ea engenharia agrícola

Journal of the Brazilian Association of Agricultural Engineering

ISSN: 1809-4430 (on-line)

ANAEROBIC BIODIGESTION OF MANURE FROM FINISHING PIG SUPPLEMENTED WITH RACTOPAMINE OVER DIFFERENT PERIODS

Doi:http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v36n3p399-407/2016

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ABSTRACT: This study aimed to evaluate the efficiency of anaerobic digestion and biogas production by manure from pig supplemented with ractopamine over different periods. We used manure of 50 finishing pigs according to the following diets: without supplementation and supplemented with 20-ppm ractopamine during 7, 14, 21, 28 and 35 days pre-slaughter. Continuous bench biodigesters were used in the experiment. We measured biogas production and made analyses of total (TS) and volatile solids (VS), pH, total phosphorus (total P), total nitrogen (total N) and ammoniacal nitrogen (ammoniacal N) in samples of manure, affluent and effluent from biodigesters. The pH and ammoniacal N as indicator parameters of the process balance and stability were satisfactory. Moreover, there was no difference in the reduction of TS and VS (50.04 and 56.51%, respectively). Manure of animals supplemented with ractopamine for 35 and 28 days presented higher biogas production (0.0722 and 0.0603 $m^3\ kg^{-1}$, respectively). The supplementation with 20-ppm ractopamine for finishing pigs from 7 to 35 days does not present collapse risks for the anaerobic biodigestion process.

KEY WORDS: β-adrenergic, biogas, ractopamine hydrochloride, environmental impact and nutrient partitioning

BIODIGESTÃO ANAERÓBIA DE DEJETOS DE SUÍNOS EM TERMINAÇÃO SUPLEMENTADOS COM RACTOPAMINA POR DIFERENTES PERÍODOS

RESUMO: Objetivou-se avaliar a eficiência do processo de biodigestão anaeróbia e produção de biogás por dejetos de suínos suplementados com ractopamina em diferentes períodos. Utilizaram-se dejetos de 50 suínos em terminação, segundo as dietas recebidas: sem suplementação e com suplementação de 20 ppm de ractopamina durante sete, 14; 21; 28 e 35 dias pré-abate. Foram utilizados biodigestores contínuos de bancada, mensuradas as produções de biogás e realizadas as análises de sólidos totais (ST) e voláteis (SV), pH, fósforo total (P total), nitrogênio total (N total) e nitrogênio amoniacal (N amoniacal) em amostras de dejetos, afluentes e efluentes dos biodigestores. O pH e N amoniacal como parâmetros indicadores do equilíbrio e da estabilidade do processo apresentaram-se satisfatórios. Não houve diferença nas reduções de ST e SV (50,04 e 56,51%, respectivamente). Dejetos de animais suplementados com ractopamina por 35 e 28 dias apresentam maiores produções de biogás (0,0722 e 0,0603 m^3/kg, respectivamente). A suplementação com 20 ppm de ractopamina para suínos em terminação, por sete a 35 dias, não oferece riscos de falência do processo de biodigestão anaeróbia.

PALAVRAS-CHAVE: β-adrenérgico, biogás, cloridrato de ractopamina, impacto ambiental e repartidor de nutrientes.

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Recebido pelo Conselho Editorial em: 14/07/2015

Aprovado pelo Conselho Editorial em: 23/02/2016

INTRODUCTION

Ractopamine hydrochloride, a beta-adrenergic agonist with nutrient partitioning function, has been used in pig farming due to its ability to redistribute nutrients that would be destined for lipids synthesis and deposition for muscular tissue deposition (Ferreira et al., 2013).

It is a synthetic drug and its use as a growth promoter is approved in countries with significant pig production, as the United States, Brazil and Canada, which currently occupy respectively the third, fourth and seventh positions in the ranking of world production. However, its use is prohibited in countries such as China and the European Union (Alemanno and Capodieci, 2012; Almeida et al., 2012).

Among the factors that affect the ractopamine efficiency in pig diets are the supplementation level, which can vary from 5 to 20 ppm, and administration time, which is generally performed in the finishing phase for periods ranging from 7 to 41 days pre-slaughter (Neill et al., 2010).

The benefits of ractopamine as a feed additive for pigs are well known in the scientific literature regarding the improvements in the performance and quantitative characteristics of carcass (Kutzler et al., 2011; Pereira et al., 2011; Marçal et al., 2015). However, it is scarce the information about the effects of this additive on the manure characteristics and resulting effects on the environment and/or manure treatment systems.

In the United States, studies have reported environmental benefits brought by ractopamine in pig feeds, since a same amount of meat could be produced with reduction of 5.3 to 6.3% in slaughtered animals; hence, reducing the amounts of consumed soybean and corn. This may indirectly decrease the use of fertilizers, pesticides and water in those crops, besides of decreasing manure generation and nutrient concentration (Woods et al., 2011).

According to Decamp et al. (2001), pigs fed with ractopamine had a decrease in the total N excretion of 43% via urine and of 33% via feces. According to the authors, the amount of retained N increases by 38% with the use of ractopamine due to increasing lean tissue deposition.

When evaluating four levels (0, 5, 10 and 15 ppm) and four periods of supplementation with ractopamine (1 to 4 weeks) in female finishing, Watanabe et al. (2013) observed that there was no effect on the contents of dry matter (DM) and organic matter (OM) of feces. However, the authors observed quadratic effect for the weekly production of DM and OM, with higher values in the second and third week, respectively, therefore, manure quality depends on the period of ractopamine supply.

Since the use of ractopamine may interfere in the manure composition, it has been hypothesized the possible interference of it in the anaerobic biodigestion process, currently the main way of manure treatment and/or recycling generated in the Brazilian pig farming. Thus, the aim of this study was to evaluate the physical and chemical manure characteristics, anaerobic biodigestion process efficiency and biogas production by manure from finishing pig supplemented with ractopamine over different periods.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory of Animal Waste Management in the *Universidade Estadual de Mato Grosso do Sul – UEMS* (State University of Mato Grosso do Sul), campus in Aquidauana - MS, Brazil. The local geographic coordinates are 20° 28'S and 55° 48'W, with an altitude of 184 meters. The average air temperature in the laboratory during the experimental period was 29.44 °C.

Six continuous bench biodigesters were used for evaluations. Each equipment was composed of one fermentation chamber and one gasometer, being built in polyvinyl chloride (PVC) and with a volume of 7.8 L of fermentation substrate.

The manure was derived from 50 barrows with initial and final weight respectively of 65.85 ± 4.34 and 96.55 ± 5.17 kg, housed in stalls at the Swine Sector of the Experimental Farm located at the Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil.

The animals were submitted to experimental corn and soybean meal-based diets supplemented with minerals and vitamins, formulated as in Rostagno et al., (2011), with 20% adjustment for amino acids. Diets formulation was based on the ideal protein concept, since the nutritional requirements of amino acids in diets are higher for supplemented animals (Table 1).

The animals received food and water ad libitum. Daily feed intake was determined and carried out initial and final weight animal measurements to obtain the weight gain and feed conversion. In addition, the animals with diet supplemented with 20-ppm ractopamine received it for periods of 7, 14, 21, 28 and 35 days pre-slaughter. The animals that took the control diet received the same diet (without ractopamine supplementation) throughout the experimental period.

TABLE 1. Compositions and calculated values of the experimental diets.

Ingredients	Ractopamine (ppm)	
	0	20
Corn	537.97	537.97
Soybean meal 45%	213.25	213.25
Millet	200.00	200.00
Soybean oil	17.92	17.92
Dicalcium phosphate	8.15	8.15
Calcitic limestone	6.29	6.29
Regular salt	1.00	1.00
Vitamin supplement[1]	4.00	4.00
Mineral supplement[2]	3.60	3.60
L-lysine HCl	4.13	4.13
L-Threonine	1.44	1.44
DL-Methionine	1.17	1.17
L-Tryptophan	0.09	0.09
Inert (kaolin)	1.00	0.00
Ractopamine	0.00	1.00
Calculated composition*		
Crude protein (g kg^{-1})	168.6	168.6
Metabolizable energy (kcal, kg)	3.230	3.230
Net energy (kcal, kg)	2.447	2.447
Total lysine (g kg^{-1})	11.09	11.09
Digestible lysine (g kg^{-1})	10.16	10.16
Digestible methionine + cystine (g kg^{-1})	6.10	6.10
Digestible threonine (g kg^{-1})	6.81	6.81
Digestible tryptophan (g kg^{-1})	1.83	1.83
Digestible valine (g kg^{-1})	7.01	7.01
Calcium (g kg^{-1})	5.10	5.10
Available phosphorus (g kg^{-1})	2.50	2.50
Total phosphorus (g kg^{-1})	4.68	4.68
Sodium (g kg^{-1})	1.60	1.60

[1]Content kg^{-1} of product: iron, 100 g; copper, 10 g; cobalt, 0.2 g; manganese, 30 g; zinc, 100 g; iodine, 1.0 g; selenium, 0.3 g; excipient QSP, 1,000 g.

[2]Content kg^{-1} of feed: Vitamin A, 6,000,000 IU; Vitamin D3, 1,000,000 IU; Vitamin E, 12,000 IU; Vitamin B1, 0.5 g; Vitamin B2, 2.6 g; Vitamin B6, 0.7 g; pantothenic acid, 10 g; Vitamin K3, 1.5 g; nicotinic acid, 22 g; Vitamin B12, 0.015 g; folic acid, 0.2 g; biotin, 0.05 g; choline, 100 g; excipient QSP 1,000 g.

*Calculated composition according to the recommendations proposed by Rostagno et al. (2011), increased by 20% for the amino acid levels.

The manure was collected in the last two days of the experimental phase (from 8:00 to 16:00 h), after excretion, avoiding to collect it near the drinking trough and feed trough. Samples were taken to determine the levels of total and volatile solids and total nitrogen and phosphorus. Subsequently, the manure were placed in labeled plastic bags and stored in a freezer until the experiment of anaerobic biodigestion, when it was defrosted at ambient temperature for 12 hours.

Starting affluents were formulated to contain approximately 4% of TS. Each biodigester received 1.10 kg of manure and 6.40 kg of water and the starting-up time was 28 days. After the starting-up time, the biodigesters were operated with hydraulic retention time of 30 days, with daily charges of 65 days. Within this period, in the first 30 days, the biodigesters were supplied daily with control affluent with 0.03 kg of manure, 0.22 kg of water and 0.5% (mass/volume) of sodium bicarbonate for pH correction, according to pretests conducted.

In the following 35 days, assessments were carried out, with differentiated supply of the biodigesters according to animal supplementation with ractopamine, as follows: 0 day - control affluent throughout the experimental period (without ractopamine); 7 days - control affluent for 28 days and supplementation affluent for 7 days; 14 days - control affluent for 21 days and supplementation affluent for 14 days; 21 days - control affluent for 14 days and supplementation affluent for 21 days; 28 days - control affluent for 7 days and supplementation affluent for 28 days; and 35 days - supplementation affluent for 35 days.

Affluent and effluent samples were collected daily for TS and VS content determination and weekly for pH and ammoniacal nitrogen (ammoniacal N) determination, following the methodologies described by APHA, AWWA and WPCF (2012). Fortnightly affluent and effluent analyses were performed for the quantification of total nitrogen (N) content using the semi-micro Kjeldahl method, and total phosphorus content (P) by the colorimetric method, according to APHA, AWWA and WPCF (2012).

Biogas production were calculated based on the gasometer displacement and biodigester area (0.00785 m^2). Biogas volume correction to the conditions of 1 atm and 25 °C was made through the expression that results from a combination of Boyle and Gay-Lussac laws:

$$\frac{V_0 P_0}{T_0} = \frac{V_1 P_1}{T_1} \quad (1)$$

where,

V_0 is the corrected biogas volume (m^3);

P_0 is the corrected biogas pressure (10,332.72 mm H_2O);

T_0 is the corrected biogas temperature (298.15 K);

V_1 is the biogas volume in the gasometer;

P_1 is the biogas pressure at the time of reading (10,315 mm H_2O), and

T_1 is the biogas temperature at the time of reading (K).

Considering the average atmospheric pressure in Aquidauana in the period equal to 10,293 mm H_2O, it has as result the following expression for the biogas volume correction:

$$V_0 = \frac{V_1}{T_1} \times 297.6515 \quad (2)$$

For the variance analysis of pH, ammoniacal nitrogen, total nitrogen, total phosphorus and TS and VS reduction, during all the experimental period, we adopted a randomized block design, in which weeks (nine blocks) were considered as a cofactor. The means were compared by the Tukey's test at 5% probability.

The data of accumulated biogas production obtained during the last 35 days were submitted to regression analysis as a function of the animal supplementation period (in days) with ractopamine.

After this analysis, the GLM procedure of SAS was used to compare the estimated means by linear equations generated for each supplementation period using the test at 5% probability.

RESULTS AND DISCUSSION

Daily animal weight gain was not affected (p > 0.05) either by ractopamine inclusion in the diet or by supplementation period. However, the increased supplementation period provided a linear improvement (P <0.05) of feed conversion (equation: $Y = 3.08461 – 0.01144x$, $R^2 = 0.72$) (Table 2).

Although not significant, the improvement in weight gain for animals supplemented for 28 days pre-slaughter, combined with low feed conversion, may result in benefit to the producer when considering the total of animals slaughtered, feed cost savings and carcass quality, especially if there is a bonus for lean carcasses.

Supplementation periods with ractopamine influenced the composition of the animals' fresh manure (Table 2). There was difference (P <0.05) in the total (TS) and volatile solids (VS) percentage in the different supplementation periods with ractopamine.

Manure from animals supplemented with ractopamine during 0, 7, 28 and 35 days showed lower total N concentrations (P <0.05). Increased protein synthesis may be associated with improved dietary protein utilization efficiency and may justify the reduction of the total N concentrations in the manure.

TABLE 2. Average daily weight gain (WG), feed conversion (FC) and average values of total solids (TS), volatile solids (VS), total nitrogen (total N) and total phosphorus (total P) in the manure and reductions of total (TS) and volatile solids (VS), in percentage, obtained from anaerobic biodigestion of pig manure supplemented with ractopamine over different periods.

Supplementation periods	0 day	7 days	14 days	21 days	28 days	35 days	P	CV (%)
Variables	Animal performance							
WG, g dia^{-1}	810	823	878	900	938	914	0.006	12.59
FC	3.13	2.93	3.04	2.77	2.65	2.77	0.028	10.37
	Physicochemical characteristics of manure							
TS[1], mg	39.07^{ab}	39.49^{a}	35.46^{d}	37.08^{c}	38.64^{ab}	37.81^{bc}	<0.001	1.39
VS[2] %	52.80^{c}	58.46^{b}	59.21^{b}	65.79^{a}	59.94^{b}	56.95^{bc}	<0.001	1.94
Total N[2], %	2.02^{b}	2.18^{ab}	2.40^{a}	2.40^{a}	2.03^{b}	2.09^{ab}	0.0082	3.69
Total P[2], %	5.10^{b}	5.78^{ab}	5.59^{ab}	5.16^{b}	6.04^{a}	5.85^{a}	0.0073	3.06
	Solids reduction post anaerobic biodigestion							
TS, %	47.41	49.24	42.55	52.44	55.32	53.26	0.339	18.84
VS, %	54.49	54.02	48.73	59.23	62.23	60.37	0.386	18.83

Means followed by the same letter in the row do not differ by the Tukey's test at 5% probability. [1] In percentage of fresh matter. [2]In percentage of dry matter. Tukey's test at 5% probability. P: statistical probability. CV: coefficient of variation.

The highest concentrations of organic matter (VS) in the manure were observed when the animals were supplemented during 21 days. There was no effect (p > 0.05) of treatments in TS and VS reductions, obtaining averages of 50.04 and 56.51%, respectively (Table 2), which is possibly due to the high coefficient of variation (18.83%). The reduction in the TS and VS contents after anaerobic biodigestion indicates the process efficiency, both in removing solids and producing biogas.

Although the same amount of sodium bicarbonate has been added to all affluents, there was difference (P <0.05) among the pH values of affluents that contained manure of animals supplemented with ractopamine during 35 days and those non-supplemented or supplemented during only 7 days (Table 3). Lower pH values in manure of animals supplemented for a long period indicate the presence of higher amounts of acids from organic matter degradation.

However, despite the difference between the affluents pH values, there was no difference among the pH values of effluents from biodigesters. In stable biodigesters, the production and consumption of volatile fatty acids are balanced, resulting in an environment with adequate tamponade capacity. Thus, affluents slightly acids can result in neutral or alkaline effluents depending on the biodigesters operating conditions. The main components responsible for the tamponade power, and thus for the pH control in manure, are the inorganic carbon, ammoniacal N and volatile fatty acids (Christensen and Sommer, 2013).

The pH values of effluents were higher than the pH range recommended by Yadvika et al. (2004) (between 6.8 and 7.2) probably due to nitrogenous compounds degradation and free ammonia formation during the anaerobic biodigestion process, as can be verified by the difference between the initial and final ammoniacal N concentrations. The ammoniacal N is beneficial for the anaerobic biodigestion process, serving as nitrogen source and as a tamponade, avoiding pH changes. However, high concentrations (above 8.5) inhibit the process to be toxic to methanogenic archaea (Yenigün and Demirel, 2013).

The concentration of ammoniacal N in the effluent ranged from 761 to 824 mg L^{-1}, which is below the levels of inhibition of the process, which according to Rajagopal et al. (2013) it is above than 1,500 mg L^{-1}. When the manure was from animals that received ractopamine for a long period (35 days), lower concentrations of ammoniacal N were observed in affluents and effluents compared to animals non-supplemented or supplemented for only 7 days.

TABLE 3. Values of pH, ammoniacal nitrogen (ammoniacal N), total nitrogen (total N) and total phosphorus (total P) in the affluents (Aff.) and effluents (Eff.) from continuous biodigesters operated with manure from pigs supplemented with ractopamine over different periods.

	pH		Ammoniacal N mg L^{-1}		Total N[1] %		Total P[1] %	
	Aff.	Eff.	Aff.	Eff.	Aff.	Eff.	Aff.	Eff.
0 day	7.67^{a}	7.68	245^{a}	822^{a}	2.82	3.91	3.33	5.12
7 days	7.66^{a}	7.69	235^{ab}	824^{a}	2.53	3.65	2.83	5.01
14 days	7.60^{ab}	7.68	227^{ab}	804^{ab}	2.47	3.67	2.87	5.09
21 days	7.60^{ab}	7.62	222^{ab}	805^{ab}	2.43	3.56	3.25	5.08
28 days	7.62^{ab}	7.66	226^{ab}	784^{ab}	2.25	3.21	2.34	4.26
35 days	7.53^{b}	7.65	207^{b}	761^{b}	2.26	3.30	2.46	3.67
P	0.017	0.367	0.029	0.002	0.173	0.477	0.322	0.093
CV (%)	1.26	0.89	10.84	4.18	15.96	16.67	30.75	19.24

Means followed by the same letter in the column do not differ by the Tukey's test at 5% probability. [1]In percentage of dry matter. P: statistical probability. CV: coefficient of variation.

Considering that ractopamine may alter the animal metabolism, improving their feed conversion, changes in the composition of manure can be expected since feed polymers were better used. The lower ammoniacal N concentrations may be explained by the manure composition, which depends on the use of nutrients in the feed consumed by the animals. The longer period of animals supplementation with ractopamine could lead to the production of manure with lower nutrient concentrations, such as nitrogen compounds which, when degraded, may form ammonium ions or free ammonia. Thus, prolonged animal supplementation may contribute to lower ammoniacal N concentration in the biodigester effluent.

Thus, supplementation with ractopamine can be seen not only as a nutritional tool to increase animal performance, but also as a mitigation tool of the manure environmental impact since it provides the decrease of N content excreted in the manure, and consequently its volatilization, and in biofertilizers, when the manure is treated in biodigesters.

In this study, the results of pH and ammoniacal N as indicator parameters of the process balance and stability were satisfactory, offering no collapse risks for the anaerobic biodigestion process.

The different periods of animal supplementation with ractopamine did not interfere in the average concentrations of total N and P of the affluents and effluents from the biodigesters, being 2.46 and 3.55% of N and 2.80 and 4.70% of P, respectively.

Although the amount of N was not different in the affluents and effluents, the ammoniacal N concentrations differed. This fact is due to the ammoniacal N formation from different N sources in the manure (organic and inorganic N). Ammoniacal N formation occurs mainly in an anaerobic environment and remains dissolved in the manure liquid phase; in pig manure, the ammoniacal N concentrations are high since the animals receive high amounts of organic N in their diets, being more readily converted into ammoniacal N (Christensen and Sommer, 2013).

Probably the differences among the ammoniacal N concentrations in the manure were due to differences in the relations ammoniacal N: total N according to different periods in which the animals were supplemented with ractopamine, which may be related to the use of N from the diet.

There was difference (P <0.05) in the daily cumulative production of biogas according to different periods of supplementation (Table 4). In addition, although the highest VS levels in the manure have been observed for the 21-day supplementation period, the animals supplemented for a longer period (28 and 35 days) showed higher biogas production; the lowest productions were obtained from manure of animals non-supplemented or supplemented with 7 days before slaughtering, with no difference between these periods.

Once the volatile solids include two fractions, the biodegradable and refractory (Kangle et al., 2012), being the biodegradable fraction related to the organic matter contained in the manure and that can be transformed into biogas by the methanogenic bacteria, biogas production were consistent with the VS reductions, despite they were not significant. The results suggest that by improving use-efficiency of nutrients, the manure quality is also improved, resulting in higher biogas production.

TABLE 4. Regression and statistics equations of the daily accumulated biogas production (y) from manure of pigs supplemented with ractopamine over different periods (x).

Supplementation period	Equation	P value	R^2	Average estimation (m^3)	CV (%)
0 day	Y= 0.00063+0.00120x	<0.0001	0.99	0.0006 d	6.92
7 days	Y= 0.00001+0.00122x	<0.0001	0.98	0.0086 d	7.79
14 days	Y= 0.00070+0.00093x	<0.0001	0.98	0.0138 c	7.96
21 days	Y= 0.00074+0.00114x	<0.0001	0.99	0.0247 b	6.27
28 days	Y= -0.00015+0.00216x	<0.0001	0.99	0.0603 a	6.27
35 days	Y= 0.00077+0.00204x	<0.0001	0.99	0.0722 a	5.15

P: statistical probability. R^2: coefficient of determination. Means followed by the same letter in the column do not differ by the t test at 5% probability. CV: coefficient of variation.

In addition to the benefits of animal performance reported in the literature, ractopamine supply for long periods can add other benefits related to lower nutrient excretion and greater biogas production when the anaerobic biodigestion process is adopted as a manure treatment system.

CONCLUSIONS

The period of 28 days of supplementation with 20-ppm ractopamine is the most recommended since it combines good animal performance, manure production with lower N content, and increased biogas production by the manure.

The anaerobic biodigestion efficiency, in terms of pH, ammoniacal N and solids reduction, is not affected by the pig diet supplementation with 20-ppm ractopamine.

ACKNOWLEDGEMENTS

The authors want to thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES* (Coordination for the Improvement of Higher Education Personnel - CAPES) for granting a Master's scholarship and to the *Fundação de Apoio ao Desenvolvimento do Ensino e a Ciência e Tecnologia do Estado de Mato Grosso do Sul – FUNDECT* (Foundation of Support for Education, Science and Technology of the Mato Grosso do Sul State - FUNDECT) for financial support.

ETHICS COMMISSION: Proceeding No. 425/2012, filed with the Ethics Committee on Animal Use of the Universidade Federal de Mato Grosso do Sul (Federal University of Mato Grosso do Sul) - CEUA/ UFMS.

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