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Qualitative and quantitative characterization of waste layers fed diets containing mineral sources and rosemary oil levels

Caracterização qualitativa e quantitativa dos dejetos de poedeiras comerciais alimentadas com dietas contendo minerais e óleo de alecrim

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Abstract: This study aimed to evaluate the effects of mineral sources and rosemary oil levels in the diet of commercial layers on the qualitative and quantitative characteristics of the waste generated. Manure was obtained from 288 commercial laying hens of the HyLine Brown lineage (semi-heavy layers) at 30 weeks of age over a period of 112 d (four cycles of 28 d). A completely randomized design was used with a 2×3 factorial scheme with repeated measures over time (16 repetitions), with two mineral sources (inorganic and organic) and three levels of rosemary oil (0, 100, and 200 mg kg^{-1}). The waste was collected weekly, weighed, and the calculations of waste production, residue coefficient (RC) were performed. Also analyses of total solids (TS), volatile solids (VS), pH, macro-, and micro-minerals were performed. Mineral sources affect the quality characteristics of the manure. The amount of rosemary oil affected the quantitative and qualitative characteristics of the waste generated. It was concluded that diets containing organic minerals resulted in waste with less polluting power, as it had a lower TS, pH, total N, and total P values. Rosemary oil increased manure production in natural matter (NM) and increased the TS and VS values in the manure layer, causing a greater environmental impact.

Keywords: additives; organic mineral; residue; *Rosmarinus officinalis*

Resumo: Realizou-se este estudo com o objetivo de avaliar fontes de minerais e de níveis do óleo de alecrim na dieta de poedeiras comerciais sobre as características qualitativas e quantitativas dos dejetos gerados. Os dejetos foram provenientes de 288 poedeiras comerciais da linhagem HyLine Brown (poedeiras semipesadas) com 30 semanas de idade, durante o período de 112 dias (4 ciclos de 28 dias). Foi utilizado delineamento inteiramente casualizado com esquema fatorial 2x3 com medidas repetidas no tempo (16 repetições), sendo duas fontes minerais (inorgânica e orgânica) e três níveis de óleo de alecrim (0, 100 e 200 mg kg^{-1}). Semanalmente os dejetos foram coletados, pesados e realizados os cálculos de produção de dejetos, coeficiente de resíduo (CR) e as análises de sólidos totais (ST), sólidos voláteis (SV), pH, macro e microminerais. As fontes de minerais afetaram as características qualitativas dos dejetos. Os níveis de óleo de alecrim afetaram as características quantitativas e qualitativas do dejeto gerado. Concluiu-se que dietas contendo mineral orgânico resultam em dejetos com menor poder poluente por apresentar menores valores de ST, pH, N total e P total. O óleo de

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alecrim aumenta a produção de dejetos na matéria natural (MN) e aumenta os valores de ST e SV nos dejetos das poedeiras causando maior impacto ambiental.

Palavras-chave: aditivos; mineral orgânico; resíduo; *Rosmarinus officinalis*

1. Introduction

Chelated minerals have been described as a bottleneck in poultry farming because they promote a greater absorption of nutrients owing to their high bioavailability, which can improve poultry performance [(1)]. These minerals are a mixture of elements that can be bound to amino acids or polysaccharide molecules and can be included in small quantities in the diet without negatively affecting performance or reducing environmental pollution by reducing mineral excretion [(2)].

Similarly, aromatic plants have also been studied with the aim of improving nutrient utilization by promoting better intestinal health in poultry [(3)]. *Rosemary (Rosmarinus officinalis*) contains phenolic compounds, such as caffeic acid, carnosol, carnosic acid, and rosmarinic acid, which are capable of promoting antioxidant activity, modifying intestinal microbiota, and improving digestibility and nutrient absorption by stimulating the synthesis of digestive and pancreatic enzymes [(4)].

Poultry production generates large amounts of waste daily because of the high density of housing, which, in turn, can have a negative impact on the environment if this situation is managed and treated badly through the excessive excretion of nitrogen, phosphorus, and other minerals into the environment. This would lead to the deterioration of aquatic systems, greenhouse gas emissions, and ammonia responsible for the eutrophication and acidification of ecosystems [(5)].

The digestibility, availability, and level of nutrients in the diet can influence the amount of nutrients excreted by birds. In this way, the quantification and characterization of manure provide important information for directing the management and nutritional strategies to be adopted, making it possible to make intelligent decisions regarding their applicability [(6)]. In addition, there is no information in the literature on the combined use of different mineral sources and rosemary oil for layers, and their impact on waste. Therefore, this study has aimed to evaluate the effects of two mineral sources and different levels of rosemary oil in the diets of commercial layers on the qualitative and quantitative characteristics of waste generated during production.

2. Material and Methods

The experiment was conducted in the laying poultry sector and the Animal Waste Laboratory at the State University of Mato Grosso do Sul/Aquidauana/MS, latitude 20º28'S, longitude 55º48'W and with an altitude of 184 meters.

The waste came from 288 commercial layers of the Hyline Brown strain (semi-heavy layers) at 30 weeks of age over a period of 112 days (four 28-day cycles). The birds were

housed two-by-two in galvanized wire cages with four rooms measuring 25×40×45 cm in a conventional experimental laying house with a fiber cement roof.

A trough-type drinking fountain was used, with water running along the entire front of the cages. The feeders were placed under the drinkers, with one for each experimental unit. The drinkers were washed daily and the birds were fed *ad libitum*.

A completely randomized design was used, with a 2×3 factorial scheme consisting of two mineral sources (inorganic and organic) and three concentrations of rosemary oil (0, 100, and 200 mg kg^{-1}), with repeated measures over time. The repetitions consisted of 16 weeks, during which the waste was collected (16 weeks). The experimental diets were based on corn and soybean meal and they were formulated to be isonutritive (Table 1) to meet the nutritional requirements of the birds according to the nutritional requirement tables [7]. The minerals used in organic form were Cu, Fe, Mn, Zn (metal-amino acid complex), and Se (yeast selenium). Rosemary oil was supplemented by adding mineral and vitamin supplements to the diets.

The different levels of inclusion of mineral supplements containing inorganic or organic minerals were due to the differences in the mineral concentrations of the sources used. The birds were adapted to the following experimental diets for 15 days: D1, diet with inorganic minerals (conventional) without rosemary oil; D2, diet with inorganic minerals and rosemary oil (100 mg kg^{-1}); D3, diet with inorganic minerals and rosemary oil (200 mg kg^{-1}); D4, diet with organic minerals without rosemary oil; D5, diet with organic minerals and rosemary oil (100 mg kg^{-1}); and D6, diet with organic minerals and rosemary oil (200 mg kg^{-1}).

In order to collect the waste, plastic sheets were placed under the cages every week for 24 h. To determine the dry matter (DM) and natural matter (NM) production of manure, excess broken eggs, feathers, and feed were removed and weighed. The hydrogenionic potential (pH) of the "in natura" manure was determined and the total solids (TS) and volatile solids (VS) and macro and micro-minerals contents of each sample were then analyzed.

Table 1 Percentage and calculated composition of the baseline experimental diets

Ingredients, %	Inorganic mineral**	Organic mineral**
Corn	62.08	62.08
Soybean meal, 45%	25.34	25.34
Soybean oil	0.45	0.45
Limestone	9.97	9.97
Dicalcium phosphate	1.09	1.09
L-lysine HCl	0.01	0.01
DL-methionine	0.22	0.22
Salt	0.49	0.49
Mineral/vitamin premix*	0.15	0.35
Inert (kaolin)	0.20	0.00
Nutritional composition calculate		
Metabolizable energy, Kcal/kg	2.750	2.750
Crude protein, %	17.00	17.00

Digestible Meth + cyst, %	0.704	0.704
Digestible lysine, %	0.774	0.774
Calcium, %	4.200	4.200
Disponible phosporus, %	0.300	0.300
Linoleic acid, %	1.600	1.600

**Composition per kg of diet: Vitamin A, 7,500 IU; Vitamin D_3, 2. 000 IU; Vitamin E, 10 IU; Vitamin 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.*
*** Rosemary oil (0, 100 and 200 mg kg^{-1}) was added on top and mixed with the mineral/vitamin premix.*

The total solid (TS) and volatile solid (VS) contents were determined according to the methodology described in APHA [8]. The samples of the manure collected were taken to an oven with forced air circulation, at a temperature of 65°C until they reached a constant weight to obtain the TS content. To determine VS, the oven-dried material was taken to the muffle furnace in previously weighed porcelain crucibles and kept at a temperature of 575°C for 2 hours. The resulting material was weighed on an analytical balance (±0.0001g) in order to obtain the weight of ash or mineral matter.

Manure production, expressed in kg TS $bird^{-1}$ day^{-1}, was calculated using manure weighing data (kg), number of birds housed, number of days, and TS content found in the manure as follows: TS production.$bird^{-1}$. dia^{-1} = [(manure weight (kg)/bird)/day) × TS(%). To verify the efficiency of the animals in transforming the feed into a final product (kg eggs) to the detriment of the excreted product, the residue coefficient (RC) was calculated, by considering the total amount of manure produced (dry basis) in relation to mass egg production (kg kg^{-1}).

For the quantification of macro-and micronutrients, the samples were subjected to nitroperchloric digestion, and the extracts obtained were used to determine the levels of C, Ca, Mg, Fe, Mn, Cu, and Zn using an atomic absorption spectrophotometer, according to the methodology described by Bataglia et al. [9]. Total N was obtained using a micro Kjeldahl distiller, according to the methodology described by Silva and Queiroz [10] and total P levels were determined using a colorimetric method, according to the methodology described by Malavolta et al. [11]. The data were statistically analyzed using the free R software, and the means were compared using Tukey's test at a 5% significance level.

3. Results and Discussion

The mineral sources and levels of rosemary oil did not affect (P>0.05) manure production in DM and the RC (P>0.05; Table 2). For the production of droppings in the natural matter (NM), there was an interaction (P<0.05) between the factors studied, in which the diet with inorganic minerals associated with 200 mg kg^{-1} of rosemary oil promoted a greater production of droppings in NM (0.118 kg $bird^{-1}$ day^{-1}).

Table 2 Manure production (DM and MN) and residue coefficient (RC) by commercial layers fed mineral (M) sources and rosemary oil (RO) levels

Variables	Mineral	RO levels, mg kg^{-1}				Valor de P			CV, %
		0	100	200	Média	M	RO	M x RO	
Production kg day^{-1} bird^{-1} (DM)	Inorganic	0,025	0,025	0,027	0,026	0,689	0,123	0,118	13,99
	Organic	0,025	0,026	0,025	0,025				
	Mean	0,025	0,025	0,026	0,026				
Production kg day^{-1} bird^{-1} (MN)	Inorganic	0,111b	0,107b	0,118a	0,112	0,381	0,041	0,017	11,13
	Organic	0,109	0,117	0,114	0,114				
	Mean	0,110	0,112	0,116	0,113				
RC (kg of manure kg eggs^{-1})	Inorganic	0,460	0,450	0,480	0,470	0,869	0,843	0,268	6,40
	Organic	0,460	0,480	0,460	0,460				
	Mean	0,460	0,465	0,470	0,465				

Different letters on the same line differ according to the Tukey test (P<0.05).

It can be inferred that the observed results were lower than those found by Garcia et al. [12], who observed an average fresh dropping production of 0.136 kg bird^{-1} day^{-1} in commercial layers. On the other hand, the production of manure in NM was found to be higher than that found by Augusto & Kuntz [13] whose study involved working with the manure of layers in different rearing systems (0.06 kg bird^{-1} day^{-1}). According to Augusto and Kuntz [13], for layers reared in a conventional system, the average production was 0.017 kg of waste per bird day^{-1}, which was lower than that found in this study. On the other hand, the RC value obtained in this study (0.46 kg of manure kg eggs^{-1}) was close to that found by Orrico Jr. et al.[14] whose study involved working with manure from commercial layers (0.40 kg of manure kg eggs^{-1}), as well as by Garcia et al. [12] who found a value of 0.474 kg of manure kg eggs^{-1}.

There was no significant interaction (P>0.05) between the mineral sources and rosemary oil levels on TS, VS, and total N. Different mineral sources did not affect the composition of the fresh bird waste (P>0.05). However, there was an isolated effect between the levels of rosemary oil on TS and VS (Table 3), with levels of 100 and 200 mg kg^{-1} showing the highest TS values of 22.67 and 22.40%, respectively. The 100 mg kg^{-1} level had the highest VS content of 17.27%.

Table 3 Physico-chemical characteristics of the waste of commercial layers fed diets containing mineral (M) sources and levels of rosemary oil (RO)

Variables	M	RO levels, mg kg^{-1}				P value			CV, %
		0	100	200	Mean	M	RO	M x RO	
TS, %	Inorganic	21.72	22.91	22.53	22.39	0.052	0.005	0.174	2.06
	Organic	21.75	22.43	22.28	22.15				
	Mean	21.73b	22.67a	22.40a	22.27				
VS, %	Inorganic	16.35	17.22	17.04	16.87	0.492	0.009	0.858	2.42
	Organic	16.56	17.32	17.05	16.98				
	Mean	16.45c	17.27a	17.05b	16.92				

pH	Inorganic	5.62aA	5.45bA	5.65aA	5.58				
	Organic	5.52aB	5.37cB	5.45bB	5.45	<0.001	<0.001	0.001	1.86
	Mean	5.57	5.42	5.56	5.51				
Total N, %	Inorganic	5.51	5.83	6.05	5.79				
	Organic	5.62	5.32	5.80	5.58	0.090	0.054	0.138	4.95
	Mean	5.56	5.57	5.92	5.58				
Total P, %	Inorganic	2.09	2.15A	2.01	2.08				
	Organic	2.05	2.01B	2.07	2.04	0.044	0.138	0.006	2.67
	Mean	2.07	2.08	2.04	2.06				

Lower case letters in the row/column differ by Tukey test (P<0.05).
TS (total solids), VS (volatile solids), pH (hydrogenionic potential), N (nitrogen) and P (phosphorus).

Averages of 22.27% TS and 16.92% VS were observed, which were lower than the 31% reported by Augusto and Kuntz [13] and Ortiz et al. [15] with values of 26.50% TS, and 19.83% VS, respectively. There was no isolated effect (P>0.05) of the factors studied on total N. Ortiz et al. [15] whose study involved working with the manure of layers fed different corn particle sizes. They obtained a value of 5.37% total N, a value close to that found in the present study (5.59%). The average TS values observed in this study were characterized by a high concentration of solids. Generally, manure with very high TS levels can affect the efficiency of biodigestion by increasing the retention time of the material. According to Zahan and Othman [16], waste should have a concentration of no more than 8% TS to facilitate circulation inside the fermentation chamber and avoid clogging the material inlet and outlet pipes in the biodigesters.

The VS content found in this study showed that the manure produced contained a large amount of organic matter in all treatments, as 76% of the VS content was volatile. This high OM content can cause environmental impacts when not treated properly, as large amounts of OM in water bodies can cause the death of aquatic organisms due to the oxygen present in the water being used to degrade the organic matter present in the waste [17].

There was an interaction (P<0.05) between the mineral sources and levels of rosemary oil for pH and total P (Table 3), where the pH values for the inorganic minerals were found to be higher than those for the organic minerals at all of the levels of rosemary oil. When the interaction was broken down, the pH values between the levels of rosemary oil and inorganic minerals showed that the levels of 0 and 200 mg kg^{-1} had the highest values of 5.62 and 5.65, respectively. Regarding the organic minerals in relation to rosemary oil levels, the 0 mg kg^{-1} level showed the highest pH value of 5.52.

These values differ from those found by Sanches et al. [18], who, when assessing the pH of fresh droppings from commercial layers fed conventional diets, obtained a pH of 7.70. Interestingly, regardless of the source of the minerals used to feed the commercial layers in this study, the inclusion of 100 mg kg^{-1} rosemary oil reduced the pH of the droppings; however, when the level was increased, a subtle increase in pH was observed.

According to Kiehl [19], the raw OM of plant or animal origin is naturally acidic unless it is contaminated with alkaline materials such as limestone or ash. Therefore, one possible

explanation for the higher pH values observed in the manure of birds fed with inorganic minerals could be the inclusion of an inert material (kaolin), as this ingredient was only used in diets containing inorganic minerals. pH is a critical parameter in poultry excreta and must be analyzed and controlled in order to avoid environmental acidification. The pH of poultry waste influences the volatilization of ammonia in the atmosphere. The volatilization rate is low when the pH is below 7.5, which favors the accumulation of H+ ions in the manure and increases the conversion of ammonia into non-volatile ammonium ions [20]. However, volatilization is high when the pH is above 8.0, and under humid conditions and high excreta temperatures [21].

Thus, pH values are important in regard to characterizing the waste produced in poultry farming because they interfere with the decomposition of waste through the growth of microorganisms responsible for degradation, either naturally or through some treatment methods.

Regarding total P, there was an interaction ($P<0.05$) between the factors studied (Table 3). Birds fed inorganic minerals and supplemented with 100 mg kg^{-1} of rosemary oil had a higher concentration of total P in the feces (2.15%) than birds supplemented with organic minerals and the same level of rosemary oil. When evaluating the total P in the droppings of commercial layers, Ortiz et al. [15] found a value of 1.32%, which was lower than that found in the present study.

The higher excretion of P in the waste of birds fed diets containing inorganic minerals can be explained by the fact that these minerals are more prone to antagonism in the gastrointestinal tract because of their chemical composition as well as the negative interactions with other components of the diet. This makes it difficult for the animal to use these minerals, thus reducing the bioavailability of other nutrients and often leading to their excretion and nutritional deficiencies [22].

The mineral sources and levels of rosemary oil did not alter ($P>0.05$) the C, Ca, Mg, Fe, and Cu content of the droppings in the layers (Table 4). However, there was an effect ($P<0.05$) of diet on the Zn content of the droppings, where the diet containing organic minerals and no rosemary oil showed the highest level of Zn excretion (528 mg kg^{-1}), and the diet containing inorganic minerals with 200 mg kg^{-1} of rosemary oil showed the lowest Zn content (462 mg kg^{-1}). Previous studies have shown that dietary supplementation with low doses of organic minerals (iron, copper, manganese, and zinc) in commercial layers reduces mineral excretion [23]. This was not observed in the present study, considering that birds fed a diet with organic minerals without rosemary oil excreted higher levels of Zn in their droppings, leading to the hypothesis that this effect may be related to the higher level of organic mineral inclusion used in the present study.

Table 4 Macro and micro-minerals content of the waste of commercial layers fed diets containing mineral (M) sources and levels of rosemary oil (RO)

Diets	C	Ca	Mg	Fe	Cu	Zn	Mn
	g 100g^{-1}			mg kg^{-1}			
Inorganic minerals (IM)	38.58	1.68	0.18	1,959	111	470ab	566
IM + RO 100 mg kg^{-1}	39.20	1.58	0.18	2,219	97	475ab	566
IM + RO 200 mg kg^{-1}	39.75	1.51	0.18	2,213	83	462b	560
Organic minerals (OM)	39.67	1.88	0.21	1,949	92	528^{a}	554
OM + RO 100 mg kg^{-1}	39.59	1.55	0.17	2,998	139	526ab	522
OM + RO 200 mg kg^{-1}	40.14	1.54	0.18	2,351	91	515ab	558
P value	0.146	0.195	0.330	0.504	0.513	0.016	0.669
CV, %	1.96	13.33	11.35	35.49	42.77	6.01	7.43

Lowercase letters in the row/column differ by Tukey test (P<0.05).

4. Conclusion

Mineral sources did not affect the amount of manure excreted by commercial layers. Diets containing organic minerals resulted in less polluting waste as they had lower TS, pH, total N, and total P values. Rosemary oil increased manure production in NM and increased the TS and VS values in the manure layer, causing a greater environmental impact.

Author contributions

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

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