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ANIMAL PRODUCTION

Anaerobic digestion of manure from laying hens fed diets containing different mineral sources and rosemary oil levels

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ABSTRACT: This study evaluated the stability of anaerobic digestion and biogas production from the manure of laying hens fed diets containing different mineral sources and rosemary oil concentrations. Manure was obtained from 288 semi-heavy laying hens, 30 weeks old, and collected for 112 days. A completely randomized design was used in a 2×3 factorial scheme with repeated measures, considering two mineral sources (inorganic and organic) and three concentrations of rosemary oil (0, 100, and 200 mg kg^{-1}). Twelve continuous bench digesters (7.5 kg) were operated with a hydraulic retention time of 30 days and daily loads of 5% total solids, for 90 days. A weekly analysis of influents and effluents was performed by measuring the values of total solids (TS), volatile solids (VS), pH, ammonia nitrogen (ammonia N), intermediary alkalinity (IA), partial alkalinity (PA), and the relationship IA:PA; biweekly analysis of total nitrogen (N) and total phosphorus (P) concentrations; and daily measurement of biogas production data to calculate the biogas production potential of the manure. Manure from laying hens fed with organic minerals has greater potential for biogas production and greater TS and VS reduction than manure from hens supplemented with inorganic minerals. Laying hen diets containing up to 200 mg kg^{-1} of rosemary oil do not present the risk of anaerobic digestion failure.
Key words: biogas, continuous digesters, *Rosmarinus officinalis.*

Digestão anaeróbia de dejetos de poedeiras alimentadas com dietas contendo diferentes fontes minerais e níveis de óleo de alecrim

RESUMO: O objetivo deste estudo foi avaliar a estabilidade no processo de digestão anaeróbia e a produção de biogás dos dejetos de poedeiras alimentadas com dietas contendo diferentes fontes de minerais e níveis de óleo de alecrim. Os dejetos foram provenientes de 288 poedeiras semipesadas, com 30 semanas de idade, colhidos por 112 dias. Utilizou-se o delineamento inteiramente casualizado com esquema fatorial 2x3, com medidas repetidas no tempo, sendo as dietas com duas fontes minerais (inorgânica e orgânica) e três níveis de óleo de alecrim (0, 100 e 200 mg kg^{-1}). Foram utilizados 12 biodigestores contínuos (7,5 kg), operados com tempo de retenção hidráulica de 30 dias, com cargas diárias com 5% de sólidos totais, por 90 dias. Foram realizadas análises semanais do afluente e efluente mensurando-se os valores de sólidos totais (ST), sólidos voláteis (SV), pH, nitrogênio amoniacal (N amoniacal), alcalinidade parcial (AP), alcalinidade intermediária (AI) e a relação AP:AI; quinzenalmente análises das concentrações de nitrogênio total (N) e de fósforo total (P) e diariamente mensuradas as produções de biogás para calcular o potencial de produção de biogás do dejeto. Dejetos de poedeiras alimentadas com minerais orgânicos apresentam maior potencial de produção de biogás, maiores reduções de ST e SV em relação àquelas alimentadas com minerais inorgânicos. A adição de óleo de alecrim na dieta de poedeiras não oferece riscos de falência para o processo de digestão anaeróbia.
Palavras-chave: biodigestores contínuos, biogás, *Rosmarinus officinalis*.

INTRODUCTION

Minerals play a fundamental role in all biochemical processes, metabolism, and other vital functions required for maintaining growth and production in poultry (RICHARDS et al., 2010). Unlike inorganic minerals, organic minerals are easily absorbed and stored by birds because they do not dissociate at acidic pH and remain intact through chemical reactions and interactions with other molecules in the intestine. They remain electrically neutral, and even during the process of ionic competition, no insoluble complexes are formed in the lumen, which favors their absorption and increases their bioavailability compared to inorganic sources (SWIATKIEWICZ et al., 2014). Additionally, the use of organic minerals can reduce the excretion of potentially polluting minerals into the environment (CARVALHO et al., 2015).

In turn, aromatic plants can be used as growth promoters, capable of improving the digestion and absorption of nutrients by acting as antimicrobials and favoring the animal's immune

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system (KOIYAMA et al., 2014; MADRID-GARCÉS et al., 2017). Improving digestion and absorption can also contribute to the reduction of nutrient excretion and the negative environmental impacts of animal waste.

One alternative for mitigating the environmental impacts of animal waste is anaerobic digestion, which also has the advantage of generating biogas and biofertilizers that can be used as promoters for animal production. However, anaerobic digestion can be influenced by factors such as: physicochemical quality of the manure, presence of inhibitory compounds, and pH (AMARAL et al., 2019).

Therefore, this study was to evaluate the stability of the anaerobic digestion process and the production of biogas from the waste of commercial layers fed with diets containing different sources of minerals and three concentrations of rosemary oil (*Rosmarinus officinalis*).

MATERIALS AND METHODS

We used 288 semi-heavy Brown Hy line layers at 30 weeks of age. Two birds were housed in each galvanized wire cage measuring 25 × 40 × 45 cm, in a conventional laying house with fiber cement roofing tiles. Trough-type drinking fountains were used, providing running water along the entire front of the cages.

The experimental diets were isonutritive, formulated from corn and soybean meal (Table 1), according to ROSTAGNO et al. (2011). The minerals used in organic form were: Cu, Fe, Mn, Zn (metal - amino acid complex) and Se (yeast selenium). The Rosemary oil supplementation was carried out together with mineral and vitamin supplementation. The diet was provided ad libitum.

The excreta were collected on tarpaulins placed under the cages once a week for 24 h, with a collection period of 112 days, totaling 16 weeks. After weighing and identification, the manure was stored in a freezer at -12 °C for subsequent anaerobic digestion tests.

The manure had an average TS content of 22.27% and a VS content of 16.92%. It was further diluted so that the biodigester effluents contained 5% TS. Each biodigester received 1,450 kg of manure and 6,050 kg of water, and the start-up time was 30 days. Subsequently, the biodigesters were operated with daily loads of 0.250 kg, containing 0.057 kg of manure and 0.193 kg of water, for 90 days.

During the test, daily samples of the effluent were collected to determine the TS and VS levels, following the methodology described by APHA/AWWA/AEF (2023). The effluent was analyzed weekly, and the pH values were measured using a digital potentiometer. Ammoniacal nitrogen (N ammoniacal) was determined according to the methodology described by APHA/AWWA/AEF (2023), and partial alkalinity (PA) concentrations, intermediate alkalinity (IA), and total alkalinity (TA) were determined according to RIPLEY et al. (1986) and JENKINS et al. (1991). The tributaries and effluents were analyzed for the concentration of total nitrogen (N) using the semi-micro Kjeldahl method while the concentration of total phosphorus (P) was measured using the colorimetric method, according to APHA/AWWA/AEF (2023).

Biogas production was calculated based on the daily displacement of the gasometer (measured using a 50 cm ruler) and the area of the biodigester (0.00785 m2). After reading the volume, the temperature was measured using a digital thermometer placed at the biogas outlet. Using Excel® spreadsheets, the volumes of biogas were corrected for normal temperature and pressure conditions (1 atm and 25 ºC).

At the beginning of the anaerobic digestion test, biogas burning tests were performed daily, and after 12 d, all treatments began showing biogas burning. The potential biogas production per kilogram of added manure (MN) was calculated using the total biogas production data for each treatment and the quantities of manure, TS, and VS added to the biodigesters. The values were expressed in m^3 of biogas per kg of manure, TS, or VS added. A completely randomized 2 × 3 factorial design was adopted, and the two experimental units received the same treatment (manure from the same diet). The treatments consisted of combinations of two mineral sources (inorganic and organic) and three concentrations of rosemary oil (0, 100, and 200 mg kg^{-1}). The data were statistically analyzed using the free software R in a repeated-measures scheme over time (weeks). Subsequently, means were compared using Tukey's test at a 5% significance level.

RESULTS AND DISCUSSION

There was no significant effect of combining ($P > 0.05$) the mineral sources with the concentrations of rosemary oil supplied to the animal diet on the PA and IA values of the

Table 1 - Percentage and calculated composition of the experimental diets.

Ingredients	Inorganic mineral			Organic mineral		
	--------------------------------------Rosemary oil, mg kg^{-1}--------------------------------------					
	0	100	200	0	100	200
Corn		62.08			62.08	
Soybean meal, 45%		25.34			25.34	
Soybean oil		0.45			0.45	
Calcitic limestone		9.97			9.97	
Bicalcium phosphate		1.09			1.09	
L-Lysine HCl		0.01			0.01	
DL-Methionine		0.22			0.22	
Salt		0.49			0.49	
Mineral and vitamin supplements		0.15			0.35	
Inert		0.20			0.00	
Calculated values						
Metabolizable energy, Kcal kg^{-1}		2.750			2.750	
Crude protein, %		17.00			17.00	
Digestible methionine + cystine, %		0.704			0.704	
Dibestible lysine, %		0.774			0.774	
Calcium, %		4.20			4.20	
Available phosphorus, %		0.30			0.30	
Linoleic acid, %		1.60			1.60	

Composition per kg of diet: vitamin A, 7,500 UI; vitamin D_3, 2,000 UI; vitamina E, 10 UI; vitamina K_3, 1.8 mg; nicotinic acid, 25 mg; pantothenic acid, 10 mg; vitamin B_6, 1.7 mg; vitamin B_{12}, 0.0013 mg; biotin, 0.05 mg; choline, 220 mg; Cu, 11 mg; Fe, 35 mg; I, 1.1 mg; Mn, 77 mg; Se, 0.33 mg; Zn, 72 mg.

biodigester effluent (Table 2). In isolation, the mineral sources in the diet interfered with the PA and IA values of the tributaries. Therefore, the inorganic minerals supplied in the diet increased these parameters without changing the IA: PA ratio in the affluents. The differences between manure from animals that received organic and inorganic minerals may be linked to the bioavailability of nutrients during the digestion and absorption processes in animals. Since the nutrients in the diet are well utilized by animals, manure may contain lower amounts of nutrients (MUTUNGWAZI et al., 2023) and, when they are part of biodigester effluents, they vary in behavior during the anaerobic digestion process.

The PA and IA in the effluent from the biodigesters were influenced by the combination of mineral source and rosemary oil concentrations ($P < 0.05$). The highest average PA occurred when manure was obtained from animals that received inorganic minerals and 100 mg of rosemary oil per kilogram of diet. Similarly, the IA was higher when the manure came from animals fed diets containing inorganic minerals and rosemary oil (100 or 200 mg kg^{-1}). The IA:PA ratio was influenced only by the mineral source in the animal diet; the inorganic diet provided higher IA:PA ratios. However, the lower the IA:PA ratio, the better the conditions for anaerobic digestion, owing to the presence of a greater amount of PA. Despite this difference, both mineral sources showed IA:PA ratios close to ideal for proper development of the anaerobic digestion process, which is 0.3 (AMARAL et al., 2019).

There was no significant effect of diet ($P > 0.05$) on the pH of the biodigester effluents and tributaries (Table 3). The initial pH values were suboptimal, and despite the tributaries not being corrected, the ecosystems inside the biodigesters were balanced in terms of the production and consumption of volatile acids, resulting in increased pH values in the effluent. This is corroborated by the low IA:PA ratios in the effluents.

The concentration of ammoniacal N in the effluent containing waste from animals that received more rosemary oil and organic minerals from their diet was higher than that of inorganic minerals. In the effluent, 100 mg kg^{-1} of rosemary oil in the diet

Table 2 - Partial alkalinity (PA), intermediate alkalinity (IA) and intermediate alkalinity: partial alkalinity ratio (IA:PA) in the affluents and effluents of continuous biodigesters operated with manure from layers fed different sources of minerals (M) and levels of rosemary oil (RO).

Mineral	---------------Rosemary oil, mg kg^{-1}--------------				----------------------------P value----------------------------			CV, %
	0	100	200	Média	M	RO	M*RO	
---Affluent PA mg $CaCO_3$ L^{-1}---								
I	24.75	25.62	50.37	33.58A				
O	23.63	0.00	6.50	10.04B	0.008	0.301	0.126	14.39
Mean	24.19	12.81	28.44	21.81				
---Effluent PA mg $CaCO_3$ L^{-1}---								
I	7108	7119[a]	6956	7061				
O	6872	6536B	7056	6821	0.022	0.290	0.030	5.59
Mean	6990	6827	7006	6941				
---Affluent IA mg $CaCO_3$ L^{-1}---								
I	3029.1	2927.8	2799.9	2928.9A				
O	2640.1	2469.4	2704.6	2604.7B	<0.001	0.214	0.052	10.02
Mean	2834.6	2698.6	2752.4	2761.8				
---Effluent IA mg $CaCO_3$ L^{-1}---								
I	2568.3	2751.1A	2800.4A	2706.6				
O	2492.9	2281.5B	2313.1B	2362.5	<0.001	0.900	0.044	12.31
Mean	2530.6	2516.3	2556.8	2534.54				
---Affluent IA:PA---								
I	32.78	33.76	20.09	28.86				
O	27.55	0.00	26.31	17.95	0.223	0.476	0.176	13.93
Mean	30.16	16.88	23.30	23.41				
---Effluent IA:PA---								
I	0.36	0.39	0.40	0.39A				
O	0.37	0.35	0.33	0.35B	0.004	0.903	0.058	13.18
Mean	0.36	0.37	0.37	0.37				

I: inorganic; O: organic. CV: coefficient of variation.
Different letters in the same column differ according to the Tukey test ($P < 0.05$).

containing inorganic mineral provided a higher concentration of ammoniacal N than that containing organic mineral.

It is worth noting that rosemary oil and mineral sources do not contribute nitrogenous compounds to the diet, but because of their nature, they can contribute to variations in the quality of manure and, therefore, in the affluents studied. This was reflected in the statistical differences found in the concentrations of ammoniacal N.

The ammoniacal N resulting from the degradation of nitrogen compounds during the anaerobic digestion process is beneficial and serves as a source of nitrogen and a buffering agent, avoiding pH changes. However, high concentrations inhibit this process because they are toxic to methanogenic archaea (AMARAL et al., 2019; MUTUNGWAZI et al., 2023). According to CHEN et al. (2008), ammoniacal N concentrations of up to 1,000 mg L^{-1} may have no adverse effects on anaerobic processes.

Mineral sources did not affect the total N concentration in the biodigester effluents and tributaries ($P > 0.05$) (Table 4). However, the inclusion of the highest concentration of rosemary oil in the diet of animals favored a higher concentration of total N in the biodigester effluents than in those containing waste from animals that did not receive rosemary oil. As previously stated, mineral sources and rosemary oil do not provide nitrogen compounds, but the differences in total N concentrations may also be due to problems often observed in experimental setups such as: the possible entry of eggs into the collection system, the addition of nitrogen compounds, and masking the true total N content of the manure.

Biodigester effluents containing layer manure with lower TS levels (from 2.9 to 4.3%) were

Table 3 - Average pH and N-ammonia concentrations in the affluents and effluents of continuous biodigesters operated with manure of layers fed different sources of minerals (M) and levels of rosemary oil (RO).

Mineral	-----RO, mg kg^{-1}-----				-----P value-----			CV, %
	0	100	200	Mean	M	RO	M*RO	
-----Affluent pH-----								
I	5.74	5.73	5.72	5.73				
O	5.73	5.65	5.71	5.69	0.260	0.502	0.613	1.86
Mean	5.73	5.69	5.71	5.71				
-----Effluent pH-----								
I	7.20	7.20	7.19	7.20				
O	7.20	7.18	7.18	7.18	0.649	0.929	0.917	0.98
Mean	7.20	7.19	7.18	7.19				
-----N-amoniacal mg L^{-1} affluent-----								
I	558.6	599.4	484.8B	547.6				
O	594.9	511.9	624.8A	577.2	0.249	0.725	0.002	17.24
Mean	576.8	555.6	554.8	562.38				
-----N-amoniacal mg L^{-1} effluent-----								
I	2681.5	2753.0A	2786.6	2749.4				
O	2550.0b	2550.1bB	2701.1a	2638.4	<0.001	<0.001	<0.001	3.61
Mean	2615.8	2651.6	2743.9	2634.4				

I: inorganic; O: organic. CV: coefficient of variation.
Lowercase letters in the same row and uppercase letters in the same column differ according to Tukey's test (P < 0.05).

used by FARIAS et al. (2012) in batch biodigesters with lower percentages of total N, from 2.3–3.0%, without harming the anaerobic digestion process. This suggests that to avoid the risk of biodigester failure due to ammonia inhibition, manure with high levels of total N can be diluted further.

The different mineral sources and concentrations of rosemary oil in the animal diets did not affect the concentration of total P in the effluents (P > 0.05). In the effluents, inorganic minerals contributed to higher concentrations of total P. As there was no difference in the initial concentration of this element, and it was not lost in the biogas, it was assumed that the difference between the values might be due to precipitation in the biodigesters.

Effluents containing manure from animals fed diets containing organic minerals showed greater reduction in TS and VS (P < 0.05) than those from animals fed diets containing inorganic minerals (Table 5). These reductions indicated that anaerobic digestion was efficient in degrading organic compounds. The high reduction in VS is linked to the high biogas production that occurred in this study because VS is the organic matter present in the waste, which can be transformed by methanogenic archaea (WANG et al., 2018). Lower reductions of 61.96% for TS and 79.94% for VS were reported by FARIAS et al. (2012).

There was no interaction (P > 0.05) between the factors studied for biogas production; however, there was an isolated effect of mineral sources (P < 0.05) on biogas production (Table 6). Manure from layers fed with organic minerals showed higher (P < 0.05) total biogas production, whereas the concentrations of rosemary oil had no effect (P > 0.05) on biogas production.

An interaction (P < 0.05) was observed for the potential biogas production per kilogram of manure between mineral sources and rosemary oil concentrations, where the organic mineral at zero concentrations of rosemary oil showed a higher manure potential than the inorganic mineral at the same concentration. There was no effect (P > 0.05) of diet on the biogas production potential per kg of TS or VS added.

As observed, the use of organic minerals resulted in higher total biogas production and higher biogas production potentials per kilogram of manure, TS end VS, as a result of the manure containing more organic matter, resulting in an increase in VS. Microminerals in the form of an organic complex or chelates, proteinates or polysaccharides have greater biological value

Table 4 - Average concentrations of total N and total P, as a percentage of total solids, in the affluents and effluents of continuous biodigesters operated with manure from layers fed mineral sources (M) and rosemary oil (RO).

Mineral	--------------------RO, mg kg^{-1}--------------------				----------------------------P value----------------------------			CV, %
	0	100	200	Mean	M	RO	M*RO	
Total N affluent								
I	5.14	5.17	5.95	5.42				
O	5.10	5.38	5.43	5.30	0.381	0.004	0.085	8.76
Mean	5.12b	5.28ab	5.69a	5.36				
Total N effluent								
I	2.10	2.09	2.33	2.17				
O	2.14	2.05	1.96	2.05	0.112	0.742	0.076	11.23
Mean	2.12	2.07	2.14	2.11				
Total P affluent								
I	2.54	2.54	2.33	2.47				
O	2.48	2.29	2.45	2.41	0.248	0.150	0.181	7.35
Mean	2.51	2.41	2.39	2.44				
Total P effluent								
I	1.72	2.35	2.36	2.14A				
O	1.51	1.52	1.53	1.52B	<0.001	0.057	0.072	27.57
Mean	1.62	1.93	1.95	1.83				

I: inorganic; O: organic. CV: coefficient of variation.
Lowercase letters in the same row and uppercase letters in the same column differ according to Tukey's test ($P < 0.05$).

(ARAÚJO et al., 2019), resulting in greater bioavailability and utilization by the animal and are therefore less excretable (SWIATKIEWICZ et al., 2014). It is likely that organic minerals in the diets contributed to poultry waste being less prone to conditions that form compounds that inhibit anaerobic digestion or even increase the availability of nutrients in the waste for microorganisms involved in the process.

CONCLUSION

The utilization of organic and inorganic mineral sources, as well as the addition of up to 200 mg kg^{-1} of rosemary oil to laying hen feed, did not pose a risk of failure in the anaerobic digestion process. Waste from laying hens fed with organic minerals has a greater potential for biogas production.

Table 5 - Reductions in total solids (TS) and volatile solids (VS), in percentages, obtained in the anaerobic digestion of layer manure fed with different sources of minerals (M) and levels of rosemary oil (RO).

Mineral	--------------------RO, mg kg^{-1}--------------------				----------------------------P value----------------------------			CV, %
	0	100	200	Mean	M	RO	M*RO	
TS reduction, %								
I	83.16	80.34	79.95	81.15B				
O	84.56	85.93	85.76	85.42A	<0.001	0.680	0.121	4.77
Mean	83.86	83.13	82.86	83.28				
VS reduction, %								
I	89.92	87.71	86.83	88.15B				
O	89.48	91.11	90.59	90.39A	0.005	0.549	0.052	3.26
Mean	89.70	89.41	88.71	89.27				

I: inorganic; O: organic. CV: coefficient of variation.
Different letters in the same column differ according to the Tukey test ($P < 0.05$).

Table 6 - Total biogas production over 90 days of daily loading and biogas production potential of manure from continuous biodigesters operated with layer manure fed various mineral sources (M) and rosemary oil levels (RO).

Mineral	-------------------RO, mg kg^{-1}	-------------------			---------------------------P value	---------------------------		CV, %
	0	100	200	Mean	M	RO	M*RO	
---------------Total biogas production (m^3)---------------								
I	0.16	0.16	0.16	0.16B				
O	0.18	0.17	0.17	0.18A	0.011	0.973	0.396	4.47
Mean	0.17	0.17	0.17	0.17				
---------------Biogas production potential of manure (m^3 kg^{-1})---------------								
I	0.046b	0.049a	0.050a	0.048				
O	0.054	0.054	0.051	0.053	0.001	0.373	0.035	6.14
Mean	0.050	0.051	0.051	0.051				
---------------Potential of added TS (m^3 kg^{-1})---------------								
I	0.007	0.007	0.007	0.007				
O	0.008	0.008	0.008	0.008	0.267	1.00	1.00	15.57
Mean	0.008	0.008	0.008	0.008				
---------------Potential of added VS (m^3 kg^{-1})---------------								
I	0.009	0.009	0.009	0.009				
O	0.011	0.010	0.010	0.010	0.154	0.850	0.850	13.48
Mean	0.010	0.010	0.010	0.010				

I: inorganic; O: organic; TS: total solids; VS: volatile solids. CV: coefficient of variation.
Different letters in the same column differ according to the Tukey test ($P < 0.05$).

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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