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Additional effect of coated sodium butyrate 30% in the piglets feeding during the nursery phase

Efeito adicional do butirato de sódio 30% revestido na alimentação de leitões durante a fase de creche

Newton Tavares Escocard de Oliveira[1]; Paulo Levi de Oliveira Carvalho[1*]; Jansller Luiz Genova[2]; Osíris Antunes de Caxias Junior[3]; Eliseu Carlos Cristofori[3]; Fábio Henrique Rodrigues Silveira[3]; Ana Lúcia Almeida Santana[4]

Abstract

In this study, the objective was to assess the growth performance, rectal microbiological profile, serum and immunological standards, and incidence of diarrhoea in piglets in the nursery phase fed diets with and without the addition of coated sodium butyrate 30% (SB). A total of 80 crossbred piglets (40 entire males and 40 females), with an average initial body weight of 9.47 ± 1.03 kg, were distributed in a randomised block design, with four treatments repeated twice in the two blocks and five piglets of the same gender per experimental unit. The treatments comprised a combination of two feeds, formulated based on corn and soybean meal with and without 0.1% SB inclusion as an additional product, and both genders, fed from of the pre-starter I diet. SB did not affect ($p > 0.05$) the growth performance and rectal microbiological profile of the piglets, but efficiently ($p = 0.05$) controlled the incidence of diarrhoea. The average values of the characteristics measured in the blood of the piglets were within the normal physiological parameters of the species. The inclusion of SB in commercial feed did not alter the growth performance and rectal microbiological profile of nursery piglets under low sanitary challenge conditions. However, the organic acid efficiently reduced gastrointestinal disorders that cause diarrhoea, with diarrhoea incidence rates of 2.68 and 4.46% for piglets fed with and without the inclusion of the additional organic acid, respectively.
Key words: Blood parameters. Butyric acid. Diarrhoea incidence. Growth performance. Microbiological profile. Sanitary challenge.

Resumo

Neste estudo, o objetivo foi o de avaliar o desempenho zootécnico, o perfil microbiológico retal, padrões imunológicos e no soro, e a incidência de diarreia em leitões na fase de creche alimentados com dietas adicionadas ou não do butirato de sódio 30% revestido (BS). Um total de 80 leitões mestiços (40 machos inteiros e 40 fêmeas), com um peso corporal médio inicial de 9,47 ± 1,03 kg, distribuídos

[1] Profs. Drs., Curso de Zootecnia, Universidade Estadual do Oeste do Paraná, UNIOESTE, Marechal Cândido Rondon, PR, Brasil. E-mail: newtonescocard@hotmail.com; paulolevi@yahoo.com.br
[2] Discente de Doutorado, Programa de Pós-Graduação em Zootecnia, UNIOESTE, Marechal Cândido Rondon, PR, Brasil. E-mail: janskllerg@gmail.com
[3] Discentes de Mestrado, Programa de Pós-Graduação em Zootecnia, UNIOESTE, Marechal Cândido Rondon, PR, Brasil. E-mail: osiriscaxiasjr@gmail.com; eliseu_crf@hotmail.com; fabiohenrique.vet@hotmail.com
[4] Discente de Doutorado, Programa de Pós-Graduação em Zootecnia, Universidade Federal da Bahia, UFBA, Salvador, BA, Brasil. E-mail: zootecana@gmail.com
[*] Author for correspondence

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em um delineamento experimental de blocos casualizados, com quatro tratamentos repetidos duas vezes nos dois blocos e cinco suínos de mesmo sexo por unidade experimental. Os tratamentos foram constituídos pela combinação de duas rações formuladas à base de milho e farelo de soja, com e sem inclusão de 0,1% de BS como produto adicional, e dois sexos, alimentados a partir da dieta pré-inicial I. Não houve efeito (p > 0,05) do BS no desempenho zootécnico e no perfil microbiológico retal dos leitões, mas houve eficiência do BS (p = 0,05) no controle da incidência de diarreia. Os valores médios das características mensuradas no sangue dos leitões estão dentro dos parâmetros fisiológicos normais da espécie. A inclusão de BS em rações comerciais não altera o desempenho zootécnico e o perfil microbiológico retal de leitões na fase de creche sob condições de baixo desafio sanitário. Entretanto, o ácido orgânico é eficiente na redução de distúrbios gastrointestinais causadores de diarreia, com proporções de incidência de diarreia de 2,68% e 4,46% para leitões alimentados com e sem inclusão do ácido orgânico adicional, respectivamente.

Palavras-chave: Parâmetros sanguíneo. Ácido butirico. Incidência de diarreia. Desempenho zootécnico. Perfil microbiológico. Desafio sanitário.

Introduction

The nursery phase is considered to represent the greatest challenge for nutritionists because it is a critical stage for pig production due to various stress factors (TSE et al., 2006). These stressors lead to a fall in immunity, reduced feed intake and growth rate, and the onset of diseases. Furthermore, after weaning, there is a transition between passive and active immunity, and an adequate acquisition of immunity is dependent on the challenges present in the breeding environment.

Post-weaning inappetence results in changes to the intestinal integrity and initially affects the metabolism, growth performance and health of the animals. Consequently, ingested and undigested nutrients serve as nutritive substances for the colon bacteria and, therefore, increase the pathogenic intestinal microbiota, resulting in diarrhoea and even mortality. The occurrence of diarrhoea may involve various mechanisms, such as changes in the water and electrolyte absorption, increased permeability of the epithelial barrier and activation of inflammatory processes (VANUCCI; GUEDES, 2009). The occurrence of post-weaning diarrhoea, allied with the European Union's prohibition on the use of antibiotics and chemotherapeutics as performance enhancers, may intensify the occurrence of enteric problems in the nursery phase and increase economic loss in pig production.

Given this, the search for new nutritional and ingredient strategies that contribute to the efficient functioning of the gastrointestinal tract of the animals, thereby favouring good performance, has become essential for the intensive production of piglets. In particular, there is a need to seek alternatives to conventional additives. Among them, the addition of organic acids in the piglet diet is a recognised way to overcome problems in the post-weaning period (BIAGI et al., 2007).

The inclusion of sodium butyrate (SB) in diets has been beneficial for the performance of piglets in the nursery phase and also for the reduction of pathogenic microorganisms, such as *Escherichia coli* and *Clostridium* spp., without causing a decrease in the number of beneficial microorganisms, like lactic acid bacteria and *Bifidobacterium* (LU et al., 2008). Organic acids can decrease gastrointestinal pH, thus creating an environment, which is hostile to Salmonella while favouring the growth of beneficial bacteria such as lactobacilli (WALIA et al., 2016). Besides, SB is an energy substrate for the enterocytes (BIAGI et al., 2007) and can, therefore, enable better development of the intestinal villi and absorption of the nutrients contained in the diet.

SB has an important regulatory role in cell proliferation and differentiation (SENGUPTA et al., 2006), as well as modulation of the intestinal microflora (CASTILLO et al., 2006). These actions depend on the bacterial adaptation to changes in chyme acidity (MROZ et al., 2006). In addition, the bactericidal/bacteriostatic action of SB in the intestine leads to dynamic changes in the microorganism population, as well as direct or indirect effects on the regulation of gastrointestinal development, morphological structure, permeability, mucin production, enzymes, motility, digestive and absorptive capacity, mucosal immunity and biliary and pancreatic secretory activities (MROZ et al., 2006).

However, the literature shows variability between the results of published studies (LINCH et al., 2017). In this context, this study aimed to evaluate the effect of the inclusion of coated SB 30% in commercial feeds for piglets during the nursery phase on zootechnical indices, diarrhoea incidence, rectal microbiological profile and haematological parameters.

Material and Methods

The experiment was carried out at the Swine Sector of the Experimental Station Nucleus of the State University of Western Paraná, Unioeste, Campus of Marechal Cândido Rondon, Paraná.

Experimental design, animals, housing and diets

The performance trial included 80 crossbred piglets (40 entire males and 40 females), weaned at 21 days old, with an average initial body weight (IBW) of 9.47 ± 1.03 kg. The animals were distributed in an experimental randomised block design, in a 2 × 2 factorial arrangement, with four replicates and five animals per experimental unit. The treatments consisted of a combination of two experimental feeds, with and without the inclusion of coated SB 30% (the content of sodium butyrate is 30%), and both genders.

The animals were identified by numbered earrings and housed in suspended nursery pens (1.54 m²), with a polyethylene plastic floor and equipped with nipple-type drinking fountains and semi-automatic front feeders, in a masonry shed with concrete floors and ceramic roof tiles.

The piglets received four types of commercial feeds. The amounts offered per animal were: weaning (0.5 kg), provided for one week to adapt the animals to the environment and management; pre-starter I (3 kg); pre-starter II (6 kg) and starter (until the end of the experimental period, seven weeks in total). Diets and water were provided *ad libitum* throughout the trial. The feed was provided for the animals at least six times per day.

The experimental feed with 30% SB inclusion, provided from the pre-starter I, presented the same composition as the reference feed (commercial feed). SB 30% was added in the proportion of 0.1 kg per 100 kg of feed (0.1%), as recommended by the manufacturer. The feed was provided by a local company and the guaranteed levels of nutrients were close to the levels reported by Rostagno et al. (2011).

Commercial feeds based on corn and soybean meal were used, formulated to meet the requirements of the commercial line of piglets. The antimicrobial composition of the feeds was as follows: weaning to starter (40 mg kg^{-1} of colistin); pre-starter I and pre-starter II (450 mg kg^{-1} of amoxicillin, 55 mg kg^{-1} of lincomycin and 55 mg kg^{-1} of spectinomycin), and starter (380 mg kg^{-1} of amoxicillin and 40 mg kg^{-1} florfenicol) (Table 1).

Table 1. Nutritional composition of the commercial feeds and their respective levels of warranty[1].

Minimum levels of warranty (g/kg)	Weaning	Pre-starter I	Pre-starter II	Starter
Humidity (Max.)	90.0	100.0	100.0	120.0
Crude protein (Min.)	180.0	190.0	200.0	200.0
Ether extract (Min.)	75.0	70.0	60.0	50.0
Mineral matter (Max.)	50.0	55.0	55.0	60.0
Average Chain Fatty Acid (Min.)	12.5	3.80	3.80	-
Lysine (Min.)	16.0	14.5	13.0	13.5
Methionine (Min.)	5.6	5.0	4.0	4.85
Crude fiber (Max.)	20.0	30.0	50.0	35.0

[1]Trace (-): Value not reported on the label of the feeds.

Sample collection and preparation

The daily weight gain (DWG), average daily feed intake (ADFI), feed conversion (FC) and final body weight (FBW) was determined. At 14 and 28 days after the start of the experiment, the log (base ten) of the counts of *E. coli* (Logcoli14, Logcoli28), *Clostridium* (Logclost14, Logclost28) and lactic acid bacteria (Logacid14, Logacid28) were measured, as well as the incidence of diarrhoea, erythrocytes (millions mm^{-3}), haemoglobin (g dL^{-1}), haematocrit (%), mean corpuscular haemoglobin (MCH; pg), mean corpuscular haemoglobin concentration (MCHC; %), total proteins (g dL^{-1}), platelets (thousand mm^{-3}), leukocytes (cells mm^{-3}), metamyelocytes (%), rods (%), segmented (%), eosinophils (%), basophils (%), lymphocytes (%) and monocytes (%).

The FBW was measured at the end of the experimental period (35th day). DWG was obtained from the difference between the FBW and the IBW of the piglets divided by the number of days of the experiment. The ADFI was obtained by computing the sum of feed intake from day 1 to day 35, divided by 35. FC was obtained by dividing DWG by ADFI.

The incidence of diarrhoea was evaluated daily, in the morning and evening, throughout the experimental period. A single observer recorded the physical characteristics of the piglets' faeces in each pen. The faeces were classified according to consistency, with scores 1, 2 and 3 representing normal, pasty and liquid faeces, respectively (SOBESTIANSKY; BARCELLOS, 2007). The occurrence of diarrhea in a piglet characterised the presence of diarrhoea in the pen; the value "one" was assigned (score of 3) for faeces that typically had 20% or less dry matter (SOBESTIANSKY; BARCELLOS, 2007). The absence of liquid faeces for a given experimental unit was indicated by assigning the value "zero" (scores 1 and 2).

The rectal microbiological profile of the animals was measured at the outset of the experiment (day 0) and at two weeks (day 14) and four weeks (day 28) after onset, totalling 48 samples. Plastic, flexible and sterile swabs of 15-cm length were opened at the moment of collection, introduced into the rectum of the same piglet from each experimental unit, conditioned in capped tubes containing sterile saline, labeled and stored under refrigeration until quantitative analysis. Samples were analysed for *E. coli*, sulphite-reducing clostridia and lactic acid bacteria between 1 and 2 h after collection (SOBESTIANSKY et al., 2005) at the Laboratory of Food and Water Analysis, of Federal University of Paraná, Palotina.

For enumeration of *E. coli*, the vial of saline solution containing the swab was considered to be a dilution of 100. Serial dilutions were then performed until attaining a 10^{-2} dilution. Then, 1 mL of the 10^{-2}

dilution was pipetted onto Petrifilm™ *E. coli* plates, which were subsequently incubated at 35 °C for 48 h. The blue colonies with gas, which were observed at 48 h, were considered positive *E. coli* colonies, according to the 3M Petrifilm™ method.

The sulphite-reducing clostridia enumeration was based on the guidelines of the IN-62 (BRASIL, 2003). The vial of saline solution containing the swab was considered to be a 10^0 dilution. Serial dilutions were then performed until attaining a dilution of 10^{-2}. Then, 1 mL of the 10^{-2} dilution was pipetted into sterile Petri dishes, followed by addition of Shahidi-Ferguson perfringens (SFP) agar. After solidification, a second layer of the same agar was added. The plates were incubated at 36 °C under anaerobic conditions for 18 to 24 h. Black colonies were considered to be sulphite-reducing clostridia and were counted.

For enumeration of the lactic acid bacteria, the vial of saline solution containing the swab was considered to be a 10^0 dilution. Serial dilutions were then performed until attaining a dilution of 10^{-5}. Next, 1 mL of the 10^{-2} and 10^{-4} dilutions were pipetted onto sterile Petri dishes and de Man–Rogosa–Sharpe (MRS) agar then added. After solidification, a second layer of the same agar as added. The plates were incubated at 30 °C for 48 h. All colonies that grew on the plates were counted (APHA, 1998).

Serum and immunological standards of piglets fed with or without SB addition, respectively, were determined in random blood samples obtained by cranial vena cava puncture of two piglets per pen at the end of the experimental period, totalling 32 animals. After collection, the blood samples were carefully transferred to tubes containing anti-coagulant solution (ethylenediaminetetraacetic acid), conditioned in a thermal box and immediately forwarded to the Laboratory of Clinical Analysis of the Veterinary Hospital of the Federal University of Paraná, Campus de Palotina for haemogram and leukogram evaluations.

The counts of red cells and leukocytes were undertaken using an automatic cell counter (BC2800-Vet™). Total plasma proteins were counted in a refractometer, and cell differentiation was performed by blood smear with panoptic staining and under magnifying glasses (1000× magnification).

Statistical analysis

For the performance characteristics, the statistical model used included the covariate effect, expressed by the equation: $Y_{ijkl} = m + F_i + G_j + FG_{ij} + b_k + \beta\ (X_{ijkl} - \bar{x}....) + \varepsilon_{ijkl}$. In this equation, Y_{ijk} = average observation of the dependent variable in each plot, measured in the i^{th} feed class, j^{th} gender class and k^{th} replication; m = effect of the overall average; F_i = effect of the feed classes, for i = (1 and 2); G_j = effect of gender classes, for j = (1 and 2); FG_{ij} = effect of interaction between the i^{th} feed class and the j^{th} gender class; b_k = block effect, for k = (1 and 2); β = regression coefficient of Y on X; X_{ijkL} = average covariate observation (average initial weight for performance characteristics) in each plot, measured in the i^{th} class of feed, j^{th} gender class and k^{th} replication; $\bar{x}$.... = overall average for the covariate X, and ε_{ijkL} = random error of the plot associated with each observation $Y_{ijkl.}$ For the characteristics measured in blood and the microbiological profile, we adopted the same statistical model, without the use of the covariate effect.

The effects of covariate and the other factors included in the statistical model on growth performance were verified by the F-test of covariance analysis (ANCOVA). The effects of feed, gender and the interaction between feed and gender on microbiological and haematological variables were verified by the F-test of variance analysis (ANOVA). With respected to the significance ($p \leq 0.05$) in the ANCOVA and ANOVA, the least squares means related to the effect of interaction were compared using the Student's t-test.

In order to evaluate the incidence of diarrhoea, a 2 × 2 frequency table was generated and classified, based on the feed classes (with or without additional SB) and the categories of binary responses (0 and 1). For a given feed class, the response proportion of the category “one” was calculated by the ratio between the response frequency for the class under study and sum of the response frequencies in all measures for the class under study, which totalled 560 measures (8 pens × 35 days × 2 measures per day [morning and afternoon]).

To compare the proportions between the two feed classes for the responses with value “one”, the calculated statistic used was $Z = [p_1 - p_2]/[p_1(1 - p_1)/n_1^{-1} + p_2(1 - p_2)/n_2^{-1}]^{0.5}$, where p_1 = ratio of response “one” for the class “with use of SB”; p_2 = response ratio “one” for class “without use of SB”; $n_1 = n_2 = 560$ = total number of responses in all measures for the classes of feed under study.

The rejection of the null hypothesis $p_1 = p_2$ occurred if $Z_{cal}^{-} \leq Z_{tab}^{-}$. The value of Z_{tab}^{-} was -1.645 and the unilateral hypothesis ($p_1 < p_2$) was the alternative hypothesis adopted. The 5% level of significance was considered in all statistical analyses, which were performed using R Core Team software (2013).

Results and Discussion

Growth performance

There was no interaction ($p > 0.05$) between feed class and gender on FBW, ADFI, DWG and FC (Table 2). There was no effect ($p > 0.05$) of gender on FBW and DWG. Females showed higher average values ($p < 0.05$) of ADFI and FC than males (Table 2).

Table 2. Least squares means adjusted for initial body weight (IBW), related to the final body weight (FBW), daily feed intake (ADFI), daily body weight gain (DBWG) and feed conversion (FC), according to the combinations of feed and gender classes[1].

Items	Females		Males		Gender		P *value*			CV (%)
	With butyrate	Without butyrate	With butyrate	Without butyrate	Female	Male	Feed	Gen-der	F*G	
IBW (kg)	9.195	9.345	9.705	9.650	9.270	9.677	-	-	-	-
FBW (kg)	26.642	26.136	26.194	26.754	26.389	26.474	0.952	0.893	0.260	3.28
ADFI (kg)	0.547	0.533	0.510	0.518	0.540*	0.514	0.719	0.034	0.179	2.87
DBWG (kg)	0.396	0.382	0.382	0.392	0.389	0.387	0.800	0.839	0.264	4.76
FC (kg:kg)	1.384	1.396	1.334	1.327	1.390*	1.330	0.872	0.022	0.572	2.27

[1]Lsmeans followed by asterisks in the line differ from each other, by the F-test, at a 5% probability level; CV: coefficient of variation.

The absence of difference between treatments may be associated with the high quality of the commercial diet, containing added acidifiers and antimicrobial promoters, as well as the strict control of management at the experimental site, affected the action of the SB reducing the sanitary challenges that could provide conditions for the action of the product by greater requirement of the recovery potential of cells of the intestinal mucosa. Similarly, Boas et al. (2016) also noted that the addition of SB to diets containing other organic acids did not affect the performance of piglets during the nursing phase.

Piva et al. (2002) documented a significant improvement in the piglets’ performance until day 14 of the nursery phase. During this period, the animals that consumed the diet containing SB presented a higher FBW, DWG and ADFI, but without differences ($p > 0.05$) in feed efficiency (FE) between the treatments. However, from days 15 to 35, the animals fed the control diet had a higher ($p < 0.05$) FE (549 g kg^{-1} ingested) than the pigs fed SB (524 g kg^{-1} ingested). Thus, the addition of SB facilitated only the initial phase of adaptation to a solid diet in piglets. The authors reported that the efficacy of SB in the post-weaning period was associated with a higher requirement for recovery of the intestinal architecture because there was a decrease in villus height due to weaning stress. In subsequent periods, the organism could stimulate the intestinal changes with the normal feed intake. Thus, the incoherence regarding the effects of SB on the growth performance of animals during the nursery phase could be directly related to the nutritional status and maturation of the intestinal morphology of the piglets (BIAGI et al., 2007).

Although some studies demonstrate a decrease in the growth performance of nursery piglets fed diets containing added SB during some or all of the treatment period (BOAS et al., 2016; COSTA et al., 2011; PIVA et al., 2002), other research workers report a positive effect, both in exclusive use as an associate (LU et al., 2008; MAZZONI et al., 2008; PIVA et al., 2007). These experimental variations in piglet performance concerning the use of SB in feed can be directly connected to the existing sanitary control in the different experimental environments. Well-conducted cleaning and disinfection protocols can reduce the sanitary challenge in the environment, explaining the lack of effect of the product (organic acid) on the animals' performance.

Silva et al. (2000) observed that nursery-aged females had better performance characteristics, but emphasised that there was an improvement in the performance of males in the subsequent phases of production. Such observations were also described by Alberton and Zotti (2010) and Vasquez Bruno et al. (2013), who reported that immunologically castrated male pigs had superior performance compared to females in the growing phase. The data presented herein and found in the literature demonstrate the importance of the procedures reported by Sakomura and Rostagno (2016), who considered gender to be a source of systematic variation, to control the experimental error and also a factor to be studied in the experiments with non-ruminants.

Rectal microbiological profile

There was an interaction between feed and gender ($p < 0.05$) for *E. Coli* 14. Males who received feed containing SB had a higher average *E. Coli* 14 value than their female counterparts (Table 3). For the other characteristics of the rectal microbiological profile, there were no differences observed between the averages in the unfolding of the interaction between gender and feed (Table 3).

This result may indicate a beneficial action of SB intake on the colonisation of intestinal microbiota in females. The presence of antimicrobial and acidifier components in the diets may have interfered with the counts of the microorganisms, mitigating the effect of SB on the intestinal microbiota. Kasprowicz-Potocka et al. (2009) reported that the use of different salts and organic acids could improve the composition of the intestinal microbiota. Likewise, Biagi et al. (2007) also did not find differences ($p > 0.05$) between the average values of the intestinal microbiota when investigating the addition of SB to the feed of nursery piglets.

Lu et al. (2008) found that piglets fed diets containing SB had a greater villus height and significantly reduced *E. coli* and Clostridium populations in the small intestine and colon compared to those fed diets without SB inclusion. However, there were no differences ($p > 0.05$) between the population averages of lactic acid bacteria. The authors highlighted the connection between the action of SB and the maintenance of the intestinal morphology and sanity conditions of piglets in the nursery phase.

Table 3. Mean values of the logarithm (base ten) of the *Escherichia coli*, *Clostridium* and lactic acid bacteria counts on the 14th and 28th day after the start of the trial, according to the combinations of feed and gender classes[1].

Items	Females		Males		Gender		P *value*			CV (%)
	With butyrate	Without butyrate	With butyrate	Without butyrate	Female	Male	Feed	Gender	F*G	
***E. Coli* 14**	3.31[B]	4.52	5.51[A]	3.89	3.92	4.70	0.741	0.219	0.038	29.09
***Clost* 14**	3.18	3.96	4.54	3.33	3.57	3.94	0.818	0.691	0.291	49.82
Lab 14	7.50	8.20	7.90	7.29	7.85	7.60	0.908	0.521	0.112	10.28
***E. Coli* 28**	5.34	5.23	4.86	5.27	5.29	5.07	0.676	0.543	0.488	13.99
***Clost* 28**	3.39	4.36	4.32	4.28	3.87	4.30	0.431	0.465	0.395	28.84
Lab 28	7.36	7.35	7.11	7.61	7.35	7.36	0.435	0.985	0.429	8.70

[1]Lsmeans followed by different capital letters in the row, differ by the Student's *t*-test, at a 5% probability level; CV: coefficient of variation.

Serum and immunological standards

Except for blood haemoglobin, no interaction effect ($p > 0.05$) was observed on the other haematological variables analysed (Table 4). There was an effect ($p = 0.0118$) of gender on the platelet count of blood samples. Females presented a lower average count than males. The estimated average values were 494,312 (females) and 618,500 (males).

Table 4. Mean values of hematological parameters of pigs, according to the combinations of feed and gender classes[1].

Items	Females		Males		Gender		P *value*			CV (%)
	With butyrate	Without butyrate	With butyrate	Without butyrate	Female	Male	Feed	Gender	F*G	
Red blood cells	5.99	6.75	6.51	6.29	6.37	6.39	0.285	0.914	0.069	7.62
Haemoglobin	11.1 [B]	11.8 [A]	11.4	10.8	11.43	11.12	0.801	0.303	0.041	5.17
Hematocrit	35.1	40.6	38.4	36.9	37.86	37.70	0.301	0.931	0.084	9.70
MCV	58.2	60.2	59.0	58.9	59.19	58.95	0.521	0.860	0.455	4.62
MCH	18.6	17.5	17.4	17.2	18.07	17.33	0.297	0.271	0.480	7.20
MCHC	32.2	29.1	29.1	29.2	30.67	29.16	0.344	0.334	0.293	9.99
Total proteins	5.90	5.80	5.90	6.20	5.87	6.07	0.903	0.630	0.630	13.5
Platelets	487,375	501,250	608,000	629,000	494,312[B]	618,50[A]	0.680	0.011	0.932	14.8
Leukocytes	15,050.0	16,525.0	16,438.0	19,213.0	15,787.5	17,825	0.210	0.228	0.691	18.9
Metamyelocytes	0	0	0	0	0	0	-	-	-	-
Rods	0	0	0.12	0	0	0.062	0.338	0.338	0.338	400.0
Segmented	34.0	36.4	35.7	37.0	35.18	36.37	0.722	0.815	0.912	27.8
Eosinophils	1.75	1.07	0.75	1.25	1.41	1.00	0.860	0.415	0.253	80.8
Basophils	0.25	0.25	0.25	0	0.25	0.12	0.543	0.543	0.543	212.7
Lymphocytes	55.7	55.6	57.2	54.5	55.43	55.87	0.758	0.936	0.846	19.2
Monocytes	6.50	7.25	5.87	7.25	6.87	6.56	0.565	0.864	0.864	53.3

[1]Lsmeans followed by different capital letters in the row differ by the Student's *t*-test, at a 5% probability level; CV: coefficient of variation; red blood cells (millions/mm^3), haemoglobin (g/dL), hematocrit (%), MCV (µ^3), MCH (pg), MCHC (%), MCV: Mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, total proteins (g dl^{-1}), platelets (thousand/mm^3), leukocytes (cells/mm^3), metamyelocytes (%), rods (%), segmented (%), eosinophils (%), basophils (%), lymphocytes (%) e monocytes (%).

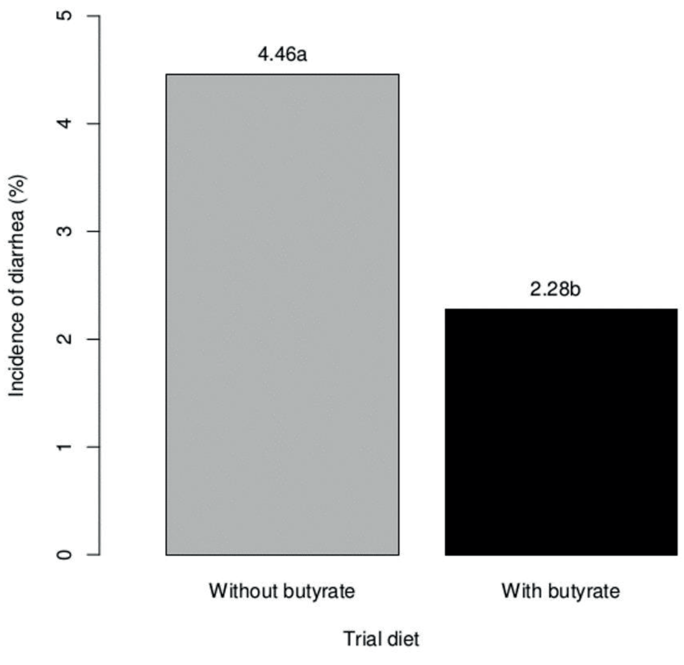
Both average values were within the normal reference standard, which ranges from 5.0-8.0 × 10^6 μL for red cells and 10–16 gdL^{-1} for haemoglobin (JAIN; SCHALM, 2010). No references were found in the scientific literature to explain the effect of gender on average platelet values. The haematological standard of platelets of swine of both genders remained within the normal reference values, which range from 5.2 ± 1.95 × 10^5 μL^{-1} (JAIN; SCHALM, 2010), indicating that the animals showed a stable physiological condition during the trial period.

Silva (2013) reported that SB might offer a therapeutic option for diseases involving platelet dysfunction. Unlike the present study, Silva (2013) demonstrated a reduction in the number of platelets in blood from nursery pigs that received a basal diet with the inclusion of 0.3% SB. Furthermore, acetylation modification of the promoter region of the mannosyl transferase-encoding gene PIGM by SB, a histone deacetylase inhibitor, increases PIGM transcription and glycosylphosphatidylinositol expression *in vivo*, as well as *in vitro*, and is of great therapeutic value (ALMEIDA et al., 2007).

Incidence of diarrhoea

There was a significant effect ($p = 0.05$) of feed classes on the incidence of diarrhoea in piglets, with an incidence of 2.68% in piglets fed diets containing SB and 4.46% in piglets that received the additional organic acid-free feed (Figure 1). The results indicated that the inclusion of coated SB 30% in the diet reduced the incidence of this disease in piglets.

Figure 1. Incidence of diarrhea according to the feed classes. Means followed by distinct letters differ from each other, through of the test of the difference between proportions, at the 5% probability level.



The higher proportion of normal faecal scores in animals that received the coated SB 30% in the diet can be explained by the relationship between mucosal integrity and nutrient absorption in the intestinal mucosa. It is known that SB acts as a substrate for the renewal, growth and differentiation of intestinal cells, and it can also modulate the intestinal microflora (CASTILLO et al., 2006), besides showing bactericidal and bacteriostatic activity in the intestine, with dynamic changes in the population of microorganisms (MROZ et al., 2006). Furthermore, by preventing the development of pathogenic bacteria, SB stimulates an increase in the number of beneficial bacteria, reducing the occurrence of diarrhoea and, in turn, promoting an increase in intestinal villus height.

Mazzoni et al. (2008) obtained similar results with the use of the SB, in which there was greater morphological and physiological development (P = 0.05) of the gastrointestinal tract mucosa in weaned piglets, promoting an improvement in the functioning of the cells involved in the digestive process.

We can also associate the best result on the incidence of diarrhoea of the animals who received the coated SB 30% in the feeds due to an improvement in the integrity of the mucosa and the defence functions of the intestinal cells against aggressive agents (LU et al., 2008). In addition, SB may have acted to reduce the intestinal pH. According to Rocha et al. (2008), who tested the efficiency of the use of organic acid in nursery piglets, the lower incidence of diarrhoea may be linked to a greater nutrient digestibility caused by enhanced enzyme activity or protein hydrolysis, resulting from a reduction in the pH of the gastrointestinal tract.

With the increased challenges for piglet production which occur under field conditions, it is possible that SB would have a relatively greater visible effect on the reduction of multifactorial diarrhoea under such conditions than seen in the current study. Costa et al. (2011) attributed the absence of significant effects of SB on piglet faecal scores to the high digestibility of the feed used in the trial period, which in the absence of health challenges, avoided the passing of indigestible nutrients.

Conclusion

In conclusion, the inclusion of coated SB 30% in commercial feed containing antimicrobials did not alter the growth performance and the rectal microbiological profile of nursery piglets submitted to low sanitary challenge conditions. SB lowered the occurrence of gastrointestinal disorders that cause diarrhoea in nursery piglets. In the nursery phase, entire males showed better feed conversion than females.

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