

Chemical composition, ruminal fermentation and greenhouse gases of diets with different levels of poultry manure

Composición química, fermentación ruminal y gases de efecto invernadero de dietas con diferentes niveles de pollinaza

Composição química, fermentação ruminal e gases com efeito de estufa de dietas com diferentes níveis de dejetos de aves

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Abstract

The use of poultry manure in tropical cattle farming is common and although many aspects of this resource have been studied, it is important to generate information on the effects of including it in comprehensive diets. The objective of this work was to know the chemical, fermentative, greenhouse gas (GHG) production and costs characteristics of diets that include poultry manure. Three diets (treatments) made with local ingredients (30, 15 and 0 % poultry manure) were evaluated. For each treatment, crude protein values and ruminal degradation, ash, fiber fractions, fermentation kinetics and fractions, GHG production and cost per kilogram were estimated. The results, except costs, were analyzed using the GLM procedure. Protein and fiber values were similar ($P>0.05$) in all treatments while ash was higher ($P<0.05$) in those containing manure. The values of maximum volume (V_m), medium fermentation (MF) and total fermentation (TF) were similar ($P>0.05$) in all treatments. In relation to GHG, the highest methane values occurred in T3 ($P<0.05$) and those diets that included poultry manure were up to 20 % more economical. The inclusion of poultry manure in diets achieves protein contributions to the rumen similar to those achieved with diets based on which this byproduct was not included, as well as a greater contribution of ash without affecting ruminal fermentation activity, but it does reduce methane production and diet costs.

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Resumen

El uso de pollinaza en la ganadería bovina tropical es común y aunque se han estudiado muchos aspectos sobre este recurso, es importante generar información sobre los efectos al incluirla en dietas integrales. El objetivo de este trabajo fue conocer las características químicas, fermentativas, de producción de gases de efecto invernadero (GEI) y costos de dietas con inclusión de pollinaza. Se evaluaron tres dietas (tratamientos) elaboradas con ingredientes locales (30, 15 y 0 % de pollinaza). A cada tratamiento se le estimó valores de proteína cruda y su degradación ruminal, cenizas, fracciones de fibra, cinética y fracciones de fermentación, la producción de GEI y el costo por kilogramo. Los resultados, excepto costos, se analizaron utilizando el procedimiento GLM. Los valores de proteína y fibras fueron similares ($P>0,05$) en todos los tratamientos mientras que la ceniza fue mayor ($P<0,05$) en los que contenían pollinaza. Los valores de volumen máximo (Vm), fermentación media (MF) y fermentación total (TF) fueron similares ($P>0,05$) en todos los tratamientos. En relación a GEI, los valores más altos de metano se produjeron en el T3 ($P<0,05$) y aquellas dietas que incluyeron pollinaza fueron hasta un 20 % más económicas. La inclusión de pollinaza en dietas logra aportes proteicos en rumen similares a los alcanzados con dietas basadas en las que no se incluyó este subproducto así como un mayor aporte de cenizas sin afectar la actividad fermentativa ruminal, pero sí disminuyendo la producción de metano y los costos de la dieta.

Palabras clave: fermentación, degradabilidad ruminal, metano, excretas avícolas

Resumo

A utilização de estrume de galinha na pecuária tropical é comum e embora muitos aspetos deste recurso tenham sido estudados, é importante gerar informação sobre os efeitos da sua inclusão em dietas abrangentes. O objetivo deste trabalho foi conhecer as características químicas, fermentativas, de produção e custos de gases com efeito de estufa (GEE) de dietas que incluem cama de galinha. Foram avaliadas três dietas (tratamentos) elaboradas com ingredientes locais (30, 15 e 0 % de estrume de galinha). Para cada tratamento foram estimados os valores de proteína bruta e degradação ruminal, cinzas, frações de fibra, cinética e frações de fermentação, produção de GEE e custo por quilograma. Os resultados, exceto os custos, foram analisados utilizando o procedimento GLM. Os valores de proteína e fibra foram semelhantes ($P>0,05$) em todos os tratamentos enquanto as cinzas foram superiores ($P<0,05$) nos que continham estrume. Os valores de volume máximo (Vm), fermentação média (MF) e fermentação total (TF) foram semelhantes ($P>0,05$) em todos os tratamentos. Em relação ao GEE, os valores mais elevados de metano ocorreram no T3 ($P<0,05$) e aquelas dietas que incluíam cama de galinha foram até 20 % mais económicas. A inclusão de estrume de aves nas dietas proporciona contribuições proteicas para o rúmen semelhantes às obtidas com dietas em que este subproduto não foi incluído, para além de uma maior contribuição de cinzas sem afectar a actividade de fermentação ruminal, mas reduz a produção de metano e os custos da dieta.

Palabras-chave: fermentação, degradabilidade ruminal, metano, excrementos de aves.

Introduction

In many regions of Mexico’s dry tropics, poultry manure has been a widely used ingredient in livestock production, mainly for cattle. This co-product is used practically throughout the year, but its use becomes more important during the dry season, and studies reporting on its quality and use in ruminants indicate its importance and relevance compared to other types of excrement (Orlando and Cornejo, 2024; Vivas *et al.*, 2023; Pinto *et al.*, 2019). Similarly, various studies have measured the effect of poultry manure on the quality of meat (Chacón *et al.*, 2019) and milk (Citalan *et al.*, 2016) from animals fed with it. This means that poultry manure continues to be used in tropical livestock systems, but much of its use is still empirical and based on lack of knowledge.

Although aspects related to its chemical composition and ruminal degradation, processes to improve its biological quality, and impact on bovine animal protein production have been studied, it is still necessary to generate information on the advantages that poultry manure, when included in complete diets, can achieve and that have not been disseminated, which could manifest themselves in economic, animal utilisation, and environmental terms when using a potentially polluting resource (Pinos *et al.*, 2012) and could be equivalent to those achieved in diets supplemented with expensive and scarce ingredients, especially considering that the current increase in the price of many ingredients has led to higher feed costs and that ruminal fermentation activity could be affected by the type of diet used (Arjmand *et al.*, 2022).

The objective of this study was to determine whether the inclusion of poultry manure in cattle diets offers the same opportunities in terms of chemical and fermentation characteristics, greenhouse gas production and costs as a diet without it.

Materials and methods

Characteristics of the study area

The analyses were carried out at the Animal Nutrition Laboratory, Faculty of Agricultural Sciences of the Autonomous University of Chiapas, located in the municipality of Villaflores, Chiapas, Mexico. This region has a climate classified as Aw, with an average rainfall of 1,100 mm and an average annual temperature of 25 °C (National Institute of Statistics, Geography and Informatics, 2020).

Treatments evaluated

Three diets (treatments) were evaluated, prepared with local ingredients commonly used in diets for finishing cattle (table 1). In the case of poultry manure, it was dried in the open air, ground with a hammer mill and sieved to remove feathers and residual lumps, and then incorporated into the experimental diets.

Table 1. Diets evaluated (% as-fed basis).

Dietary component	T1	T2	T3
Poultry manure	30	15	
Corn grain	50	56	56
Ground African star grass (<i>Cynodon nlemfuensis</i> Vanderyst)	20	28	25
Urea	0	1	0
Dried distillery grains	0	0	6
Wheat bran	0	0	10
Soybean meal	0	0	3

Variables evaluated
Chemical composition

Each treatment was analysed in triplicate to determine its dry matter (DM), organic matter (OM), ash (ASH) and crude protein (CP) content according to AOAC (1990 a y b). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated according to the technique described by Van Soest (1991).

In vitro fermentation and degradation

The technique described by Menke *et al.* (1979) was used. For each treatment evaluated, 12 vials (bottles) of 125 mL were used, in which 500 mg of the corresponding treatment was placed. Then, under a continuous flow of carbon dioxide (CO₂), 90 mL of diluted bovine ruminal inoculum (1:10) was added to each vial. The reduced and added mineral solution contained KH₂PO₄ (0.45 g.L⁻¹), NaCO₃ (0.6 g.L⁻¹), (NH₄)₂SO₄ (0.45 g.L⁻¹), NaCl (0.9 g.L⁻¹), MnSO₄ (0.18 g.L⁻¹), CaCl₂ (0.12 g.L⁻¹), L-cysteine (0.25 g.L⁻¹) and Na₂S (0.25 g.L⁻¹). The filled vials were sealed with rubber stoppers and aluminium rings and incubated at 39 °C. Three vials without substrate were included as controls.

The fermentation parameters were determined by measuring the gas pressure with a pressure gauge at 2, 4, 6, 8, 10, 12, 16, 20, 22, 24, 28, 32, 34, 36, 40, 48, 60 and 72 hours of incubation. The pressure values (kg.cm²) were converted to gas volume (mL.g⁻¹ of substrate) using a linear regression equation (volume = pressure/0.019 with R² = 0.98), which was obtained by relating the pressure (P) generated by known volumes of air injected into the flasks maintained under the same handling conditions (De la Rosa-Zariñana *et al.*, 2023). The parameters of gas production kinetics: maximum volume (V_m), rate (S) and lag phase (L) were estimated using the logistic model $V=V_m/(1+e^{-(2.4 \times S \times (T-L))})$ proposed by Schofield and Pell (1995) using SAS version 9.1 (2004).

Fermentation fractions were also estimated through the fractional volume (V_f) of fermentation gas at time intervals of 0 to 8 (V_{f₀₋₈}), 8 to 24 (V_{f₈₋₂₄}) and 24 to 48 (V_{f₂₄₋₄₈}) hours of incubation, transforming them into fractions (g.kg⁻¹ DM) of fast (RF), medium (MF) and slow (SF) fermentation using the regression equations; FR (g.kg⁻¹) = V_{f₀₋₈}/0.4266 (R² = 0.9441), MF (g.kg⁻¹) = V_{f₈₋₂₄}/0.6152 (R² = 0.998) and SF (g.kg⁻¹) = V_{f₂₄₋₄₈}/0.3453 (R² = 0.9653) (Ramírez *et al.*, 2020). RF, MF and SL refer to sugars, starch and structural components (cellulose and hemicellulose), respectively.

Crude protein degradation (CPDeg) was calculated as the ratio between the initial incubated material and the residue obtained, which was analysed for its crude protein content.

In vitro estimation of greenhouse gases

To determine CH₄ and CO₂, a third series of fermentation was carried out using a syringe (50 mL) to measure gas production at 6, 12, 18, and 24 hours of incubation. To determine methane, the methodology described by Ramírez *et al.* (2023), which is based on the separation of CO₂ from the fermentation gas using a trap containing 40 mL of potassium hydroxide (KOH) at a concentration of one molar (56.10 g of KOH per litre of distilled water), following the original procedure of Bartha and Pramer (1965). The residual volume was considered as mL of CH₄+minor gases and adjusted as theoretical methane (CH₄) by multiplying each value by 0.7714 according to the proportion of methane determined in the rumen gas sample (Zhong, 2016). Using the above values, the total gas produced during fermentation (VTGF; mL.g⁻¹ DM) and methane (%) were estimated, obtaining the percentage of CO₂ by difference.

Diet cost

To determine the cost per kilogram (as offered), the individual price of each ingredient on the local market was considered, along with its percentage inclusion in each diet, taking into account prices for the year 2025.

Data analysis

The results of each variable measured, except costs, were analysed using the GLM procedure of the SAS statistical package version 9.1 (2004) using a completely randomised experimental design. The means were compared using Tukey's test (P<0.05).

Results and discussion

Chemical composition

The DM and CP values found were similar (P>0.05) in all treatments. The NDF values differed between treatments, with those with higher poultry manure content being higher (P<0.05). The OM values differed between treatments (P<0.05), with the treatment without poultry manure having the highest OM content. The NDF and ADF values were similar (P>0.05) between treatments. Finally, with regard to CPDeg, T2 had the highest value and T3 the lowest (P<0.05) (table 2).

Table 2. Chemical and crude protein degradation characteristics of diets with different levels of poultry manure (Mean ± Standard deviation).

Nutrient	T1	T2	T3
DM	87.76 ± 0.18 ^a	88.96 ± 0.15 ^a	88.45 ± 0.12 ^a
CP	11.27 ± 0.88 ^a	10.87 ± 0.31 ^a	10.55 ± 0.31 ^a
CA	7.09 ± 0.09 ^a	5.27 ± 0.02 ^b	4.15 ± 0.05 ^c
OM	92.90 ± 0.09 ^c	94.73 ± 0.02 ^b	95.85 ± 0.05 ^a
NDF	32.10 ± 0.38 ^a	31.20 ± 0.41 ^a	30.95 ± 0.44 ^a
ADF	21.95 ± 0.23 ^a	21.45 ± 0.27 ^a	18.89 ± 0.32 ^a
CPDeg (%)	90.81 ± 0.72 ^{ab}	92.03 ± 0.63 ^a	90.46 ± 0.03 ^b

^{a, b, c} Means with different letters in the same row are statistically different (P<0.05). DM: Dry matter (%). CP: Crude protein (%). CA: Crude ash (%). OM: Organic matter (%). NDF: Neutral detergent fibre (%). ADF: Acid detergent fibre (%). CPDeg= Crude protein degradation at 24 h (%). T1: Diet with 30 % poultry manure. T2: Diet with 15 % poultry manure. T3: Diet with 0 % poultry manure.

Considering the CP and CPDeg values found, in all cases, any of the treatments provides the rumen-degradable protein necessary to meet the requirements for ruminal microbes, which are considered to be around 8.0 % (Detman *et al.*, 2014). Similarly, calculating it as proposed by Mendoza *et al.* (2022), the treatments do not provide a significant proportion of passage protein that could be important for the animal's production requirements as it is absorbed in the duodenum (1.03, 0.87 and 0.92 %, respectively) and could impact the production of animals on pastures with average production (Oliveira de Souza *et al.*, 2021). In this case, diets that include poultry manure can be considered as those that provide most of their protein as rumen-degradable, which is related to a higher concentration of ammonium present in the rumen and useful in microbial protein synthesis (Elizondo-Salazar, 2020), which could contribute significantly to the total supply of amino acids to the small intestine and to the modification of the amino acid profile of the duodenum (Castillo and López, 2019).

Poultry manure has been recognised as a co-product with relatively high ash levels of around 15-18 % (Pinto-Ruiz *et al.*, 2019), which explains the higher ash values in diets with poultry manure compared to diets without poultry manure and which may be important in terms of a greater contribution to the animal's mineral requirements when this co-product is part of the diet. The NDF values obtained in diets with poultry manure were similar to those reported by Bórquez-Gastelúm *et al.* (2018), who evaluated diets made with silage poultry manure together with corn stover, corn grain, wheat bran, soybean meal, and fish meal, finding an NDF content of 30.6 %. Likewise, when compared to other food sources, the NDF and ADF values are similar to those recorded for tree species (34.64 and 28.99 %, respectively), but lower than those reported for grasses such as *Cenchrus ciliaris* (73.71 and 49.62 %, respectively), according to Bernal *et al.* (2022).

In vitro fermentation and degradation

In vitro gas production is a simple technique that helps characterise the kinetics and fermentation fractions of feed substrates (Aragadvay-Yungán *et al.*, 2022), which could explain the effect of diet composition on ruminal fermentation activity (Cui *et al.*, 2019), an aspect of interest in defining strategies for the optimal use of ingredients, such as poultry manure. Table 3 shows the results of the fermentation kinetics of the treatments evaluated in this study. For L (h), similarities ($P>0.05$) were found in T1 and T3. The value of S (h^{-1}) found was equal ($P>0.05$) between T1 and T2 and different for T3 ($P<0.05$). However, for the Vm values, these were similar ($P>0.05$) in all the treatments evaluated.

Table 3. Fermentation parameters of diets with different levels of poultry manure (Mean $\pm$ Standard deviation).

Parameter	T1	T2	T3
L (h)	7.34 $\pm$ 0.35 ^b	8.72 $\pm$ 0.83 ^a	6.95 $\pm$ 1.25 ^b
S (h^{-1})	0.038 $\pm$ 0.00055 ^a	0.038 $\pm$ 0.00285 ^a	0.033 $\pm$ 0.00358 ^b
Vm (mL.g^{-1} MS)	375.9 $\pm$ 17.11 ^a	367.49 $\pm$ 27.97 ^a	396.28 $\pm$ 35.91 ^a

^{a, b}Means with different letters in the same row are statistically different ($P<0.05$). L= Lag phase (h). S= Gas production rate (h^{-1}). Vm= Maximum gas production volume (mL.g^{-1} DM). T1: Diet with 30 % poultry manure. T2: Diet with 15 % poultry manure. T3: Diet with 0 % poultry manure.

The higher L value found in T2 (15 % poultry manure) indicates that the adaptation of microbes to ruminal conditions and, therefore, the degree of colonisation of the substrate takes longer. However, T1, the diet that also contained poultry manure (30 %), was similar ($P>0.05$) in its L value to the diet that did not contain it (T3). These differences could be explained by the lower soluble and readily available fraction (FR) found in the diet corresponding to T2 (table 4), which is a result of its composition.

On the other hand, the high S values for the diets with poultry manure (T1 and T2) indicate that these treatments were fermented more quickly, an activity that could be associated with a more rapid multiplication of microbes and that, at this rate, they consume the nutrients in the environment provided by the diets compared to the diet without poultry manure (T3). With regard to the fermentation potential represented by the metabolic state of ruminal microbes (Vm), although T3, the treatment without poultry manure, had the highest Vm value due to its formulation with a higher proportion of concentrated ingredients, statistically it was similar ($P>0.05$) to those formulated with poultry manure.

The L values of the diets evaluated in this study were higher than those reported by De la Rosa *et al.* (2023) for diets for fattening

sheep with different protein and energy levels (4.24 h) but similar to those reported for *P. maximum* (6.75 h) (Díaz *et al.*, 2023). The Vm values were lower than those obtained in diets with grains or high in concentrates (462 to 493 mL.g^{-1} DM) reported by Sánchez *et al.* (2019) and higher than those produced by sorghum silage and pastures (294.5 and 194.45 mL.g^{-1} DM, respectively) (Villalba *et al.*, 2025; Amparo-Holguín *et al.*, 2021). On the other hand, it is known that the gas produced during *in vitro* ruminal fermentation depends, but not exclusively, on the concentration of highly fermentable carbohydrates and the components of the feed cell wall (Villalba *et al.*, 2021), so table 4 shows the results of the fermentation fractions of the treatments evaluated. For the FM and FT values found, similarities were found in all the treatments evaluated ($P>0.05$). For RF, T2 was statistically different ($P<0.05$) from T3. For SF, T3 had the highest values and was significantly different ($P<0.05$) from the other treatments.

Table 4. Fermentation fractions of diets with different levels of poultry manure (g.kg^{-1}) (Mean $\pm$ Standard deviation).

Treatment	RF	MF	SF	TF
T1	100.44 $\pm$ 7.22 ^{ab}	330.27 $\pm$ 15.00 ^a	350.00 $\pm$ 21.49 ^b	780.71 $\pm$ 36.88 ^a
T2	70.62 $\pm$ 23.25 ^b	319.22 $\pm$ 18.53 ^a	373.98 $\pm$ 30.39 ^b	763.82 $\pm$ 60.20 ^a
T3	118.94 $\pm$ 29.95 ^a	298.97 $\pm$ 54.98 ^a	408.88 $\pm$ 57.90 ^a	826.79 $\pm$ 76.45 ^a

^{a, b}Means with different letters in the same column are statistically different ($P<0.05$). RF: Rapid fermentation fractions. MF: Medium fermentation fractions. SF: Slow fermentation fractions. TF: Total fermentation. T1: Diet with 30 % poultry manure. T2: Diet with 15 % poultry manure. T3: Diet with 0 % poultry manure.

The lower RF value ($P<0.05$) found in T2 and the higher SF value ($P<0.05$) in T3 could be associated with the ingredients considered in each of the diets. In the case of T2, this could be associated with the lack of soluble carbohydrates, and in the case of T3, with higher structural carbohydrates, specifically the difference in the fractions of this type of carbohydrate (more cellulose) (Ramírez *et al.*, 2020). With regard to TF, it can be seen that although the treatment without poultry manure (T3) produced the highest gas production, due to the greater amount of concentrated ingredients in its formulation (Miranda-Romero *et al.*, 2020), this value was statistically similar ($P>0.05$) to that obtained in the diets with poultry manure.

The values found in this study for RF and MF in all treatments are similar to the ranges published by De la Rosa *et al.* (2023) for diets for pen-raised sheep with different protein and energy levels. All of the above reaffirms the importance that, although ruminal fermentation activity could be affected by the type of diet used (Arjmand *et al.*, 2022), the use of poultry manure does not cause significant changes in this activity compared to diets that do not contain this ingredient.

In vitro estimation of greenhouse gases

Table 5 shows that, in relation to polluting gases, the highest values occurred in T3 ($P<0.05$).

Treatments with poultry manure produced lower amounts of methane, which may be related to a higher amount of ammonia present in the rumen and contributed by the poultry manure and produced during the anaerobic digestion process. It has been shown that ammonia inhibits gas production. It is also known that there is a high production of volatile fatty acids, which add to the inhibitory effect of nitrogen (Rendón-Correa *et al.*, 2022). The results obtained in T1 and T2 are within the ranges obtained by Pereyra *et al.* (2022) with diets that also contained poultry manure in their composition (13.5 to 20 mL.g^{-1} DM).

Cost of feed

Table 6 shows the cost per kilogram of feed as offered. It can be seen that feeds containing poultry manure can be up to 20 % cheaper

(T1), a key factor in the economics of livestock farming, as feed costs can account for up to 80 % of production costs.

Table 5. Greenhouse gas estimation of diets with different levels of poultry manure.

Treatment	TV (mL.g ⁻¹ DM)	CO ₂ V (mL.g ⁻¹ DM)	CH ₄ theoretical (mL.g ⁻¹ DM)
T1	161.648 ^b	141.269 ^b	15.722 ^c
T2	160.539 ^b	134.294 ^b	20.245 ^b
T3	187.922 ^a	152.283 ^a	27.492 ^a

^{a, b, c}Means with different letters in the same column are statistically different (P<0.05). TV: Total volume (mL.g⁻¹ DM). CO₂V: Residual volume (mL.g⁻¹ DM). CH₄: Methane (mL.g⁻¹ DM). T1: Diet with 30 % poultry manure. T2: Diet with 15 % poultry manure. T3: Diet with 0 % poultry manure.

Table 6. Cost per kilogram of diets with different levels of poultry manure (as offered).

Tratamiento	\$ en BTO	\$ unidad Proteína
T1	5,75	58,11
T2	6,17	63,75
T3	7,17	76,83

Cost according to January 2025 prices. T1: Diet with 30% poultry manure. T2: Diet with 15% poultry manure. T3: Diet with 0% poultry manure, BTO: As offered.

Conclusions

The inclusion of poultry manure in diets can achieve rumen protein intakes similar to those achieved with diets that did not include this by-product, as well as a higher ash intake, all without affecting ruminal fermentation activity but reducing methane production and diet and nutrient unit costs. Although these results were obtained under *in vitro* conditions, it would be interesting to corroborate them through *in vivo* trials.

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