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Biogas production: litter from broilers receiving direct-fed microbials and an enzyme blend

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ABSTRACT: The effect of additives used in the feed of broilers on anaerobic bio-digestion of poultry litter was evaluated. Four diets were used: NC: negative control; DFM: NC + 500 ppm direct-fed microbials (DFM) containing *Bacillus subtilis* and *Bacillus licheniformis*; ENZ: diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E: ENZ + DFM. Substrates for the anaerobic bio-digestion were prepared with litter from each treatment, containing 4 % total solids (TS). These were used in 16 continuous bio-digesters with a 2 kg d^{-1} load, to determine the production and potential biogas production and composition during an 85-day period. Influent and effluent samples were collected for the amounts of TS and volatile solids (VS), fiber fraction (neutral detergent fiber [NDF], acid detergent fiber [ADF] and lignin), nutrients (N, P and K), and total and thermotolerant coliforms to be determined. For all treatments a reduction in the following effluents was observed as follows: TS (49, 48, 48 and 50 %) VS (70, 54, 55 and 62 %) NDF (91, 90, 95 and 96 %) ADF (89, 88, 93 and 94 %) and lignin (80, 76, 89 and 88 %). The efficiency of the treatment for coliforms in bio-digesters was higher than 90 % in the 85-day period in all treatment groups. There was a reduction in biogas and methane production when DFM (5500 and 4000 mL) and DFM + E (5800 and 4100 mL) were used, compared to treatments NC (6300 mL and 4400) and ENZ (6400 and 4500 mL). The potential production of reduced TS and VS was higher in ENZ (1:00 and 1.74 106 mL kg^{-1}) when compared to NC (0.88 and 1:02 106 mL kg^{-1}), DFM (0.80 and 1:40 106 mL kg^{-1}) and DFM + E (0.88 1:25 and 106 mL kg^{-1}). The additives did not affect the percentage of methane production, and all treatments showed values higher than 70 %. Adding enzymes to the diet of broilers influences the litter characteristics and, as a consequence, increases biogas production. The addition of DFM and DFM + E to broiler diets reduced biogas and methane production.
Keywords: additives, bio-digestion, bio-digester, methane, livestock residues

Introduction

The Brazilian poultry industry is considered a reference in both production and promotion of the use of technology, with an outstanding position in world meat production (FAO, 2011). As feed is the major and most important factor in poultry production, measures taken to improve diets become very important. Advances in biotechnology have led to the marketing of products which improve productivity and feed efficiency in broilers when added to feeds (Huyghebaert et al., 2011).

One of the factors that have contributed to the high productivity evident in the poultry industry has been the use of feed additives. Among additives, utilization of direct-fed microbials and exogenous enzymes has been emphasized as these can help improve animal performance and enable the use of feed ingredients that are difficult to degrade (Huyghebaert et al., 2011).

Besides feeding, droppings and waste material produced are also a matter of concern in large-scale poultry production (Bolan, 2010). With this evolution, nutritionists look for alternatives to be used in the formulation of more efficient feed, with low environmental impact which, at the same time, can provide alternative treatment of the residues generated.

Solid residues at the end of each broiler production cycle include the litter and dead animals, and are considered either a resource or a pollutant. Adequate management of these residues with high nutrient content will result in minimum environmental impact. These residues represent potential pollutants for water bodies and also underground water, increasing mineral nutrients, oxygen-demanding organic matter, suspended matters and sometimes even pathogenic organisms (Nahm and Nahm, 2004).

The purpose of using technology in residue management is to use the available nutrients, with minimum loss to the environment during processing. Studies are necessary for determining up to what point these can be used, and under which conditions and dimensions, these are actually feasible.

Anaerobic bio-digestion is an outstanding alternative for treating residues related to its sanitary aspects and potential renewable energy generation, besides offering an economic organic and nutrient recycling process (Demirer and Chen, 2005). However, little is known about the influence of diet on the composition of litter to be used in biogas production.

Thus, this study aimed to evaluate the influence of enzyme additives and direct-fed microbials used in the diet of broilers on the anaerobic bio-digestion of litter in continuous digesters.

Materials and Methods

Nine hundred day-old broiler chicks from the same breeder flock were used. In a fully randomized design these were distributed into four treatment groups with 9 replicates, 25 birds in each, and six birds per m² density. The experimental treatments were: NC: negative control diet; DFM: NC diet + 500 ppm direct-fed microbials (DFM), with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ: NC diet + 20 ppm phytase, 200 ppm protease and 200 ppm xylanase; DFM + E: ENZ diet + DFM diet.

Wood shavings were used as litter material, 0.7 kg dry matter (DM) per housed bird, so all treatments had the same amount of litter in all boxes (20 kg natural matter).

The chicks were vaccinated against Marek's disease, Gumboro (IBD), and fowl pox in the hatchery. At 5 and 21 days of age they were vaccinated against IBD, and at 7 days of age against Newcastle disease, both by ocular administration.

The environmental temperature and relative humidity of the air were recorded daily with digital thermometers placed at the near floor. Curtains and fans were used to ensure the birds' thermal comfort and a 24 hour light program. Feed and water were provided *ad libitum* throughout the experimental period that was divided into three phases: initial (1 to 21 days of age), growing (22 to 35 days of age) and final (36 to 42 days of age).

As DFM was used in certain treatments, shoe covers were used during the daily bird management to prevent litter contamination.

Feeds were formulated based on corn and soybean meal (SBM), supplemented with minerals, vitamins and amino acids, to meet each rearing phase (initial, growing and final) nutritional requirements according to NRC recommendations (1994). No growth promoter was used.

The nutritional matrix of each enzyme was taken into account when formulating the diet as follows: xylanase supplied 1676 10^2 J metabolizable energy kg^{-1} feed; phytase supplied 0.15 % available phosphorus and 0.12 % calcium; protease supplied 4 % crude protein, 4 % digestible arginine, 1 % digestible lysine, 4 % digestible methionine + cystine, 8 % digestible threonine, and 3 % digestible tryptophan. The product used as DFM contained *Bacillus* subtilis (minimum 0.735×10^8 UFC g^{-1}) and Bacillus licheniformis (minimum 0.735×10^8 UFC g^{-1}).

After 42 days, litter was removed from the boxes, identified and placed in plastic buckets. The litter produced was divided to provide the daily load (2 kg) to the continuous bio-digesters for an 85-day period. The substrate was prepared for each loading with a total solids (TS) amount close to 4 %. After preparation, solids were separated from the liquid fraction by using 3-mm sieves. The liquid fraction was added to the bio-digesters with a 30-day hydraulic retention time (HRT).

In a fully randomized design, the substrates prepared with litter from broilers fed the different diets listed above were used in the continuous bio-digesters, with four replicates.

The continuous bio-digesters are formed by two different parts: a vessel with the fermenting material and a gasometer. The vessel is made up of a straight PVC cylinder, 300-mm diameter and 1-m long, the extremities being attached to PVC plates, 1.5-m thick. In one of the plates, an inlet pipe was placed to feed the digester; the other extremity had two outlet pipes, one for the bio fertilizer, and another for the gas (Orrico Junior et al., 2010).

The gasometer was consisted of two cylinders (250 and 300 mm diameter) one inside the other, so much so that the space between the two was filled with water ("water seal") reaching a depth of 500 m. The 300 mm diameter cylinder was attached to a 2.5 m thick PVC plate. The 250 mm cylinder had one of its extremities closed by a cap that received the gas produced, the other was capsized in the water seal to store the gas produced. The gasometers were placed on a bench, at room temperature, protected from sunlight and rain (Orrico Junior et al., 2010).

Influent and effluent samples were collected every week during the anaerobic bio-digestion stage to measure the following: acidity using a pH meter; the total solids (TS) and volatile solids (VS) according to the method described by APHA (2005); the neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and nitrogen (N) according to the methods proposed by the AOAC (2005).

The amount of phosphorus (P) followed the method described by Malavolta (1989), and the potassium (K) concentrations were determined using an atomic absorption spectrophotometer.

The samples for total and thermotolerant coliform analyses were collected from the influent at the beginning of the experiment and from the effluent after 30, 60 and 85 days of treatment in the continuous biodigesters, using the technique with multiple tubes described by APHA (2005).

The vertical displacement of the gasometers was measured to determine the volume of biogas produced daily. The values were multiplied by the area of the gasometers inner cross-section (0.0507 m^2). After each reading, the gasometers were reset to zero using the biogas discharge gauge. The biogas volume was corrected to $1.013\ 10^5$ Pa and 20 °C conditions, and the accumulated and daily biogas volumes were recorded (mL).

The biogas potential production was calculated using the daily biogas volume data and the amounts of substrate, TS and VS added to bio-digesters, and the TS and

VS amounts reduced during the anaerobic bio-digestion process. The values were expressed as mL biogas kg^{-1} substrate, TS and VS added and reduced.

Composition of the biogas produced in bio-digesters processing poultry litter was analyzed every week to determine the amounts of methane (CH_4), carbon dioxide (CO_2) and other gases using a Finigan GC-2001 gas chromatography, equipped with Porapack Q and Molecular Sieve columns, and a thermal conductivity detector.

The data were analyzed by the SAS® (SAS Institute, 2002) GLM program, and statistically significant means were compared using the Tukey test at 5 % probability.

Results and Discussion

The anaerobic bio-digestion process reduced all the effluents' pH resulting in an average between treatments of 7.4 when poultry litter was used in a continuous bio-digester with a 30 day hydraulic retention time (HRT). In accordance with FAO (1996) when the level of methane production reaches stability, pH was verified when the digesters of the study reached the production of biogas and methane constant, recording values from 7.2 to 8.2.

TS amounts were found to be reduced in all effluents from treated litters in the bio-digesters. However, the diet given to the birds had no effect ($p > 0.05$) in reducing TS in the bio-digestion process, where the averages were 49, 48, 48 and 50 %, for NC, DFM, ENZ and DFM + E. Different feed additives that were used caused less VS reduction ($p < 0.05$) when compared to the control treatment, except when the additive association (DFM + E) was used. All treatments caused a reduction in VS in excess of 50 %, showing the efficiency of anaerobic bio-digestion in the degradation of resistant compounds, such as broiler litter (Table 1).

Digestion efficiency can be related to the high amounts of substrate available in VS fermentation. Thus, digestion efficiency was better and obtained a greater reduction in the quantity of VS present when compared to the influent and effluent. The broilers' litter in the substrate showed a clear reduction ($p < 0.05$) in fiber fraction due to sieving, a larger amount of NDF and ADF being found in the effluents of enzyme treatments (ENZ and DFM + E) (Table 2). This can be related to the presence of xylanase, an enzyme that degrades fibers, reduces litter particles that were difficult to degrade and facilitates the sieving process. The diet did not influence

Table 1 – Amount of Volatile Solids (VS) in influents and effluents (% and kg) and % reduction of VS from litter of broilers fed diets with direct-fed microbials (DFM) and enzymes, treated in continuous bio-digesters.

Treatments*	Volatile Solids				
	Influent	Effluent	Influent	Effluent	Reduction
	%		kg		%
NC	0.44 A	0.13 B	0.0088 A	0.0026 B	70 A
DFM	0.33 B	0.15 AB	0.0066 B	0.0029 AB	54 B
ENZ	0.34 B	0.16 A	0.0068 B	0.0031 A	55 B
DFM+E	0.38 AB	0.14 AB	0.0076 AB	0.0028 AB	62 AB
F values	6.14	4.12	6.13	4.14	7.24
P values	0.0090**	0.0032**	0.0090**	0.0314**	0.0050**
CV[1] (%)	10	7	10	7	9

[1]Coefficient of variation; *NC = negative control; DFM = NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ = diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E = ENZ + DFM; **$p \leq 0.05$; [NS]non-significant; A-B = means followed by different letters within a column are significantly different ($p \leq 0.05$) according to Tukey test.

Table 2 – Amounts of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin in influents, effluents and % degraded fiber from litter of broilers fed diets with direct fed microbials (DFM) and enzymes, treated in continuous bio-digesters.

Treatments*	Influent g 100 g^{-1} DM %			Effluent g 100 g^{-1} DM %			% Degraded		
	NDF	ADF	Lignin	NDF	ADF	Lignin	NDF	ADF	Lignin
NC	11.84 C	4.92 C	1.89 C	2.25	1.12	0.75	91 B	89	80
PRO	18.59 B	7.65 B	1.68 D	3.41	1.76	0.76	90 B	88	76
ENZ	24.30 A	8.23 A	2.63 A	2.22	1.10	0.55	95 A	93	89
P+E	24.98 A	8.35 A	2.03 B	2.11	0.97	0.40	96 A	94	88
F values	793.93	74.94	178.18	1.14	1.38	0.61	3.65	2.17	0.374
P values	<0.0001**	<0.0001**	<0.0001**	0.372[NS]	0.2969[NS]	0.623[NS]	0.044**	0.144[NS]	1.13[NS]
CV[1] (%)	1	2	3	45	48	60	3	4	14

[1]Coefficient of variation;*NC = negative control; DFM = NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ = diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E = ENZ + DFM; **$p \leq 0.05$; [NS]non-significant; A-B = means followed by different letters within a column are significantly different ($p \leq 0.05$) according to the Tukey test.

the fiber fraction, which explains the higher degradation achieved in treatments that included enzymes (Table 2).

The different diets interfered ($p < 0.05$) in the average concentration of nutrients (N, P and K) present in the effluents of poultry litter treated in continuous bio-digesters. Effluents from the litter of broilers fed enzymes and DFM combination had higher average concentrations of N (4.01 g 100 g^{-1}), followed by the diet with enzymes (3.86 g 100g^{-1}). The control diet and the diet with DFM had the lowest concentrations (3.72 and 3.71 g 100 g^{-1}) in relation to P, the effluent from DFM + E treatment (4.65 g 100 g^{-1}) resulted in the lowest concentrations, and these were no different from those found in the effluents from the control (5.09 g 100 g^{-1}) diet and enzyme treatments (5.44 g 100 g^{-1}). However, a higher concentration of P was in the treatment containing DFM (5.77 g 100 g^{-1}). The lowest K concentrations were reported in the enzymes and enzymes + DFM treatments (2.16 and 2.18 g 100 g^{-1}), followed by treatment control and DFM (2.51 and 3.23 100 g^{-1}) (Table 3).

The increase in N and K amounts in the biofertilizers (effluents) when compared to the amounts in the material used to load the bio-digesters was expected. Residues in general undergoing anaerobic bio-digestion lose carbon as CH_4 and CO_2, resulting in concentration of the other nutrients, in disagreement with the phosphorus data in the present study. This result can be associated with the physical-chemical removal by means of these compounds' precipitation.

The number of total and thermotolerant groups of coliforms in influents and effluents of litters from animals receiving feed additives were lower when compared to the control treatment in all analyzed time periods analyzed (30, 60 and 85 days) (Table 4). In all the treatments, efficiency of treatment in continuous bio-digesters was higher than 90 % for fecal and thermotolerant coliforms only in the final sample collection period (85 days) (Table 4).

The potential contamination of soil and water by effluent pathogens should be better studied as the level of fecal coliforms present in bio-digester effluents depend on a higher hydraulic retention time (HRT) and temperature for the treatment to become more efficient. This treatment is more restricted in continuous bio-digesters than in batch bio-digesters. According to Côté et al. (2006), a reduction in efficiency of pathogenic microorganisms is associated with the fermentation temperature and TRH used; therefore, the higher the values, the more effective the production of pathogens.

According to CONAMA Resolution 357/05, that classifies waters to be used in irrigation as class 2, the maximum limit is 1000 thermotolerant (fecal) coliforms in a 100 mL sample (CONAMA, 2005). Therefore, the effluent produced by the continuous bio-digester during the experimental period would be above the standard and could not be used in fertirrigation. Total elimination of both fecal and thermotolerant coliforms stresses the importance and efficiency of the anaerobic bio-digestion process in reducing the organisms responsible for fecal pollution.

Diet influenced ($p < 0.05$) the substrates in biogas production and potential production (Table 5). Biodigesters with litter from birds that were only fed diets with enzymes (ENZ) had the highest biogas production both daily and accumulated (m^3), and methane (m^3) (Table 5), with no difference in relation to the control treatment. In relation to the other treatments, the same happened with biogas production potential per kg of added and reduced TS and VS (Table 5).

Another explanation for the reduction in biogas production is that these bacteria, when added to the feed, increase degradation of the substrate, using nutrients present in the litter during the period these were stored. Thus, the use of *Bacillus* bacteria in the broilers' diet and their influence on biogas production will depend on the time the litter was stored and the production of antimicrobials (Tables 5 and 6).

However, Praes et al. (2013) did not find in their study a negative influence of the broilers' diets with DFM on the production of biogas and methane, when treating broiler droppings in batch bio-digesters. In relation to the control treatment, there was a 27 % increase

Table 3 – Mineral and macro nutrient composition, nitrogen (N), phosphorus (P) and potassium (K), of influents and effluents from litter of broilers fed diets with direct fed microbials (DFM) and enzymes, treated in continuous bio-digesters.

Treatments*	Influent g 100 g^{-1} DM %			Effluent g 100 g^{-1} DM %		
	N	P	K	N	P	K
NC	2.35	6.44	1.15	3.72 C	5.09 AB	2.51 B
DFM	2.36	6.25	1.23	3.71 C	5.77 A	3.23 A
ENZ	2.24	5.60	1.20	3.86 B	5.44 AB	2.16 C
DFM+E	2.20	6.32	1.21	4.01 A	4.65 B	2.18 C
F values	4.82	2.45	1.76	34.95	5.62	73.98
P values	0.21^{NS}	0.11^{NS}	0.21^{NS}	<0.0001**	0.012**	<0.0001**
CV[1] (%)	4	8	5	1	8	1

[1]Coefficient of variation; *NC = negative control; DFM = NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ = diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E = ENZ + DFM; **$p \leq 0.05$; ^{NS}non-significant; A-B = means followed by different letters within a column are significantly different ($p \leq 0.05$) according to the Tukey test.

Table 4 – Most probable number of total and thermotolerant coliform organisms (MPN 100 mL^{-1}) and efficiency (%) of treating litter of broilers fed diets with direct fed microbials (DFM) and enzymes, in continuous bio-digesters in 30, 60, and 85 days.

Treatments*	30 Days					
	Total coliforms (MPN 100 mL^{-1})		Efficiency (%)	Thermotolerant coliforms (MPN 100 mL^{-1})		Efficiency (%)
	Influent	Effluent		Influent	Effluent	
NC	9.30 x 10^7 A	0.780 x 10^7 A	92	9.30 x 10^7 A	0.780 x 10^7 A	92
DFM	0.17 x 10^7 B	0.068 x 10^7 B	6	0.17 x 10^7 B	0.068 x 10^7 B	60
ENZ	0.14 x 10^7 B	0.110 x 10^7 B	21	0.09 x 10^7 B	0.110 x 10^7 B	88
DFM+E	0.78 x 10^7 B	0. 680 x 10^7 B	13	0.45 x 10^7 B	0.200 x 10^7 B	56
Treatments*	60 Days					
	Total coliforms (MPN 100 mL^{-1})		Efficiency (%)	Thermotolerant coliforms (MPN 100 mL^{-1})		Efficiency (%)
	Influent	Effluent		Influent	Effluent	
NC	9.30 x 10^7 A	0.065 x 10^7 A	99	9.30 x 10^7 A	0.065 x 10^7 A	99
DFM	0.17 x 10^7 B	0.004 x 10^7 B	97	0.17 x 10^7 B	0.004 x 10^7 B	97
ENZ	0.14 x 10^7 B	0.012 x 10^7 B	91	0.09 x 10^7 B	0.008 x 10^7 B	91
DFM+E	0.78 x 10^7 B	0.020 x 10^7 B	97	0.45 x 10^7 B	0.018 x 10^7 B	96
Treatments*	85 Days					
	Total coliforms (MPN 100 mL^{-1})		Efficiency (%)	Thermotolerant coliforms (MPN 100 mL^{-1})		Efficiency (%)
	Influent	Effluent		Influent	Effluent	
NC	9.30 x 10^7 A	0.0005 x 10^7 A	99	9.30 x 10^7 A	0.0005 x 10^7 A	99
DFM	0.17 x 10^7 B	0.0002 x 10^7 B	99	0.17 x 10^7 B	0.0002 x 10^7 B	99
ENZ	0.14 x 10^7 B	0.0001 x 10^7 B	99	0.09 x 10^7 B	0.0001 x 10^7 B	99
DFM+E	0.78 x 10^7 B	0.0002 x 10^7 B	99	0.45 x 10^7 B	0.0002 x 10^7 B	99

*NC: negative control; DFM: NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ: diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E: ENZ + DFM. **$p \leq 0.05$. A-B: means followed by different letters within a column are significantly different ($p \leq 0.05$) according to Tukey test.

Table 5 – Daily and accumulated volume (mL), potential of gas production by Total Solids (TS) and Volatile Solids (VS) added and reduced (mL kg^{-1}), from litter of broilers fed diets with direct fed microbials (DFM) and enzymes, treated in continuous bio-digesters.

Treatments*	Volume		Potential			
			TS		VS	
	Daily	Accumulated	Added	Reduction	Added	Reduction
	mL		mL kg^{-1}			
NC	6.30 10^3 A	0.53 10^6 A	0.44 10^6 A	0.88 10^6 B	0.72 10^6 B	1.02 10^6 C
DFM	5.50 10^3 B	0.47 10^6 B	0.38 10^6 B	0.80 10^6 B	0.76 10^6 B	1.40 10^6 B
ENZ	6.40 10^3 A	0.55 10^6 A	0.46 10^6 A	1.00 10^6 A	0.94 10^6 A	1.74 10^6 A
DFM+E	5.80 10^6 B	0.48 10^6 B	0.41 10^6 B	0.88 10^6 B	0.75 10^6 B	1.25 10^6 B
F values	6.96	6.99	10.17	11.92	21.25	65.48
P values	0.006**	0.006**	0.001**	0.0007**	<0.0001**	<0.0001**
CV[1] (%)	5	5	5	5	5	6

[1]Coefficient of variation;*NC = negative control; DFM = NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ = diet formulated with an enzyme blend (20 ppm phytase, 200 ppm protease and 200 ppm xylanase); DFM+E = ENZ + DFM; **$p \leq 0.05$; [NS] non-significant; A-B = means followed by different letters within a column are significantly different ($p \leq 0.05$) according to Tukey test.

in biogas production per kg of added TS. Therefore, data were contradictory to those in the present study, where treatments containing DFM obtained lower production of methane and biogas. Highest biogas production was obtained in the litter of birds fed with diet containing enzyme complex (Table 5).

According to Kocher et al. (2003), the feed enzymes cause the rupture of fiber cell walls, degrade proteins, and decrease the effects of antinutritional factors, so that nutrients become more available both for the animal and the anaerobic bacteria present in biodigesters. The higher nutrients' availability increases biogas production.

Conclusions

When added to the diet of broilers, enzymes increase the amount of organic matter and nutrients in poultry litter. DFM and DFM + E inhibited the production of biogas and methane.

Table 6 – Daily volume (mL) and composition of biogas, methane (CH_4), carbon dioxide (CO_2) and other gases (mL and %) produced from litter of broilers fed diets with direct fed microbials (DFM) and enzymes, treated in continuous bio-digesters.

Treatments*	Volume (mL)			Proportion (%)		
	Biogas	CH_4	CO_2	CH_4	CO_2	Other gases
NC	6.30 10^3 A	4.40 10^3 A	1.70 10^3 AB	71	28	1.18
DFM	5.50 10^3 B	4.00 10^3 B	1.05 10^3 B	72	27	1.28
ENZ	6.40 10^3 A	4.50 10^3 A	1.80 10^3 A	71	28	1.11
DFM+E	5.80 10^6 B	4.10 10^3 B	1.60 10^3 AB	71	28	1.08
F values	6.96	7.77	5.25	0.83	1.89	0.17
P values	0.006**	0.004**	0.015**	0.50^{NS}	0.19^{NS}	0.91^{NS}
CV[1] (%)	5	5	7	1	3	4

[1]Coefficient of variation; *NC = negative control; DFM = NC + 500 ppm of direct fed microbials (DFM) with *Bacillus subtilis* and *Bacillus licheniformis*; ENZ = diet formulated with an enzyme blend (20 ppm phytase, 200 ppm de protease and 200 ppm de xylanase); DFM+E = ENZ + DFM; **$p \leq 0.05$; ^{NS}non-significant; A-B = means followed by different letters within a column are significantly different ($p \leq 0.05$) according to the Tukey test.

Treating broiler litter in continuous bio-digesters reduces pH, total solids, volatile solids, fibers that are difficult to degrade, and fecal and thermotolerant coliforms present in their effluents, thus reducing the pollutant potential of residues from poultry production. More non-complexed nutrients become available for use in soil, particularly nitrogen and potassium.

However, despite the bacteria being removed after the digestion process, a significant number of pathogens were found, which means that auxiliary treatment processes are needed in order to minimize environmental pollution.

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