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Alternatives to antibiotic growth promoters for weanling pigs

Alternativas aos antibióticos melhoradores de desempenho para leitões desmamados

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ABSTRACT

An experiment was conducted to evaluate the addition of sodium butyrate, plant extracts and nucleotides on weanling pig performance, digestive content pH, organ morphometry, and intestinal epithelial histology. A total of 90 piglets at 21 days of age and an average initial weight of 6.35±0.34kg were used. The piglets were distributed in a randomized complete block design with five treatments, six replicates, and three animals per experimental unit. The treatments consisted of the following: Control: basal diet without antibiotic; Antibiotic: basal diet with 40mg kg^{-1} colistin sulfate and Additive: 1000, 1500 and 2000mg kg^{-1} of a combination of sodium butyrate + plant extracts + nucleotides. The experiment lasted 35 days, at which time one animal was slaughtered to assess pH of the digestive contents, morphometry of the organs and histology of the intestinal epithelium. No differences were found (P>0.05) in the performance, pH of the digestive contents, morphometry of the organs or histology of the intestinal epithelium by the analysis of orthogonal polynomials or contrasts. The combination of sodium butyrate, plant extracts and nucleotides not improved the productive characteristics of weanling pigs.

Key words: *herbal extracts, nucleotides, nutrition, sodium butyrate.*

RESUMO

Foi conduzido um experimento para avaliar a adição de butirato de sódio, extratos vegetais e nucleotídeos sobre desempenho, pH do conteúdo digestório, morfometria dos órgãos e histologia do epitélio intestinal de leitões desmamados. Foram utilizados 90 leitões, com idade média de 21 dias e peso médio inicial de 6,35±0,34kg. Os leitões foram distribuídos em um delineamento em blocos casualizados completos, com cinco tratamentos, seis repetições por tratamento e três animais por unidade experimental. As dietas experimentais consistiam de: Controle - dieta basal sem antibiótico; Antibiótico – dieta basal com 40mg kg^{-1} de sulfato de colistina; Aditivo: 1000, 1500 e 2000mg kg^{-1} de uma combinação de butirato de sódio + extrato vegetal + nucleotídeos. No 35º dia de experimentação, foi eutanasiado um animal por gaiola (unidade experimental) para avaliar o pH do conteúdo digestório, morfometria de órgãos e histologia do epitélio intestinal. Não foram observadas diferenças (P>0,05) para desempenho, pH do conteúdo digestório, morfometria de órgãos e histologia do epitélio intestinal por meio das análises de polinômios ortogonais ou mesmo pelos contrastes. A combinação de butirato de sódio, extratos vegetais e nucleotídeos não melhorou as características produtivas de leitões desmamados.

Palavras-chave: *extratos vegetais, nucleotídeos, nutrição, butirato de sódio.*

INTRODUCTION

In intensive pig farming, the post-weaning phase is critical because the animals are predisposed to enteric problems by a variety of factors. ***Escherichia coli*** strains are responsible for diarrhea, morbidity and mortality and therefore generate the greatest economic losses in production. Traditionally, antibiotics are used in pig feed as growth promoters and for the treatment of gastrointestinal infections. However, possible bacterial cross-resistance has limited the use of the antibiotics as growth promoters and alternatives such as herbal extracts, organic acids and nucleotides (ANDRADE et al., 2011; COSTA et

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al., 2011) that have shown beneficial effects for pig production (YAN et al., 2011) are been studied.

Many plants have multifunctional beneficial properties due to their specific bio-active components. Most biologically active plant constituents are secondary metabolites, such as terpenoids (mono- and sesquiterpenes, steroids, etc.), phenolics (tannins), glycosides, and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers, lactones, etc.) (HUYGHEBAERT et al., 2011). These herbal extracts improve feed palatability, stimulate the secretion of endogenous enzymes, enhance nutrient absorption, modulate the intestinal microbiota and improve performance (WINDISCH et al., 2008). According to LAMBERT et al. (2001), the antimicrobial activity of extracts lies in their ability to disrupt bacterial cells by altering membrane permeability.

Organic acids are widely distributed in nature as normal components of plant or animal tissues. Organic acids are also formed by microbial fermentation of carbohydrates in the intestines of pigs and other intermediary metabolic processes (COSTA et al., 2011). The sodium butyrate ($C_4H_7O_2Na$) derived from butyric acid has been studied because it acts reducing gastric pH and dietary buffering capacity, which stimulates the production of pancreatic secretions and reduces colonization by pathogenic microorganisms, both in feed and in the gastrointestinal tracts of animals (KNARREBORG et al., 2002).

Another additive used in piglet nutrition is nucleotides, which are intracellular compounds involved in numerous metabolic processes that are essential for cell growth (MATEO et al., 2004). Nucleotides are precursors of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which are key sources of energy, especially in the form of ATP. The benefits of nucleotide supplementation may be related to improvements in histological features and organ morphometry (ANDRADE et al., 2011). Furthermore, dietary nucleotides benefit gastrointestinal morphology and function, the immune system and the intestinal microbiota of monogastric species (SAUER et al., 2011).

However, results describing the true effects of these additives on pig development remain inconsistent. Moreover, determining the effect of combinations of the additives tested in this study and the appropriate concentrations for piglets may contribute to the search for alternatives to antibiotic use as a growth promoter in pig production. The aim of the present study was to evaluate a combination of sodium butyrate, plant extracts and nucleotides as a substitute for antibiotic growth promoters on the weaned pig performance, digestive content pH, organ morphometry and intestinal epithelial histology.

MATERIALS AND METHODS

The experiment was conducted at the Swine Research Station (CES) of the Federal University of Lavras in Lavras, MG. A total of 90 piglets (18 pigs treatment^{-1}) from 21 to 56 days of age with an average initial weight of 6.35±0.34kg were assigned to five treatments according to a randomized complete block design: Control, diet with no antibiotic; Antibiotic, diet with 40mg kg^{-1} of colistin sulfate; Additive, diets with 1000, 1500 or 2000mg kg^{-1} of a mixture of sodium butyrate + plant extracts + nucleotides. The additive was composed of 5% plant extracts, including 2% ***Glycyrrhiza glabra*** (sweet root), 1.5% ***Rosmarinus officinalis*** (rosemary) and 1.5% ***Peumus boldus*** (boldo); 32% sodium butyrate; 3% nucleotides prepared from ***Saccharomyces cerevisiae*** yeast hydrolysis and carrier (60%). Three animals were assigned to each pen (two males and one female or two females and one male) and six replicates per treatment were conducted.

The experimental diets (Table 1) were isocaloric and isonitrogenous and were formulated to meet the requirements of piglets in the pre-initial (day 21 to day 35) and initial (day 35 to day 56) phases of the experiment, as recommended by ROSTAGNO et al. (2005). The animals received water and feed *ad libitum* during the experimental period. To assess the performance variables, the animals were weighed at the beginning of the experiment, at day 28, at day 35 and at day 56, and feed intake and orts were quantified.

After day 35 of the experiment, one animal per pen was slaughtered. The animal was chosen according to body weight; the animal chosen possessed the closest to the average weight of the three animals in each pen, independent of gender. After slaughter, the abdominal cavity was opened by longitudinal incision and the digestive organs (stomach, jejunum and cecum) were removed to measure the pH of their respective contents. The empty stomach, liver and cecum were then weighed for morphometric analysis. For the intestinal histology analyses, samples of approximately 3cm in length from the duodenum (removed at 15cm from the pyloric sphincter) and the jejunum (removed at 1.5m from the ileocecal junction) were washed with saline solution (NaCl 0.9%) and fixed in buffered formalin (10.0%) (OETTING et al., 2006; ANDRADE et al., 2011). Subsequently,

Table 1 - Percent composition and calculated values of basal diets.

Ingredients (%)	Pre-initial diet (day 21 to day 35)	Initial diet (day 35 to day 56)
Corn	48.88	52.35
Soybean meal (46%)	27.10	21.94
Milk replacement[1]	15.27	5.60
Micronized soybean	1.57	6.86
Soybean oil	1.11	2.56
Sugar	1.11	1.01
L-Lysine HCl (78%)	0.77	0.31
DL-Methionine (99%)	0.18	0.03
L-Threonine (98.5%)	0.37	0.07
L-Tryptophan (98%)	0.08	0.02
Dicalcium phosphate	1.99	1.43
Limestone	0.85	0.67
Common salt	0.41	0.35
Vitamin supplement[2]	0.05	0.05
Mineral supplement[3]	0.05	0.05
Kaolin and/or growth promoter[4]	0.20	6.69
Calculated values		
Metabolizable energy (kcal/kg)	3363	3270
Crude protein (%)	19.76	17.92
Calcium (%)	0.98	0.72
Total phosphorus (%)	0.75	0.62
Digestible lysine (%)	1.52	1.08
Digestible threonine (%)	0.96	0.62
Digestible tryptophan (%)	0.26	0.18
Digestible methionine (%)	0.43	0.28
Lactose (%)	10.92	4.00

[1]Commercial product, Prius L68 - crude protein (10.5%), lactose (71.5%), spray-dried fat (4.5%), and metabolizable energy (3780kcal kg^{-1}).
[2]Amount per kg of feed: vit. A - 11,500UI; vit. D3 - 5,850UI; vit. E - 45UI; vit. K3 - 3mg; thiamine - 1.8mg; riboflavin - 5.1mg; pyridoxine - 3.5mg; vit. B12 – 24µg; folic acid - 0.82mg; pantothenic acid - 18mg; niacin - 37.5mg; biotin - 0.14mg; selenium - 0.35mg; ethoxyquin - 0.042mg.
[3]Amount per kg of feed: manganese - 60mg; zinc - 150mg; iron - 100mg; copper - 10mg; iodine - 1.2mg.
[4]Growth promoter; commercial product.

the tissues were embedded in paraffin and sectioned using a microtome before being mounted on slides in the Histopathology Laboratory of the Department of Veterinary Medicine, State University of Santa Cruz (Universidade Estadual de Santa Cruz – UESC). The villus height (VH) and crypt depth (CD) were measured from images taken with a light microscope and the ratio VH:CD was calculated.

The data were subjected to analysis of variance using the PROC GLM of SAS (Statistical Analysis System, 2001). The degrees of freedom of the factor level were broken down into their individual components (linear, quadratic and cubic) using orthogonal polynomials. The following contrasts were also tested using a significance level of 5%: C1 (Control treatment x Antibiotic treatment); C2 (Control treatment x the mean of 1000, 1500 and 2000mg kg^{-1} treatments) and C3 (Antibiotic treatment x the mean of the 1000, 1500 and 2000mg kg^{-1} treatments).

RESULTS AND DISCUSSION

The body weight of the piglets at 21, 35 and 56 days of age; average daily feed intake (ADFI); average daily gain (ADG) and feed conversion (FC) were not affected (P>0.05) by the treatments (Table 2). In the literature, there is no information where herbal extracts, sodium butyrate and nucleotides were added as a compound in the animal feed. This is the first article supplementing piglets feed with a combination of these additives.

The data from this study are consistent with those found by COSTA et al. (2011), who also found no improvement in the performance of piglets (21 to 55 days of age and BW of 6.10 to 20.56kg) that were supplemented with essential oil of thyme (***Thymus vulgaris***), cinnamon (***Cinnamonum zeylanicum***), eucalyptus (***Eucalyptus urophylla***), ***Melaleuca alternifolia***, ***Echinacea angustifolia***, extract of ginger (***Zingiber officinale***), pepper (***Capsicum frutescens***) and sodium butyrate which have the same mechanisms of action of the extracts and acids studied in this research. The lack of improvement in the performance of the piglets from the antibiotic or additive treatments in this experiment may be due to rigorous sanitary control of the facilities compared with commercial conditions, which may influence the effects of these additives on animal performance (NRC, 1998; OETTING et al., 2006). Additionally, broilers which have been challenged with ***Salmonella*** Enteritidis supplemented with a specific blend of essential oil components and sodium butyrate in the feed, had no beneficial effect on growth performance (CERISUELO et al., 2014).

In the literature, there is great disagreement regarding the effects of plant extracts, organic acids and nucleotides on pig performance (PIVA et al., 2002; WANG & KIM, 2010). Some studies show results suggesting that the inclusion of such supplements has favorable effects (OETTING et al., 2006; YAN et al., 2012) and others show no effects of supplementation (BHANDARI et al., 2008; COSTA et al., 2011).

Table 2 - Means of initial live weight (P1), live weight at day 35 (P35), live weight at day 56 (P56), average daily feed intake (ADFI), average daily gain (ADG), and feed conversion (FC) from days 21 to 28, day 21 to day 35, and day 21 to day 56 of age.

Variable	Treatments[1]					Contrasts[2]			
	Control	Antibiotic	1000 mg kg^{-1}	1500 mg kg^{-1}	2000 mg kg^{-1}	C1	C2	C3	SE[3]
Initial BW (kg)	6.36	6.35	6.35	6.35	6.36	-	-	-	-
28 day BW (kg)	7.32	7.14	7.17	7.14	7.08	0.26	0.15	0.93	0.08
35 day BW (kg)	9.10	8.94	8.96	9.03	8.79	0.48	0.35	0.94	0.09
56 day BW (kg)	17.19	16.76	17.31	17.49	16.69	0.58	0.53	0.97	0.23
ADG (g)									
Days 21 to 28	138	113	118	114	123	0.28	0.31	0.77	0.01
Days 21 to 35	196	185	187	192	173	0.51	0.38	0.94	0.01
Days 21 to 56	309	294	315	318	285	0.50	0.52	0.82	0.01
ADFI (g)									
Days 21 to 28	180	154	163	187	134	0.31	0.37	0.71	0.01
Days 21 to 35	318	298	310	318	302	0.34	0.60	0.51	0.01
Days 21 to 56	591	544	589	618	542	0.30	0.30	0.86	0.01
FC (g)									
Days 21 to 28	1.37	1.37	1.59	1.71	1.18	0.99	0.56	0.57	0.08
Days 21 to 35	1.63	1.63	1.68	1.66	1.77	0.96	0.35	0.31	0.03
Days 21 to 56	1.92	1.82	1.87	1.94	1.93	0.30	0.22	0.96	0.03

[1]Control: basal diet; Antibiotic: basal diet with 40 mg kg^{-1} colistin sulfate; Additive: basal diet with 1000, 1500, or 2000 mg kg^{-1} of additive.
[2]C1: Control X Antibiotic; C2: Control X mean of 1000, 1500 and 2000mg kg^{-1}; C3: Antibiotic X mean of 1000, 1500 and 2000mg kg^{-1}.
[3]Pooled standard error.

Nevertheless, in addition to sanitary control, the different parts of the plants used to obtain the essential oil, the concentrations of the major components (AO et al., 2011), the extraction protocols, the diet composition, the level of dietary added to herbal extracts, sodium butyrate and nucleotides, the different procedures for obtaining and drying of yeast (which containing nucleotides) and possible antagonistic or synergistic effects between additives can influence pig performance.

The pH of the digestive contents of the piglets was not affected (P>0.05) by the treatments (Table 3). The current experimental results agree with COSTA et al. (2011), who showed that supplementing piglet feed with plant extracts or organic acids did not affect the pH of the stomach, jejunal and cecal contents. In contrast, MANZANILLA et al. (2004) found an increase in the pH of the stomach contents of weanling pigs that were fed formic acid or plant extracts compared with the control treatment. A possible explanation for the lack of change in the pH of the digestive contents could be the buffering capacity of the feed (COSTA et al., 2011). The basal diet for this experiment consisted of kaolin clay (mineral), protein sources and other sources of minerals, which may have increased the buffering capacity of the feed and prevented pH variation in the contents of such organs.

The organ morphometry of the piglets was not affected (P>0.05) by the treatments (Table 3). The current experimental results agree with those found by OETTING et al. (2006), who found no difference in the relative weight of the liver, empty stomach and empty cecum while studying the effects of different levels of plant extracts in weanling pig feed. ANDRADE et al. (2011) also found no difference in the morphometry of the stomach, liver and pancreas of weaned piglets while studying the effects of treatment with nucleotides substituted for antibiotics.

In the post-weaning period, there is usually intestinal villous atrophy, given the higher rate of epithelial shedding caused by solid feed intake, the adhesion of pathogenic bacteria to enterocytes and toxin production (OETTING et al., 2006). The positive effects of additives on piglet intestinal histology may be related to the decreased turnover rate of mature cells in the intestinal epithelium, which promotes decreased turnover of enterocytes, thereby maintaining enterocyte integrity. However, in this experiment, the histology of piglet intestinal epithelia was not affected (P>0.05) by the treatments (Table 3). The results of the present study corroborate those found by ABREU et al. (2010), who found no differences in the duodenum and

Table 3 - pH values of the stomach, jejunal, and cecal contents; mean relative weights (percentage of live weight) of the stomach, liver, and cecum; mean villus height (VH, µm), crypt depth (CD, µm), and the ratio of villus height:crypt depth (VH:CD) in the duodenum and jejunum of the piglets after treatment.

Variables	Treatments[1]					Contrasts[2]			SE[3]
	Control	Antibiotic	1000 mg kg^{-1}	1500 mg kg^{-1}	2000 mg kg^{-1}	C1	C2	C3	
pH									
Stomach	1.34	1.58	1.77	1.54	1.34	0.52	0.92	0.50	0.12
Jejunum	6.69	6.14	6.16	6.45	6.68	0.14	0.33	0.40	0.12
Cecum	6.10	5.98	6.28	6.09	6.08	0.58	0.35	0.77	0.07
Relative organ weight									
Empty stomach (%)	0.71	0.72	0.74	0.79	0.78	0.17	0.93	0.08	2.47
Liver (%)	2.23	2.53	2.26	2.38	2.39	0.35	0.24	0.99	8.88
Empty cecum (%)	0.21	0.22	0.21	0.19	0.22	0.21	0.76	0.07	1.01
Histology									
Duodenum									
VH (µm)	224.72	221.24	227.27	192.49	200.27	0.48	0.16	0.56	11.80
CD (µm)	97.48	94.50	81.20	94.72	73.09	0.86	0.40	0.29	5.14
VH:CD	2.24	2.78	2.92	2.06	2.83	0.17	0.51	0.31	0.11
Jejunum									
VH (µm)	201.76	197.02	223.75	178.74	209.35	0.75	0.57	0.89	5.17
CD (µm)	87.16	83.64	95.6	79.23	83.36	0.76	0.85	0.90	3.52
VH:CD	2.47	2.43	2.40	2.29	2.60	0.91	1.00	0.88	0.09

[1]Control: basal diet; Antibiotic: basal diet with 40mg kg^{-1} colistin sulfate; Additive: basal diet with 1000; 1500, or 2000mg kg^{-1} of additive.
[2]C1: Control X Antimicrobial; C2: Control X mean of 1000, 1500, and 2000mg kg^{-1}; C3: Antimicrobial X mean of 1000, 1500, and 2000mg kg^{-1}.
[3]Pooled standard error.

jejunum in VH, CD and VH:CD while assessing the effects of glutamine, nucleotides, and swine plasma in weaned piglet feed. While studying different concentrations of nucleotides in feed for weaned piglets, ANDRADE et al. (2011) found a linear decrease ($P<0.01$) in CD and a linear increase ($P<0.01$) in VH:CD in the piglets' duodenums when the levels of nucleotides was increased. According to SAUER et al. (2012), the effect of nucleotides on the morphology of the intestine can vary according to immunological challenges and the conditions of the animals' health.

The sanitary condition of the facilities and low challenge to the animals may be the factors that limit a pronounced effect of the tested additives, as there was no difference between the control and the treatments.

CONCLUSION

The combination of sodium butyrate (32%), plant extracts (5%) and nucleotides (3%) did not yield improvements in performance or changes in pH of the digestive contents, morphometry of the organs or histology of the intestinal epithelia of weaned pigs.

ETHICS COMMITTEE ON ANIMAL USE

The experiment was approved by the Ethics Committee on Animal Use (Comissão de Ética no Uso de Animais – CEUA) of the State University of Santa Cruz (Universidade Estadual de Santa Cruz – UESC), Ilhéus – Bahia, protocol number 029/2009.

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