Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v25e-77719E

e-ISSN 1809-6891
Animal science | Research article

Characterization and anaerobic digestion of manure from pigs submitted to feed restriction or supplemented with ractopamine or chromium

Caracterização e digestão anaeróbia dos dejetos de suínos submetidos a restrição alimentar ou suplementados com ractopamina ou cromo

Elaine Mariza Rosa[1], Cristiane Almeida Neves Xavier[1], Charles Kiefer[2], Leide Daiana de Oliveira Arruda[1], Willian Rufino Andrade[1], Danilo de Souza Sanches[2], Elis Regina de Moraes Garcia[1], Tânia Mara Baptista dos Santos*[1]

1 Universidade Estadual de Mato Grosso do Sul (UEMS), Aquidauana, Mato Grosso do Sul, Brazil
2 Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso, Brazil
* corresponding author: tania@uems.br

Abstract: The aim of this study was to characterize the production and anaerobic digestion of manure from finishing pigs subjected to feed restriction or supplemented with ractopamine or chromium (Cr). The waste came from 50 barrows in the finishing phase, aged ± 154 days, with a starting weight of 99.0 ± 4.4 kg and a final weight of 117.2 ± 5.8 kg. The experimental diets were as follows: control (conventional diet), qualitative restriction (7.5% reduction in net energy compared to the control diet), quantitative restriction (15% reduction in feed supply), Cr (0.8 mg), and ractopamine (10 ppm). The data were subjected to an analysis of variance using a randomized block design, in which the weeks of analysis were considered blocks (cofactors). There were no differences in manure production between the diets regarding natural matter (NM), dry matter (DM), mineral matter (MM), or organic matter (OM). Animals fed the control diet had the highest residue coefficient, and there was no difference among the other diets. No differences were observed among the diets regarding total solids, pH, or total nitrogen in the tributaries or effluents. The highest biogas yield (574 mL g^{-1}) of added volatile solids (VS) was obtained in the digesters supplied with manure from animals fed a qualitatively restricted diet. It can be concluded that a qualitatively restricted diet results in higher manure production but with lower nitrogen and phosphorus excretion and higher biogas yields.

Keywords: biogas, digesters, organic mineral, growth promoter

Resumo: Realizou-se este estudo como o objetivo de caracterizar a produção e a digestão anaeróbia de dejetos de suínos em terminação submetidos a restrição alimentar ou suplementados com ractopamina ou cromo (Cr). Os dejetos foram provenientes de 50 suínos machos castrados, em fase de terminação, com ± 154 dias de idade, com peso inicial de 99,0 ± 4,4kg e final de 117,2 ± 5,8kg. As dietas experimentais foram: controle (dieta convencional); restrição qualitativa (redução de 7,5% de energia líquida em relação à dieta controle); restrição quantitativa (redução de 15% no fornecimento de ração); Cr (0,8 mg); e ractopamina (10 ppm). Os dados foram submetidos à análise de variância por meio do delineamento em blocos ao acaso, no qual as semanas de análises foram consideradas

Received: November 07, 2023. Accepted: April 04, 2024. Published: July 31, 2024.

como blocos (cofator). Não foram observadas diferenças entre as dietas nas produções de dejetos na matéria natural (MN), matéria seca (MS), matéria mineral (MM) e matéria orgânica (MO). Animais que receberam a dieta controle apresentaram o maior coeficiente de resíduo e não houve diferença entre as demais dietas. Não foram observadas diferenças entre as dietas para sólidos totais, pH e nitrogênio total dos afluentes e efluentes. O maior rendimento de biogás (574 mL g^{-1}) de sólidos voláteis (SV) adicionados foi obtido com os digestores abastecidos com dejetos de animais alimentados com dieta qualitativamente restrita. Conclui-se que a dieta com restrição qualitativa resulta em maior produção de dejetos, porém com menores excreções de nitrogênio e fósforo e maior rendimento de biogás.

Palavras-chave: biogás; digestores; mineral orgânico; promotor de crecimento

1. Introduction

Various nutritional strategies can be used during the finishing phase of pigs to improve growth rate, feed efficiency, and carcass quality. This includes using food restriction or supplementing diets with additives such as ractopamine and/or Cr. Feed restriction during finishing aims to improve feed efficiency and carcass quality by reducing the amount of fat deposited, and consequently, increasing the percentage of meat [(1, 2, 3)].

Dietary restrictions can be quantitative or qualitative. Energy consumption can be reduced by restricting the quantity of food and controlling the amount of feed provided to the animals. Based on this assumption, the lower the food supply, the lower the manure production. Another form of food restriction that reduces energy consumption in animals is the inclusion of ingredients with lower caloric value, known as qualitative food restriction [(4)].

Cr is a mineral component of the glucose tolerance factor that increases the fluidity of the cell membrane, allowing binding of the insulin receptor, which increases glucose uptake [(5)]. Cr participates in lipid and protein metabolism and nucleic acid synthesis [(6)], and can promote an increase in weight gain, feed consumption, and the percentage of lean meat in pig carcasses when supplemented correctly [(7, 8)].

The inclusion of ractopamine in pig feed during the finishing phase has positive effects on performance [(9)], increases lean carcass mass, and reduces the amount of carcass fat [(10, 11)]. Ractopamine is a synthetic compound that has a chemical and pharmacological structure and properties similar to that of natural catecholamines [(12)], which acts through β-specific receptors, resulting in a decrease in lipogenesis and an increase in muscle mass [(13)].

Based on these concepts, it is hypothesized that animals fed diets with a lower energy density will produce waste with fewer pollutants. From the same perspective, other recommended nutritional strategies are diets supplemented with ractopamine or Cr, which can improve performance and reduce the amount of fat deposited in the carcass.

However, information on the effects of ractopamine, feed restriction, and Cr on manure characteristics and their impact on the environment is still unclear, and information on this subject is scarce in the literature. Therefore, considering the need for information related to the composition of manure, the aim of this study was to characterize the manure and evaluate the anaerobic digestion of this waste from finishing pigs fed diets containing ractopamine or Cr, or under quantitative or qualitative dietary restrictions.

2. Material and Methods

The experiments were performed at the Animal Waste Laboratory of the State University of Mato Grosso do Sul, Brazil. The municipality has two well-defined seasons: rainy summers and dry winters. The average temperature was 29.4 °C during the experiment.

The manure came from 50 barrows in the finishing phase, aged ± 154 days, with a starting weight of 99.0 ± 4.4 kg and a final weight of 117.2 ± 5.8 kg. The animals were housed in a masonry shed and divided into five groups of ten animals each, with two animals per stall. The room was covered with ceramic tiles, had a concrete floor with shingled sides, and was fitted with curtains. The stalls, measuring 1.15 × 2.86 m, were equipped with feeders, nipple-type drinkers and water slides measuring 1.15 × 0.30 × 0.10 m.

The diets were formulated from corn and soybean meal and supplemented with vitamins and minerals to meet the nutritional requirements established by Rostagno et al. [(14)]. These were the control (conventional diet), qualitative restriction (7.5% reduction in net energy compared to the control diet), quantitative restriction (15% reduction in feed supply), Cr (0.8 mg), and ractopamine (10 ppm).

Manure was collected on the last three days of the experimental phase, immediately after excretion, according to the experimental diet to which the animals were subjected. On the days prior to collection, the water slides were thoroughly cleaned, and the water supply was turned off for 24 hours. After this period, waste from each stall was collected by scraping the floor. The collected material was weighed and stored in a freezer in labeled plastic bags. The manure generated and collected from each stall was identified, treated, and stored until it was analyzed in the laboratory. Afterward, the manure was homogenized to obtain a composite sample for each treatment to obtain the material supplied to the anaerobic digesters.

Manure production was expressed as kg of total solids (TS) animal^{-1} day^{-1} and calculated using data from the daily weighing of manure (kg) and the TS content of the manure, in which manure production (TS animal^{-1} day^{-1}) = manure, kg × TS, %. The residue coefficient (RC) was calculated from the manure production data (in DM) and weight gain during the confinement period for each animal, according to the following equation: RC = amount of manure, kg in DM / weight gain, kg. Ten semi-continuous cylindrical bench digesters made of polyvinyl chloride (PVC) with a useful volume of 7.5 L of the fermenting substrate were used (Figure 1). The digesters consisted of two distinct parts: a fermentation chamber and a gasometer, which consisted of two internal and external, whose purpose was to store and quantify the generated gas. The fermentation chamber and gasometer were connected using a silicone hose.

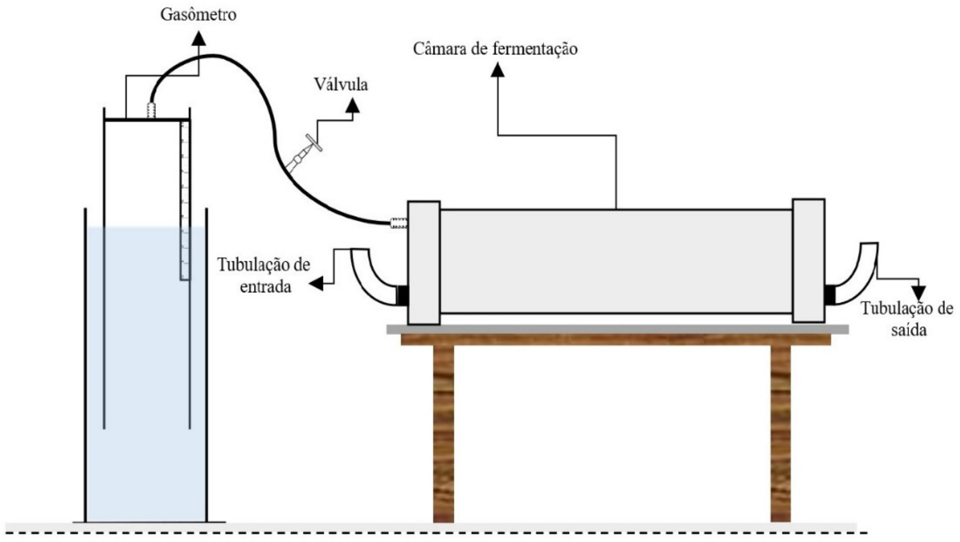

Figure 1 Schematic representation of the experimental anaerobic digester used

The initial tributaries were formulated to contain approximately 3% TS. The start-up time was 30 days; during this period, biogas was burned in all the digesters. After the start-up, the digesters were subjected to daily loads for 90 days. During this period, the digesters were supplied daily with effluent that varied according to the treatments, with the addition of sodium bicarbonate ($NaHCO_3$), used to help form and maintain alkalinity, correct pH and thus provide a suitable environment for greater reduction of volatile solids (VS), promoting greater biogas production accompanied by better quality biofertilizer.

During the test, samples of the effluent were collected daily to determine the TS and VS contents and weekly to measure the pH values using a digital potentiometer. Ammoniacal nitrogen (N ammonia) was determined according to the methodology described by APHA [(15)], and partial alkalinity (PA), intermediate alkalinity (IA), and total alkalinity (TA) were determined according to Ripley et al. [(16)] and Jenkins et al. [(17)]. Total nitrogen (N) concentration was determined using the Kjeldahl method according to Silva and Queiroz [(18)].

The effluents and tributaries were analyzed every two weeks using dry digestion and spectrophotometry (725 nm) to determine the concentration of total phosphorus (P). Dry digestion consisted of calcining the ground samples in a muffle furnace at 600 °C for 3 hours, the addition of 2 mL of concentrated hydrochloric acid, calcination for a further 3 hours, digestion in a digester block with a sand bath at 200 °C with the addition of hydrochloric acid diluted in water (1:1) for approximately 30 minutes, and dilution and storage of solutions.

The calorimetric method involved the formation of a yellow compound from the phosphoric vanodomolybdenum system with an acidity of 0.2–1.6 N [(19)]. The color development was measured in a spectrophotometer, and the P concentration of the samples were determining using a standard line drawn using known concentrations between 0 and 52 μg of P mL^{-1}. The standards were prepared as described by Malavolta [(20)].

Biogas production was calculated based on the displacement of the gasometer measured with a 50 cm tape measure. After reading the volume produced, the temperature was measured using a digital thermometer placed at the biogas outlet until it stabilized. After each reading, the gasometers were zeroed by discharging biogas. To correct for the volume of biogas, an expression resulting from the combination of Boyle's and Gay–Lussac's laws was used.

Considering that the average atmospheric pressure in Aquidauana during the experimental period was 10293 mm of H_2O, the result is the following expression was used to correct the volume of biogas:

V_0 = corrected biogas volume, m^3,

P_0 = corrected biogas pressure (mm H_2O)

T_0 = Corrected biogas temperature (297.7525 K)

V_1 = Volume of biogas in gasometer

P_1 = Biogas pressure at the time of reading (mm H_2O);

T_1 = Biogas temperature at the time of reading, K

$$\text{Corrected biogas volume}: \frac{V0\,x\,P0}{T0} = \frac{V1\,x\,P1}{T1}$$

Biogas burning tests were carried out using a Bunsen burner attached to the biogas outlet to monitor the stability of the process because overloading digesters can lead to the accumulation of volatile acids and excessive amounts of carbon dioxide in the biogas, a condition where it does not burn. The biogas production potentials were calculated using the daily biogas production data for each treatment and the quantities of VS added during the process. Values are expressed in mL of biogas per gram of added VS.

The anaerobic digestion process monitoring data were subjected to analysis of variance using a randomized block design, and the weeks of analysis were considered as blocks (cofactors). Manure production data were statistically analyzed using the Scott–Knott test, with a 5% probability. Data on the chemical characteristics of the tributaries and effluents (%) were analyzed using the Scott–Knott test at a 1% probability level.

3. Results and Discussion

There were no significant differences between the diets in the production of natural matter (NM), dry matter (DM), or organic matter (OM) (Table 1). However, there were differences between the diets in the residue coefficient (RC). Animals fed the control diet had a higher RC (1.12) than those fed the other diets, which, in turn, showed no differences between them. The data indicated that the highest amount of waste per kilogram of meat produced was generated by feeding the basal diet; that is, the supply of this diet may have a greater environmental impact than the others because the same amount of meat would be produced at the expense of greater waste generation. From a manure treatment perspective, larger systems are required for the same unit of meat produced with the supply of other diets.

Table 1 **Manure production based on natural material (NM), dry material (DM), organic matter (OM), and residue coefficient (RC) of finishing pigs fed different diets**

Diets	NM	DM	OM	RC, kg
	g animal^{-1} day^{-1}			
Control	548	169	120	1.12a
Qualitative restriction	838	306	202	0.73b
Quantitative restriction	671	231	122	0.77b
Chrome	545	160	123	0.46b
Ractopamine	754	223	169	0.82b
P-value	0.356	0.108	0.127	0.008
CV, %	20.78	21.21	20.70	34.33

Means followed by the same letter in the column do not differ by the Scott-Knott test (P<0.05).

The concentrations of ammoniacal N and alkalinity (Table 2), which indicate the balance and stability of the process, were satisfactory with no risk of failure in the anaerobic digestion process [21, 22, 23]. Ammoniacal N is beneficial for anaerobic digestion, serving as a source of nitrogen and a buffer to prevent pH changes [24]. The concentration of ammoniacal N in the effluent ranged from 525 to 771 mg L^{-1}, which were below the process inhibition levels, which are higher than 1,070 mg L^{-1} according to Agyeman et al. [25].

Table 2 Average concentrations of ammoniacal nitrogen and alkalinity in the affluent and effluent of manure from finishing pigs fed different diets

Diets	Ammoniacal N, mL	Alkalinity, mL		
		Partial	Intermediate	Total
Affluent				
Control	341 ± 166	3,448 ± 1,429	2,034 ± 369	4,520 ± 2,439
Qualitative restriction	254 ± 132	3,981 ± 591	1,758 ± 542	5,739 ± 313
Quantitative restriction	276 ± 94	3,239 ± 1,331	1,889 ± 296	5,127 ± 1,163
Chrome	260 ± 142	1,997 ± 989	1,264 ± 615	3,260 ± 915
Ractopamine	314 ± 162	5,033 ± 4,039	2,113 ± 299	5,284 ± 1,148
Effluent				
Control	771 ± 256	2,489 ± 1,443	4,060 ± 1,707	6,549 ± 2,444
Qualitative restriction	525 ± 166	3,772 ± 1,145	9,722 ± 1,864	1,328 ± 1,942
Quantitative restriction	592 ± 217	1,484 ± 540	4,107 ± 1,701	5,592 ± 1,595
Chrome	621 ± 229	733 ± 693	2,255 ± 855	2,988 ± 593
Ractopamine	708 ± 315	1,337 ± 713	4,195 ± 1,534	5,310 ± 1,711

The lower concentrations of ammoniacal N can be explained by the composition of the manure, which depends on the use of nutrients from food consumed by the animals. The formation of ammoniacal N occurs mainly in anaerobic environments, and it remains dissolved in the liquid phase of the manure; in the case of pig manure, the concentrations are high because the animals receive high amounts of organic N in their diets and are also more readily transformed into ammoniacal N [23].

The average TS content of the tributaries was 2.53%, and the total N content was 1.27%. It is possible that there was a difference in the C: N ratio of the tributaries since there was no difference in the total N content, but there was a difference in the VS content

The diets with Cr and ractopamine showed higher VS values in the effluents, which increased the chances of these tributaries producing more biogas than the other diets. The diet with qualitative restriction resulted in the lowest VS value in the affluent and the highest FS content. This tributary had the lowest total P content. The last two parameters indicated that the mineral profile of this effluent was different from that of the other diets; however, it was not possible to conclude whether its quality was superior or inferior. The minerals present in biodigester effluents can favor the growth of microorganisms and improve their metabolism, including the efficiency of the use of methane precursors [26, 27].

According to Zhang et al. [28], the pH under ideal conditions for anaerobic bacteria can vary from 6.0 to 8.0; therefore, these values are in the ideal range for microbial development, with 7.0 being considered ideal. In this range, the methanogenic phase is not compromised because the active bacteria grow better under neutral conditions [26, 27, 29, 30, 31, 32].

The effluents from the biodigesters did not differ in terms of TS, FS, or total N contents (Table 3). The average TS content of the effluents was 2.65%, FS was 0.99%, and TN was 1.26%. These values are close to those observed in the tributaries, with no apparent reduction. Differences in the levels of VS, total P, and pH of the effluents were observed.

Table 3 Chemical characteristics, in percentages, of the affluents and effluents of digesters operated with manure from finishing pigs fed different diets

Dietas	ST	SV	SF	pH	Total N	Total P
Affluents						
Control	2,44	1,50b	0,94b	7,36	1,30	2,17a
Qualitative restriction	2,40	1,22c	1,18a	7,46	1,38	1,45b
Quantitative restriction	2,44	1,58b	0,86b	7,36	1,18	1,96a
Chrome	2,57	1,70a	0,86b	7,36	1,25	2,00a
Ractopamine	2,82	1,91a	0,91b	7,39	1,23	2,00a
Mean	2,53	-	-	7,39	1,27	-
P-value	0,07	<0,01	<0,01	0,15	0,93	<0,01
CV, %	10,64	14,72	12,73	0,98	13,60	11,13
Effluents						
Control	2,19	1,38 b	0,81	6,64 b	1,46	2,28 a
Qualitative restriction	2,33	1,23 b	1,11	7,39 a	1,31	1,78 b
Quantitative restriction	3,07	1,99 a	1,10	6,51 b	1,30	2,26 a
Chrome	2,53	1,61 b	0,92	6,27 b	1,20	2,41 a

Ractopamine	3,14	2,07 a	1,07	6,56 b	1,08	2,02 b
Mean	2,65	-	0,99	-	1,26	-
P-value	0,122	0,031	0,362	<0,001	0,926	0,014
CV, %	27,73	15,30	29,11	3,62	22,67	14,09

TS: total solids; VS: volatile solids; FS: fixed solids; pH: hydrogenionic potential; Total N: total nitrogen; total P: total phosphorus. Lowercase letters in the column differ by the Scoot-Knott test (P<0.05).

Diets with quantitative restriction and ractopamine had higher VS levels (average, 2.03%. The diet with qualitative restriction showed the highest pH value in the effluent (7.39), which implied that the medium was differentiated into nutrients. Its use may have led to the development of a population of microorganisms different from those found in other digesters. Diets with qualitative restriction and ractopamine had the lowest total P levels, averaging 1.90%.

The diet with qualitative restrictions led to a higher weekly biogas production and potential biogas production per unit of VS (Table 4). There was no difference in the accumulated weekly biogas yields between the digesters operated with manure from animals fed the control and quantitative restriction diets, which were higher than those obtained from the Cr and ractopamine diets. SANTOS et al. [33] observed higher biogas production after 14 days of ractopamine supplementation than with no supplementation and supplementation for 7 days.

Table 4 Weekly accumulated production and biogas production potential of digesters operated with manure from finishing pigs fed different diets

Diets	Biogas, mL week^{-1}	Biogas, mL g^{-1} of added VS
Control	9,229b	305b
Qualitative restriction	14,670^{a}	574a
Quantitative restriction	5,286b	160c
Chrome	1,548c	104c
Ractopamine	2,335c	127c
P-value	<0.01	<0.01
CV, %	5.98	31.49

Lowercase letters in the column differ by the Scoot-Knott test (P<0.05).

However the dosage of ractopamine used by the authors was 20 ppm, whereas in the present study, it was 10 ppm (for 16 days), suggesting that the concentration may be more important than the period of supplementation. The second-highest biogas production potential (in mL g^{-1} of VS added), 47% lower, was obtained from biodigesters operated with manure from the animals that received the control diet. The diets with quantitative restriction, Cr and ractopamine showed the lowest potential for biogas production, with an average of 130 mL g^{-1} of VS added.

The diets fed to the animals were isoproteic, which might have led to similar levels of total N in the effluents and effluents of the biodigesters. The diet with qualitative restrictions was prepared with an energy content reduced by approximately 7%, which may have contributed to this diet producing more biogas than the other diets did. This may have contributed to a different nutrient profile in the manure, and an effluent pH above neutrality may have favored the anaerobic digestion process [31, 34, 35].

4. Conclusion

The control diet had a higher residue coefficient than that of the other diets, which may have had a greater environmental impact because it produced a greater amount of residue. The qualitative feed restriction provides the highest biogas yield (mL g^{-1} of added VS), followed by the control, quantitative feed restriction, Cr, and ractopamine diets. Qualitative or quantitative restrictions, as well as Cr or ractopamine supplementation, reduced manure production per unit quantity of meat produced.

Declaration of conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: T. M. B. Santos and C. Kiefer. Data curation: E. M. Rosa, C. A. N. Xavier, C. Kiefer and, T. M. B. Santos. Formal Analysis: E. M. Rosa, C. A. N. Xavier, C. Kiefer, L. D. O. Arruda, W. R. Andrade, D. S. Sanches, E. R. M. Garcia and, T. M. B. Santos. Investigation: E. M. Rosa, C. A. N. Xavier, C. Kiefer, L. D. O. Arruda, W. R. Andrade, D. S. Sanches, E. R. M. Garcia and, T. M. B. Santos. Methodology: C. Kiefer and T. M. B. Santos. Project administration: T. M. B. Santos and C. Kiefer. Supervision: T. M. B. Santos. Writing-original draft: E. M. Rosa. Writing-review & editing: E. M. Rosa, C. A. N. Xavier, C. Kiefer, L. D. O. Arruda, W. R. Andrade, D. S. Sanches, E. R. M. Garcia and, T. M. B. Santos.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES; Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade Estadual de Mato Grosso do Sul (UEMS), and Universidade Federal de Mato Grosso do Sul (UFMS) for the financial support in the execution of the research project.

References

1. Silva ADL, Moreira JÁ, Oliveira RLR, Mota LC, Teixeira ENM, Souza JG, Oliveira RGP. Qualitative feed restriction for late finishing pigs on meat quality and fatty acid profile. Semina: Ciências Agrárias. 2016; 37(4): 2343-2353. https://doi.org/10.5433/1679-0359.2016v37n4Supl1p2343

2. Njoku C, Adeyemi O, Sanwo K, Sanya B, Popoola A, Aina A. Effects of qualitative and quantitative feed restriction on carcass yield and pork quality. Polish Journal of Natural Sciences. 2018; 33(1): 29-48. http://www.uwm.edu.pl/polish-journal/sites/default/files/issues/articles/njoku_et_al_2018_0.pdf

3. Vasconcelos TS, Thomaz MC, Castelini FR, Alvarenga PVA, Oliveira JÁ, Ramos GF, Santos Ruiz U. Evaluation of pineapple byproduct at increasing levels in heavy finishing pigs feeding. Animal Feed Science and Technology. 2020; 269(1): 114664. https://doi.org/10.1016/j.anifeedsci.2020.114664

4. Santos APD, Kiefer C, Martins LP, Fantini CC. Restrição alimentar para suínos machos castrados e imunocastrados em terminação. Ciência Rural. 2012; 42(1): 147-153. https://doi.org/10.1590/S0103-84782012000100024

5. Evans GW, Bowman TD. Chromium picolinate increases membrane fluidity and rate of insulin internalization. Journal of Inorganic Biochemistry. 1992; 46(4): 243-250. https://doi.org/10.1016/0162-0134(92)80034-S

6. Vincent JB. Effects of chromium supplementation on body composition, human and animal health, and insulin and glucose metabolism. Current Opinion in Clinical Nutrition & Metabolic Care. 2019; 22(6): 483-489. https://doi.org/10.1097/MCO.0000000000000604

7. Farias TVA, Kiefer C, Nascimento KMRDS, Corassa A, Alencar SADS, Rodrigues GP, Santos APD. Chromium and energy restriction as substitutes for ractopamine in finishing gilts diet. Ciência Rural. 2022; 52(1): e20200736. https://doi.org/10.1590/0103-8478cr20200736

8. Ele T, Wei C, Lin X, Wang B, Yin G. Meta-analysis of the effects of organic chromium supplementation on the growth performance and carcass quality of weaned and growing-finishing pigs. Animais. 2023; 13(12): 2014. https://doi.org/10.3390/ani13122014

9. Leal RS, Mattos BOD, Cantarelli VDS, Carvalho GCD, Pimenta MEDSG, Pimenta CJ. Performance and carcass yield of pig fed diets containing different levels of ractopamine. Revista Brasileira de Saúde e Produção Animal. 2015; 16(1): 582-590. https://doi.org/10.1590/S1519-99402015000300010

10. Pompeu MA, Rodrigues LA, Cavalcanti LFL, Fontes DO, Toral FLB. A multivariate approach to determine the factors affecting response level of growth, carcass, and meat quality traits in finishing pigs fed ractopamine. Journal of Animal Science. 2017; 95(4): 1644-1659. Disponível em: https://doi.org/10.2527/jas.2016.118

11. Rickard JW, Allee GL, Rincker PJ, Gooding JP, Acheson RJ, McKenna DR, Carr SN. Effects of ractopamine hydrochloride on the growth performance and carcass characteristics of heavy-weight finishing pigs sent for slaughter using a 3-phase marketing strategy. Translational Animal Science. 2017; 1(1): 406–411. https://doi.org/10.2527/tas2017.0053)

12. Andretta I, Kipper M, Lehnen CR, Demori AB, Remus A, Lovatto PA. Meta-analysis of the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs. Livestock Science. 2012; 143(1):91-96. https://doi.org/10.1016/j.livsci.2011.09.004

13. Almeida VVD, Nuñez AJC, Miyada VS. Ractopamine as a metabolic modifier feed additive for finishing pigs: a review. Brazilian Archives of Biology and Technology. 2012; 55: 445-456. https://doi.org/10.1590/S1516-89132012000300016

14. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RD, Lopes DC, Euclides RF. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3ª Ed. Viçosa: UFV, 2011. 252p.

15. Apha - American Public Health Association. Standard methods for the examination of water and wastewater. 24th ed. Washington DC: APHA, AWWA, WEF, 2023, 1624p.

16. Ripley LE, Boyle WC, Converse JC. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. Journal Water Pollution Control Federation. 1986; 58(1): 406-411. https://www.jstor.org/stable/25042933

17. Jenkins SR, Morgan JM, Zhang X. Measuring the usable carbonate alkalinity of operating anaerobic digesters. Journal Water Pollution Control Federation. 1991; 63(1): 28-34. https://www.jstor.org/stable/25043948

18. Silva DJ, Queiroz AC. Análise de Alimentos (métodos químicos e biológicos), 3ª Ed. Viçosa: UFV, 2006. 235p.

19. Perkin-Elmer Corporation. Analytical methods for anatomic absorption spectrophotometry. Morwalk, 1996. 300p.

20. Malavolta E, Vitti GC, Oliveira SA. Micronutrientes, uma visão geral. In: Ferreira ME, Cruz MC. Micronutrientes na agricultura*.* Piracicaba: POTAFOS/CNPq, 1991. p.1-33.

21. González-Fernández C, García-Encina. Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry. Biomass and Bioenerg. 2009; 33(8): 1065-1069. https://doi.org/10.1016/j.biombioe.2009.03.008

22. Astals S, Ariso M, Galí A, Mata-Alvarez J. Co-digestion of pig manure and glycerine: Experimental and modelling study. Journal of Environmental Management. 2011; 92(4): https://doi.org/1091-1096, 10.1016/j.jenvman.2010.11.014

23. Coca FOCG, Xavier CAN, Andrade WR, Arruda LDO, Gonçalves LMP, Kiefer C, Santos TMB. Produção de biogás com dejetos de suínos – efeito de energia líquida e ractopamina da dieta. Archivos de Zootecnia. 2016; 65(252): 507-512. https://doi.org/10.21071/az.v65i252.1918

24. Morozova I, Nikulina N, Oechsner H, Krümpel J, Lemmer A. Effects of Increasing Nitrogen Content on Process Stability and Reactor Performance in Anaerobic Digestion. Energies. 2020; 13(5): 1139. https://doi.org/10.3390/en13051139

25. Agyeman FO, Han Y, Tao W. Elucidating the kinetics of ammonia inhibition to anaerobic digestion through extended batch experiments and stimulation-inhibition modeling. Bioresource technology. 2021; 340(1): 125744. https://doi.org/10.1016/j.biortech.2021.125744

26. Nguyen VK, Chaudhary DK, Dahal RH, Trinh NH, Kim J, Chang SW, Nguyen DD. Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. Fuel, 2021. 285(1): 119105p. https://doi.org/10.1016/j.fuel.2020.119105

27. Zamri MFMA, Hasmady S, Akhiar A, Ideris F, Shamsuddin AH, Mofijur M, Mahlia TMI. A comprehensive review on anaerobic digestion of organic fraction of municipal solid waste. Renewable and Sustainable Energy Reviews. 2021; 137 (1):110637. https://www.sciencedirect.com/science/article/abs/pii/S1364032120309217

28. Zhang W, Li L, Xing W, Chen B, Zhang L, Li A, Yang T. Dynamic behaviors of batch anaerobic systems of food waste for methane production under different organic loads, substrate to inoculum ratios and initial pH. Journal of bioscience and bioengineering. 2019; 128(6): 733-743. https://doi.org/10.1016/j.jbiosc.2019.05.013

29. Kothari R, Pandey AK, Kumar S, Tyagi VV, Tyagi SK. Different aspects of dry anaerobic digestion for bio-energy: an overview. Renewable and Sustainable Energy Reviews. 2014; 39(1): 174-195. https://doi.org/10.1016/j.rser.2014.07.011

30. Xu F, Li Y, Ge X, Yang L, Li Y. Anaerobic digestion of food waste - challenges and opportunities. Bioresource Technology. 2018; 247(1): 1047-1058. https://10.1016/j.biortech.2017.09.020

31. Li Y, Chen Y, Wu J. Enhancement of methane production in anaerobic digestion process: a review. Applied Energy. 2019; 240(1): 120-137. https://doi.org/10.1016/j.apenergy.2019.01.243

32. Atelge MR, Atabani AE, Banu JR, Krisa D, Kaya M, Eskicioglu C, Duman, FATİH. A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. Fuel. 2020; 270(1): 117494. https://doi.org/117494,10.1016/j.fuel.2020.117494

33. Santos T, Trevizan PS, Xavier CA, Kiefer C, Ferraz AL. Biodigestão anaeróbia de dejetos de suínos em terminação suplementados com ractopamina por diferentes períodos. Engenharia Agrícola. 2016; 36(1): 399-407. http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v36n3p399-407/2016

34. Mao C, Feng Y, Wang X, Ren G. Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews. 2015; 45(1): 540-555. https://doi.org/10.1016/j.rser.2015.02.032

35. Hakawati R, Smyth BM, McCullough G, Rosa F, Rooney D. What is the most energy efficient route for biogas utilization: heat, electricity or transport? Applied Energy. 2017; 206(1): 1076-1087. https://doi.org/10.1016/j.apenergy.2017.08.068