive composed by organic acids and mannan-oligosaccharide does not improve energy and nutrient digestibility but increases the N retention and weight gain in weaned piglets in the starting phase.
Key words: Nutritional additive. Intestinal challenge. Pigs. Prebiotic.

Resumo

O presente estudo foi realizado com o objetivo de avaliar os efeitos da suplementação de ácidos orgânicos, acético, propiônico e fórmico (50%) e mananoligossacarídeo (50%), sobre o desempenho, digestibilidade e escore fecal de leitões desafiados. Assim, vinte leitões (21 - 24 d idade), machos castrados, com peso inicial de 5,57 ± 0,32 kg foram alojados em gaiolas de metabolismo por 28 dias, em sala climatizada. Os tratamentos foram compostos pela inclusão (0,1%; n = 10) ou não (n = 10) do aditivo à dieta. O delineamento experimental foi inteiramente casualizado com dois tratamentos,

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10 repetições e um animal por repetição. Utilizou-se 10% de cevada e 35,9 e 34,0% de farelo de soja na matriz nutricional da dieta pré-inicial (de três a 14 dias pós desmame) e inicial (de 15 a 28 dias pós desmame), respectivamente, com o objetivo de causar um desafio intestinal para os leitões. Não foi usado nenhum tipo de antimicrobiano ou promotor de crescimento. Diariamente o escore fecal foi registrado e semanalmente foi feita pesagem das sobras de ração e dos animais para avaliação do desempenho. A digestibilidade foi avaliada por meio de coleta total de fezes e urina. Leitões suplementados com o aditivo apresentaram ganho de peso 8,7% maior ($P < 0,05$) que aqueles no tratamento controle, na fase inicial. Para as outras respostas de desempenho não houve diferença significativa entre os tratamentos. Da mesma forma, a adição de aditivo à dieta não afetou o escore fecal e a digestibilidade dos nutrientes e da energia bruta. Na fase inicial e no período total, leitões que receberam o aditivo na dieta apresentaram 18,37 e 15,07% maior nitrogênio consumido e 19,53 e 16,05% maior nitrogênio retido, respectivamente, quando comparado aos leitões do tratamento controle ($P < 0,05$). A adição do aditivo, contendo ácidos orgânicos e mananoligossacarídeo, não contribui para a melhora na digestibilidade dos nutrientes e da energia, porém aumenta a retenção de nitrogênio e o ganho de peso dos leitões.
Palavras-chave: Aditivo nutricional. Desafio intestinal. Prebiótico. Suínos.

Introduction

The higher susceptibility of weaned piglets to anorexia, with long periods among large feed intake portions, makes the nursery phase critical in the pig production system (BROOKS et al., 2001; SUIRYANRAYNA; RAMANA, 2015). The low growth performance observed in this phase is directly related to the occurrence of gastrointestinal disorders, mainly diarrhoea caused by pathogenic bacteria (ADEWOLE et al., 2016). Thus, dietary additives such as organic acids (BOAS et al., 2016; DEVI et al., 2016; ZHANG et al., 2016) and mannan-oligosaccharides (FESSELE; LINDHORST, 2013; GIANNENAS et al., 2016; WENNER et al., 2013) have been used to increase growth performance, decrease diarrhoea mortality, and modify the intestinal environment of pigs after weaning.

Organic acids act mainly in gastric pH reduction, increasing stomach acidification and improving protein digestion by pepsin. Furthermore, pH reduction prevents the colonisation of pathogenic bacteria such as *E. coli* and *Salmonella* (BUSSER et al., 2011; CALVEYRA et al., 2012; WALSH et al., 2012), promotes proliferation of intestinal cells, increases the villous size and absorptive capacity (DIAO et al., 2015, 2016), and improves immune capacity (KUANG et al., 2015) in weaned piglets.

In relation to mannan-oligosaccharide effects, previous studies have reported three distinct responses. The first concerns the beneficial modulation of the native microbiota present in the host. The second is the possible enhancing action on gut physiology, such as papillae increase. The third is a direct consequence of both the first and second through the influence of these compounds on animal performance (HALAS; NOCHTA, 2012; SILVA; NÖRNBERG, 2003).

Therefore, diet supplementation with a product based on mannan-oligosaccharide and organic acids (acetic, formic, and propionic) could improve weaned piglets' growth performance. Therefore, this study aimed to evaluate the effects of dietary supplementation with the above-mentioned additives on performance, digestibility, and faecal score in challenged piglets.

Material and Methods

The trial was carried out at the Zootechnical Laboratory at the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. All procedures used in this experiment were approved by the Animal Experimentation Ethics Committee (protocol no. 19602).

Twenty castrated male piglets (21-24 days old) with 5.57 ± 0.32 kg of BW were individually housed, after weaning, in metabolism cages for 28 days. The cages (0.48 m^2) were equipped with a feeder and drinker, and the pigs were maintained inside an air-conditioned room (28°C). Dietary treatments were supplemented with 0.1% or 0,0% of additive containing mannan-oligosaccharide (50%) and a mixture of acetic, propionic, and formic organic acids (50%). The experiment was conducted in two growing phases: pre-starter, between 3 and 14 days after weaning, and starter, between 15 and 28 days post-weaning. The data were also evaluated in the total period (3-28 days). Feed and water were provided *ad libitum* throughout the experimental period.

Two diets were formulated to be isocaloric and isoproteic (one diet for the pre-starter phase and another for the starter phase - Table 1) and either meet or exceed all other nutrient requirements (ROSTAGNO et al., 2005). The diets were supplemented with 10.0% of barley to induce a challenge in the gastrointestinal tract of the piglets. Barley contains a large amount of non-starch polysaccharides compared to corn and thus has lower digestibility. Also, a higher amount of soybean meal was used than what is recommended for the age of the piglets (35.9% and 34.0% for the pre-starter and starter phases, respectively). No lactose was included in the diets, and the levels of this nutrient were only met by the addition of whey powder in the percentages of 14.0% and 8.3% in the pre-starter and starter diets, respectively. No other growth promoter commonly used in the pre-starter and starter phases, such as zinc oxide, copper, or antimicrobial, was used in the diet. The animals and the leftovers were weighed weekly, and the average daily feed intake (ADFI), average daily gain (ADG), and gain:feed ratio (G:F) were calculated.

Table 1. Composition of the experimental diets (based on natural matter), provided in the period from 1 to 14 days (Pre-starter) and from 15 to 28 days (Starter) post-weaning.

Item	Pre-starter[1]	Starter[1]
Ingredient (g kg^{-1})		
Corn	239.20	342.00
Soybean meal	359.40	340.00
Whey powder	200.00	114.30
Barley	100.00	100.00
Coconut fat	40.00	40.00
Sugar	30.00	30.00
Monocalcium phosphate	12.20	14.40
Limestone	6.40	6.50
Salt	3.30	3.70
L-Lysine HCL (99%)	3.00	3.60
DL-Methionine (98.5%)	2.90	1.90
L-Threonine (99%)	1.60	1.70
Premix Vitamin and Mineral[1]	1.30	1.30
Choline	0.70	0.60
Nutritional composition (g kg^{-1})		
Metabolisable energy (Mcal kg^{-1})	3,530	3,510
Crude protein	200.00	200.00

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Ether extract	5.85	6.13
Calcium	7.00	7.50
Available phosphorus	5.00	4.60
Digestible lysine	13.60	13.20
Neutral detergent fiber	95.20	104.80
Digestible methionine + cystine	8.80	7.70
Digestible tryptophan	2.50	2.30
Digestible threonine	9.10	8.70

[1] Levels per kg of product: Vitamin A, 14400 IU; Vitamin D3, 2700 IU; Vitamin E, 32.40 mg; Vitamin K, 3.60 mg; Vitamin B1, 2.88 mg; Vitamin B2, 9.18 mg; Vitamin B6, 2.79 mg; Vitamin B12, 34.20 mg; Pantothenic acid, 23.40 mg; Niacin, 46.80 mg; Folic acid, 0.81 mg and biotin, 162 mcg. Selenium, 0.48 mg; Iodine, 0.56 mg; Iron, 64.0 mg; Copper, 12.80 mg; Zinc, 128.0 mg and Manganese, 48.0 mg.

The pigs were fed experimental diets for a nine-day adaptation period, followed by a five-day period of total collection of faeces and urine. Ferric oxide (Fe_2O_3) was added to the diets at 0.30% to determine the collection timeframe by the appearance of marked faeces (ADEOLA, 2001). Faecal and urine collections were performed once a day. The faeces were weighed and stored in identified plastic bags. Urine was collected in plastic containers with 25 mL of 6N H_2SO_4 to minimise NH_3 volatilisation. The volume was weighed daily, and a 10.0% aliquot was removed and refrigerated at -15°C.

The faeces were evaluated daily (4-28 days post-weaning) for consistency through subjective faecal score evaluation according to the following scale: 1 = hard and dry stools; 2 = normal stool consistency (firm); 3 = soft stools but non-diarrhoeic; 4 = watery stools with diarrhoea characteristics. A total of 500 faecal score observations were made. The values found in the first three days of the experimental period were excluded due to the absence of faeces in most experimental units.

The diet samples were collected, homogenised, sub-sampled (about 500 g), and stored at -20° C for posterior analyses. Similarly, at the end of the experimental period, the faeces and urine samples were thawed, homogenised, sub-sampled (500 g and 100 mL per replicate, respectively), and analysed. The analyses were performed according to AOAC (1995) procedures.

Diets and faeces samples were ground to pass through a 1 mm sieve (Model EDB-5, De Leo Laboratory Equipments, Porto Alegre, Brazil). The dry matter (DM) in the faeces was determined after the samples were dried in a forced air oven at 60°C for 72 hours. Subsequently, drying was done at 105°C for 12 hours (AOAC, 1995). The nitrogen (N) content in the diets (in natural matter) and faeces (pre-dried in a greenhouse at 105°C) was determined by the Micro-Kjeldhal method (AOAC, 1995, adapted by PRATES, 2007). Crude protein (CP) was calculated as N × 6.25. The gross energy (GE) of the diets, faeces, and freeze-dried urine samples was determined using an adiabatic oxygen bomb calorimeter (C2000 - IKA Werke GmbH&Co. KG, Staufen, Germany) with benzoic acid (6318 kcal kg^{-1}) used for calibration (6317± 2 kcal kg^{-1} in the assay). Crude fat was determined after ether extraction (AOAC, 1995) in an extractor apparatus. Urine samples were dried in a forced-ventilation oven at 60 °C for 72h (AOAC, 1995) and analyzed for GE content as described above. Urinary N was determined in the liquid sample (AOAC, 1995) as described above. All analyses were performed in duplicate, and the standard deviation between replicates was less than 5% for all methods and less than 1% for energy.

The experimental design was completely randomised with two treatments, 10 replicates, and one animal per replicate. Statistical analysis was performed by ANOVA using the Generalised Linear Model (SAS®, Inst. Inc., Cary, NC, USA), and the means were compared by the F test. For the faecal score analysis, the non-parametric test of Kruskall-Wallis (5%) was used. The animal was considered the experimental unit and the results were considered significant if $P \leq 0.05$ and trending if $P < 0.10$.

Results

No pigs were removed from the trial during the study. In the pre-starter and total phases, no additive effect was found on pig performance; however, in the starter phase, the piglets fed with supplemented diets had 8.7% higher ADG than the control treatment (P<0.05). There was no additive effect on ADFI and G:F (Table 2).

The additive supplementation in the diet did not influence the digestibility responses in any of the evaluated phases (Table 3). In the starter phase, piglets fed with a supplemented diet had higher N intake and absorption ($P<0.05$), which were 18.37% and 19.53% higher than in the control treatment (Table 4), respectively. Similarly, in the total phase, piglets fed with a supplemented diet had higher N intake and absorption ($P<0.05$), which were 15.07% and 16.05% higher than in the control treatment (Table 4), respectively. There was a trend ($P<0.10$) of higher N retention in the initial (25.38%) and total (19.74%) phases for piglets fed supplemented diets compared to control diets. For other responses of N balance, no additive effect was found. The use of additives in the diet did not modify the faecal score; 89% of faeces had normal to soft appearance, whereas those with a diarrhoeal appearance appeared in only 7.8% (Table 5).

Table 2. Growth performance of piglets fed the experimental diets[1].

Item	Control	Additive[2]	SEM[3]	*P*-value[4]
Body weight (kg)				
Starter	5.81	5.99	0.13	0.33
Final	17.50	18.69	0.54	0.14
Pre-starter (3 - 14 d)				
ADFI (g d^{-1})	349	374	24	0.45
ADG (g d^{-1})	280	297	19	0.54
G:F (g g^{-1})	1.24	1.29	0.04	0.57
Starter (15 - 28 d)				
ADFI (g d^{-1})	820	885	42	0.29
ADG (g d^{-1})	663b	726a	21	0.05
G:F (g g^{-1})	1.23	1.22	0.05	0.86
Total (3 - 28 d)				
ADFI (g d^{-1})	580	625	28	0.27
ADG (g d^{-1})	468	508	18	0.13
G:F (g g^{-1})	1.24	1.23	0.04	0.95

[1]ADFI: Average daily feed intake; ADG: Average daily gain; G:F: Gain:Feed.
[2]Additive compound by 50% of mannan-oligosaccharide and 50% of organic acids (acetic, propionic and formic).
[3]SEM = Standard error of mean.
[4]Means were compared by the F test *($P \leq 0.05$)*.

Table 3. Apparent total tract digestibility of nutrients and energy of piglets fed the experimental diets[1].

Item[2]	Control	Additive[3]	SEM[4]	*P*-value [5]
Pre-starter (3 - 14 d)				
DCDM (%)	87.50	87.70	0.76	0.85
DCCP (%)	85.44	85.90	1.03	0.75
DCGE (%)	85.64	85.65	0.86	0.99
DCME (%)	83.50	83.39	0.81	0.92
GE (Mcal kg^{-1})	4.89	4.86	-	-
DE (Mcal kg^{-1})	4.19	4.17	0.04	0.69
ME (Mcal kg^{-1})	4.08	4.05	0.03	0.58
ME: DE	0.975	0.974	0.001	0.67
Starter (15 - 28 d)				
DCDM (%)	86.51	88.87	1.11	0.15
DCCP (%)	82.56	84.90	1.35	0.23
DCGE (%)	84.43	86.63	1.24	0.23
DCME (%)	81.92	84.43	1.39	0.22
GE (Mcal kg^{-1})	4.80	4.80	-	-
DE (Mcal kg^{-1})	4.05	4.15	0.06	0.23
ME (Mcal kg^{-1})	3.93	4.05	0.06	0.23
ME: DE	0.968	0.974	0.01	0.17
Total (3 - 28 d)				
DCDM (%)	87.00	88.29	0.73	0.23
DCCP (%)	83.00	85.40	0.95	0.31
DCGE (%)	85.03	86.14	0.82	0.36
MCGE (%)	82.71	83.91	0.85	0.33
GE (Mcal kg^{-1})	4.84	4.83	-	-
DE (Mcal kg^{-1})	4.12	4.16	0.04	0.47
ME (Mcal kg^{-1})	4.01	4.05	0.04	0.45
ME: DE	0.972	0.974	0.01	0.71

[1]DCDM= digestibility coefficient of dry matter; DCCP = digestibility coefficient of crude protein; DCGE = digestibility coefficient of gross energy; MCGE = metabolizable coefficient of gross energy; GE = gross energy; DE = digestible energy; ME = metabolizable energy.
[2]The digestibility coefficients and DE and ME were calculated based on dry matter.
[3]Additives were compound by 50% of mannan-oligosaccharide and 50% of organic acids (acetic, propionic and formic).
[4]SEM = Standard error of mean.
[5]Means were compared by the F test *($P \leq 0.05$)*.

Table 4. Nitrogen balance of piglets fed the experimental diets[1].

Item[2]	Control	Additive[3]	SEM[4]	*P*-value[5]
Pre-starter (3 - 14 d)				
N intake (g d $^{-1}$)	16.04	17.47	1.23	0.42
N feces (g d $^{-1}$)	2.40	2.50	0.32	0.82
N urine (g d $^{-1}$)	2.65	2.89	0.23	0.48
N absorbed (g d $^{-1}$)	13.64	14.97	0.96	0.34
N retained (g d $^{-1}$)	10.99	12.08	0,99	0.45
N retained $^{-1}$ absorbed	0.79	0.80	0.02	0.87
N digestibility (%)	67.85	68.74	1.54	0.69
BVCP (%)	80.57	80.69	2.12	0.84
Starter (15 - 28 d)				
N intake (g d $^{-1}$)	29.60 b	36.26 a	2.04	0.03
N feces (g d $^{-1}$)	4.83	5.49	0.32	0.16
N urine (g d $^{-1}$)	7.53	7.67	1.21	0.94
N absorbed (g d $^{-1}$)	24.76 b	30.77 a	1.94	0.04
N retained (g d $^{-1}$)	17.23	23.09	2.14	0.07
N retained $^{-1}$ absorbed	0.66	0.75	0.06	0.30
N digestibility (%)	60.91	63.58	3.54	0.59
BVCP (%)	69.59	75.04	2.50	0.58
Total (3 - 28 d)				
N intake (g d $^{-1}$)	22.82 b	26.87 a	1.35	0.05
N feces (g d $^{-1}$)	3.62	4.00	0.28	0.35
N urine (g d $^{-1}$)	5.09	5.28	0.66	0.84
N absorbed (g d $^{-1}$)	19.20 b	22.87 a	1.18	0.04
N retained (g d $^{-1}$)	14.11	17.58	1.24	0.06
N retained $^{-1}$ absorbed	0.73	0.78	0.03	0.32
N digestibility (%)	61.34	66.16	2.88	0.25
BVCP (%)	73.49	76.87	2.30	0.95

[1]BVCP=biological value of crude protein.
[2]Nitrogen balance was calculated based on dry matter.
[3]Compound by 50% and 50% mannanoligosaccharide organic acids (acetic, propionic and formic).
[4]SEM = standard error of mean.
[5]Means were compared by the F test *(P*≤0.05).

Table 5. Faecal score observation in piglets fed the experimental diets from 4 to 28 days of age[1].

Score	Control (n)	Additive (n)[2]	Total (n)	Control (%)	Additive (%)[2]	Total (%)
1- Hard	10	6	16	4.0	2.4	3.2
2- Firm	105	116	221	42.0	46.4	44.2
3- Soft	113	111	224	45.2	44.4	44.8
4- Watery	22	17	39	8.0	6.8	7.8
Total	250	250	500	100	100	100

[1]There was no significant difference between treatments in accordance with the Kruskal-Wallis test *($P \leq 0.05$)*.
[2]Compound by 50% and 50% mannanoligosaccharide organic acids (acetic, propionic and formic).

Discussion

In the present study, we hypothesised that weaned piglets' diets supplemented with an additive composed of 50% mannan-oligosaccharide and 50% organic acids could improve the growth performance by improving the absorption/digestion capacity of nutrients and reducing the diarrhoea incidence. There was no additive effect on ADFI in any period evaluated, and it can be inferred that the additive did not influence diet palatability. Some researchers have reported that supplementation with organic acids in isolated or mixed form could negatively influence consumption by low palatability (WALSH et al., 2007), mainly mixtures with propionic and formic acids, which are considered as having an odor and unpleasant taste (SUIRYANRAYNA; RAMANA, 2015). Few studies have been able to show the effects of organic acid or manan-oligosaccharide on feed intake. Walsh et al. (2007) observed an increase in ADFI in piglets receiving diets with a mixture (0.4%) of lactic, propionic, citric, and benzoic acids. Zhao et al. (2012) reported higher feed intake in pigs fed mannan-oligosaccharide diets (0.5%). In contrast, Boas et al. (2016), working with a mixture (0.5%) of lactic, formic, and citric acids; Walsh et al. (2012), with (2.58 mL L^{1}) propionic, acetic, and benzoic acid; and Santos et al. (2010) and Marinho et al. (2007), with mannan-oligosaccharide (inclusion levels from 0.2 to 0.75%), did not observe an effect of additives on feed intake. In summary, the effects of organic acids and prebiotics such as mannan-oligosaccharide on growth performance are credited to the improvement in nutrient utilisation of the diet and not to an increase in feed intake.

In addition, the supplementation effect may significantly alter depending on the addition of one additive or the combination of several, as in the present work. Poeikhampha and Bunchasak (2011), Corassa et al. (2012), and Giannenas et al. (2016), working with different acid and mannan-oligosaccharides mixtures, were able to observe the synergistic effect of these additives, mainly on ADG and G:F. The results of the present study did not show synergy of these additives on G:F. However, there was improvement on ADG in the starter phase. This result was not repeated when analysing the whole evaluation period, showing that this gain was diluted in the total period.

There was no effect of the additives on nutrient and energy digestibility. However, animals that received additive in the diet had higher intake and absorption of N in the starter phase and in the total period. In addition, there was a trend of better N retention with the supplementation. Higher N retention was also observed by Mroz et al. (2000), working with growing pigs supplemented with a complex of formic, fumaric, and butyric acids. The beneficial effect of organic acids and mannan-oligosaccharides may be attributed to changes in the intestinal microflora, with modifications associated with the immune response (DAVIS et al., 2004; NOCHTA et al., 2009) and improvement in the small intestine morphology (SAVAGE et al., 1996). In the current experiment, the additive effect was more pronounced in the starter phase, which is consistent with the higher digestibility values observed at this stage, due to a maturation improvement in the gastrointestinal tract of the piglet. In addition, the trend of higher N retention is in accordance with the better growth rate observed at this stage. These results agree with those observed by Poeikhampha and Bunchasak (2011), which showed that dietary supplementation with mannan-oligosaccharide and organic acid salts (gluconate and diformate) reduced ammonia production in the large intestine, indicating that less N was available for fermentation. Devi et al. (2016) also observed improvement in DM, GE, and N digestibility with diets supplemented only with benzoic acid and not with an additive mix, similarly to the present trial. Blank et al. (1999), by including fumaric acid in weaned piglets' diets, observed an improvement in GE and CP digestibility and in most amino acids. Diao et al. (2016), Halas et al. (2010), and Kluge et al. (2006) also observed positive results in the nutrient digestibility of diets with organic acid supplementation.

In the present experiment, although the N digestibility increased with the use of additives, no increase in CP was observed. These results are not well elucidated but agree with those found by Omogbenigun et al. (2003) and disagree with those found by Suiryanrayna and Ramana (2015). According to Diao et al. (2016), the improvement in nutrient and energy digestibility is due to the acids' function in reducing the buffering diets' capacity, activating digestive enzymes, reducing nutrient competition with microorganisms adhered to intestinal mucosa, improving mucosal morphology with villi enlargement and crypt reduction, and improving digestibility, absorption, and nutrient retention. The jejunum is the key segment for digestion, nutrient absorption, and transport, as the function of digestion and transport is dependent on intestinal villi. Therefore, an adequate mucosal structure is essential for the improvement of these functions and, consequently, an increase in growth rate. Halas et al. (2010) supplemented the diet with 5000 mg kg^{-1} of benzoic acid and observed higher villus height and crypt depth, as observed in the Diao et al. (2016) study. Our results do not allow us to conclude about any effect of the additive on the intestinal morphology; however, the improvement in N digestive capacity could be indicative of a positive effect of the additive on the mucosal structure. However, for other nutrients, no additive effect was observed.

The inconsistent results found in the literature for growth performance and digestibility may be related to the presence or lack of presence of a nutritional challenge, intestinal or sanitary, among studies. Previous research has shown that growth promoters' effect is more pronounced when animals are under some type of challenge (GIANNENAS et al., 2016). Faecal score results corroborate the hypothesis that the use of higher soybean meal levels and barley addition in the diet was not effective to generate intestinal challenge. For example, normal-to-soft stools appeared in 89% of the observations, whereas diarrhoea appeared in only 7.8%. In addition, the score variation was similar among treatments. No animal was removed from the study because of disease, which could reflect a good health condition in the environment and high health status, which does not allow us to conclude on any additive effect in relation to diarrhoea incidence. These results agree with previous studies in which additive supplementation, such as organic acids (BOAS et al., 2016; ZHANG et al., 2016), manan-oligosaccharides (CHE et al., 2012), or a combination of these (CORASSA et al., 2012), did not influence faecal scores in weaned piglets.

In contrast, when evaluating the supplementation of diets with a mixture of 2.0 mg kg^{-1} of benzoic acid + 100 mg kg^{-1} of thymol, Diao et al. (2015) observed diarrhoea reduction. Similarly, Silva et al. (2012) and Zhao et al. (2012) supplemented the piglet diet with 0.1% and 0.2% mannan-oligosaccharide, respectively, and also observed an effect of this additive on diarrhoea reduction. The authors attributed these results to the ability of the organic acids and mannan-oligosaccharide to inhibit *E. coli* growth in the gastrointestinal tract. In fact, previous research using these additives in pigs' diets has shown that both have an effect on the inhibition of several pathogenic microorganisms that are usually responsible for the diarrhoea observed in the post-weaning phase (CALVEYRA et al., 2012; DEVI et al., 2016; SBARDELLA et al., 2015; TREVISI et al., 2012). However, reduction in the number of pathogenic organisms has not always been observed in diarrhoea reduction, and the results were dependent on the challenge type imposed on the piglets.

According to our results, intestinal challenge from the diet was not sufficiently able to exacerbate the additive effects. In addition, the growth performance and digestibility results, regardless of supplementation or not with additive, were good; little is left to improve due to the additives. However, our study may contribute to the discussion about the use of alternative antimicrobial additives in pig production. Antibiotics have been banned in the European Union since 2006, and their use has been tightening in all pig production systems around the world. Thus, over the past decades, studies on alternative additives have been given more importance. There is still no clear response to the action of organic acids, mannan-oligosaccharides, or their combination on performance in weaned piglets. The discrepancies between our results and those found in the literature are due to factors such as differences in the weaning age, animal health status, presence of health challenge, diet type, dosage of additives, and genetic potential of the animals.

Conclusions

We conclude that, in the experimental conditions under which it was tested, the inclusion of an additive with 50% acetic, propionic, and formic acid and 50% mannan-oligosaccharide in diets for weaned piglets does not improve digestibility and does not influence diarrhoea incidence. However, the weight gain and N balance results were positively influenced by the addition of additive, especially in the starter phase.

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