

## Effect of different inoculations on biogas and methane production through anaerobia biodigeston using residues from the avícola sector

Joseane Bortolini<sup>I</sup>, Ana Claudia Guedes Silva<sup>II</sup>, Maria Hermínia Ferreira Tavares<sup>III</sup>, Dayane Taine Freitag<sup>IV</sup>, Leandro Fleck<sup>V</sup>, Osvaldo Kuczma<sup>VI</sup>

### ABSTRACT

Anaerobic digestion has become a viable treatment technique for poultry litter, generating biofertilizer and biogas. Thus, the present study aimed to analyze the use of different digestates as inocula for the anaerobic digestion of poultry litter, evaluating the biogas and methane production. Two types of inocula (bovine and swine biofertilizer) were used, with feeding loads of 0.67, 1.00 and 1.67 gVS L<sup>-1</sup> day<sup>-1</sup>. Statistical analyses followed a split-plot design, where the main plot being inoculum and the feeding load as secondary. The experimental data were submitted to analysis of variance, at the level of 5% of significance. The following variable responses were considered: biogas production, specific biogas production as a function of volatile solids (VS) added to the biodigester, specific biogas production as a function of organic load, in terms of COD, and methane production. The highest rates of average biogas production, specific biogas production by VS added, specific production of biogas as a function of COD, and average methane production occurred with the use of bovine inoculum. Through the analysis of the total average percentage of methane production, productions of 63.0% and 54.5% for bovine and swine inoculum, respectively, are observed. Considering the results obtained, the use of bovine inoculum for the process of anaerobic digestion of poultry litter is recommended vinculado a produção de biogás e metano.

**Keywords:** Biofertilizer; Feeding load; Poultry production

### 1 INTRODUCTION

Within the food production sector, poultry increase in developing countries deserves special mention given the low cost of meat and eggs (EUROSTAT, 2016). According to SINDIVIAPAR (2018), the state of Paraná was responsible for 35.17% of

<sup>I</sup> Universidade Estadual do Oeste do Paraná, PR, Brasil - josi\_bortolini@hotmail.com

<sup>II</sup> Universidade Estadual do Oeste do Paraná, PR, Brasil - josi\_bortolini@hotmail.com

<sup>III</sup> Universidade Estadual do Oeste do Paraná, PR, Brasil - maria.tavares@unioeste.br

<sup>IV</sup> Universidade Estadual do Oeste do Paraná, PR, Brasil - dayane.freitag@gmail.com

<sup>V</sup> Universidade Estadual do Mato Grosso do Sul, MT, Brasil - fleckmissal@gmail.com

<sup>VI</sup> Universidade Estadual do Oeste do Paraná, PR, Brasil - osvaldokuczman@hotmail.com



chicken production in Brazil in 2017, in in this same year 1.79 billion chickens were slaughtered, which stands for 5,14% more than in 2013. As domestic chicken production increases, larger quantities of poultry litter are generated, with the need to increase process efficiency and reduce production costs (SANTOS, MALHEIROS, TAVEIRA, 2017).

Essential during broiler breeding, the poultry litter provides a healthy, safe environment to the flock, preventing direct contact with moisture and microorganisms. On the other hand, a large volume of wastes is generated annually, affecting natural resources (PAULINO et al., 2019).

As a result of this the use of residues poultry sector represent a great opportunity for biogas generation and its use as an alternative source of energy (RIBEIRO et al., 2018), in this sense one of the efficient techniques for poultry litter treatment is anaerobic digestion, which according to Peres et al., (2019) and Markou (2015) has the main advantage of generating biogas with high energy content. The anaerobic digestion consists of an ecosystem model in which different groups of microorganisms, under oxygen restricted conditions, work interactively converting complex organic matter to methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), water (H<sub>2</sub>O), hydrogen sulfide (H<sub>2</sub>S), ammonia (NH<sub>3</sub>+), and new bacterial cells (GUERI, DE SOUZA, KUCZMAN, 2017). To maximize biogas production and maintain its stability, the directly influencing factors should be monitored (TAVARES et al., 2016), among these variables are pH, temperature, alkalinity, volatile acidity, hydraulic retention time, organic load, and concentration of total volatile solids (SILVA, 2018).

Sagula, da Costa, de Lucas Junior (2017) mentioned several factors able to inhibit the anaerobic digestion, standing out the following: ammoniacal nitrogen concentration, temperature, pH, heavy metals, and inoculum acclimatization. In this regard, Shah et al. (2014) affirmed that the inoculum, which is responsible for complex organic matter degradation, determines the biogas production potential and the speed of anaerobic digestion.

As the bed of chicken is being produced in large quantity, due to the increasing increase of poultry cutting in recent years, in a comparison between the Brazilian production of chicken meat in 2018 and the one estimated for 2019, an average growth of 1.8% is estimated (USDA, 2019).

In addition, another important fact was the prohibition by the Ministry of Agriculture, Livestock and Supply (MAPA) of the use of this residue to feed ruminants (Normative Ruling N°. 15, July 17, 2001) that poultry farmers sold this waste as a nutritional input for cattle ranchers. Consequently, the producers had to seek other means of utilization and / or treatment for the bed.

Analyzing the two facts mentioned above, it is perceived that the treatment of this residue should be considered as an intrinsic action to the production of chickens, and the cost of this treatment should be included in the cost of production of the activity, in order to provide sustainability to this productive chain (KONRAD et al., 2018, MILANEZ et al., 2018). Therefore, our study aims to use bovine and swine biofertilizers as inocula in the anaerobic digestion of poultry litter, since changes in the population of microorganisms can influence the stability of the process, in the organic conversion rate, as well as the rate of variation of yield and biogas composition (DI WU et al., 2019).

## 2. MATERIAL E METHODS

### 2.1. Poultry litter

Poultry litter (PL) from a farm in Quatro Pontes – PR (Brazil) was used as a substrate for the anaerobic digestion process. First, the waste was characterized with respect to the following parameters: hydrogenionic potential (pH), moisture, total, fixed and volatile solids, chemical oxygen demand (COD), and carbon/nitrogen ratio (C: N), following the methods in Table 1.

Table 1 - Methods used in the characterization of the poultry litter

Parameter	Method
pH	Method 4500 B (APHA, 2012)

Moisture	Gravimetric method
Total, Fixed, and Volatile Solids	Method 2540B (APHA, 2012)
COD	Method 5220B (APHA, 2012)
Total Carbon	Furnace Method
Total Nitrogen	Method 4500 (APHA, 2012)

Source: authors

Before feeding biodigesters, the poultry litter underwent disaggregation and sieving pretreatments to facilitate biodigestion.

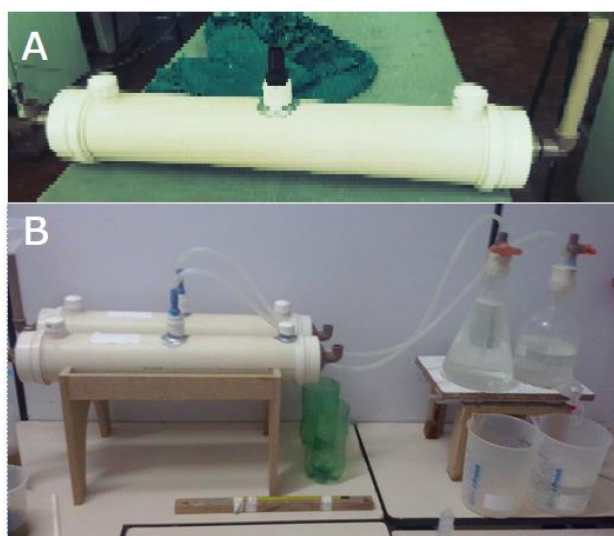
## 2.2 Swine and bovine inocula

Digestates from two biodigesters were used as inocula for the anaerobic digestion: swine inoculum (S) from a biodigester in the city of Cascavel, and bovine inoculum (B) from another in the town of Céu Azul, both in Paraná state (Brazil). These materials were characterized for pH, total, fixed, and volatile solids, following the methods in Table 1.

## 2.3 Anaerobic digestion process

The experimental tests were performed in two horizontal digesters, made of PVC, with a useful volume of 4 L (0.60 m long x 0.10 m in diameter). These reactors contained an inlet for feeding (1a) and an outlet for effluent discharging (1b).

Figure 1 - Experimental module for feeding (a) and outlet for effluent discharging (b)



Source: authors

The experimental tests occurred in a closed environment with low thermal variation. The average operating temperature of the system was  $29.5 \pm 1.5$  °C. According to HUSSAIN & DUBEY (2015), in mesophilic conditions, anaerobic biodigesters must operate between 25 and 37 °C.

The system pH was daily monitored, considering its influence on fermentation (MÉNDEZ-ACOSTA et al., 2013), it ranged from 6.8 to 7.2, which is the most favorable range for anaerobic digestion (WARD et al., 2008).

Aliquots of 1.2 L inoculum and 2.8 L poultry-litter solution ( $\text{g}_{\text{residue}}: \text{mLH}_2\text{O} - 1:20$ ) were added to the biodigesters, differing only regarding the used inoculum (swine or bovine).

After 24-hour inoculation, a daily biodigester feeding started in a semi-continuous system for full removal of dissolved oxygen at trace levels. The feeding loads were of 0.67 (L1), 1.00 (L2), and 1.67 (L3)  $\text{gVS L}^{-1} \text{ day}^{-1}$ , at 2% volatile solids (VS) concentration and operating times of 74, 69, and 66 days, respectively, also including a stabilization period.

## 2.5 Process monitoring

The process was monitored for pH, volatile acidity (VA), total alkalinity (TA), intermediate alkalinity (IA), and partial alkalinity (PA). The pH was determined daily on samples from the biodigester output. Moreover, the ratios VA/TA and IA/PA were assessed based on methods of Silva (1977), the first should be maintained between 0.1 and 0.5 (Ripley et al., 1986), whereas the second below 0.3 since above this value fermentation may be disturbed.

## 2.6 Biogas and methane production

Biogas production can be measured by volumetric methods as in a specific methanogenic activity (SMA) test. In the literature are found three commonly used methods: a) biogas volume and composition measurements, b) biogas composition, and c) methane volume direct measurement. The major difference between the first and last method is that to measure only methane volume, one should wash the

biogas using a sodium hydroxide solution to enable carbon dioxide absorption (AQUINO et al., 2007). In addition, we used Mariotte bottles for biogas collection since they are easy to handle and present reliable results.

Therefore, the biogas production was measured by volume displacement of acidified saline solution between Mariotte bottles coupled to each biodigester. This salt solution is composed of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and sodium sulfate decahydrate (Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O) and has the purpose of avoiding the dissolution of gases from the process, which makes quantification more accurate.

The biogas volume was corrected for the standard conditions of temperature and pressure (1 atm and 273.15 K) using the Clapeyron equation (1):

$$\frac{P_1 * V_1}{T_1} = \frac{P_2 * V_2}{T_2}$$

Where:

P<sub>1</sub>= Standard condition of pressure (1 atm),

T<sub>2</sub>= Standard condition of temperature (273.15 K),

V<sub>1</sub>= Corrected gas volume,

P<sub>2</sub>= Pressure at biogas reading,

T<sub>2</sub>= Temperature at biogas reading,

V<sub>2</sub>= Biogas volume reading.

For methane volume, the produced biogas was washed with a concentrated sodium hydroxide solution (NaOH) with the objective of dissolving carbon dioxide (CO<sub>2</sub>). Thus, while the solution passes through the bottle, it retains the CO<sub>2</sub>, allowing the passage of other gases such as the methane (CH<sub>4</sub>). According to Aquino et al. (2007), this procedure assumes that carbon dioxide and methane are the main constituents of the biogas. This consideration is valid since at neutral pH most of the ammonia and half of the hydrogen sulfide, if present, will be ionized and dissolved in the liquid phase.

## 2.7 Inhibitions

A limiting factor for the anaerobic digestion of poultry litter is biogas production inhibition as a function of the ammoniacal nitrogen concentration (YENIGÜN & DEMIREL, 2013). In this context, we monitored the concentration of ammoniacal nitrogen, in the form of free ammonia, according to APHA (2012).

## 2.8 Statistical analyses

With the aid of the Minitab Statistical Software, version 18 (MINITAB, 2018), the experimental data were subjected to normality test at 5% level of significance and, when necessary, Box-Cox transformations were performed.

Statistical analyses followed a split-plot design, with inoculum level as the main factor (two levels: B and S) and feeding load as the secondary one (with three levels: L1, L2, and L3), using the SISVAR free software, version 5.3 (FERREIRA, 2010). The following response variables were considered: biogas production, specific biogas production as a function of volatile solids, specific biogas production as a function of organic matter (in terms of COD), and methane production.

Data were submitted to analysis of variance (ANOVA) at 5% significance level, and means compared by the Tukey's test at a 95% confidence interval.

## 3 RESULTS AND DISCUSSION

### 3.1 Substrate and inoculum physicochemical characterizations

Table 2 shows the physicochemical characterization of both poultry litter and inocula (bovine and swine). The pH ranged between 7.34 and 7.90 for both factors. This is an important fact for an anaerobic digestion where pH exerts a significant influence (Latif et al., 2017). Methane-producing microorganisms showed optimum growth within a pH range of 6.6 to 7.4, however, stability could be reached within a wider range, from 6.0 to 8.0 (CHERNICHARO, 1997).

The concentration of solids in the bovine inoculum was higher than that in the swine one, which can be explained by the diet of each species. For Orrico et al., (2016), the animal diet interfere significantly with the composition of its droppings. The COD concentration of the substrate was 7306.36 mg L<sup>-1</sup>. According to Kawai et al., (2016), COD has been used as an indicator of anaerobic digestion efficiency in terms of organic matter reduction.

Table 2 - Physicochemical characterization of the substrate and the inoculum.

Material	Parameter	Poultry litter	
Substrate	pH	7.90	
	TS (%)	85.00	
	FTS (%)	36.00	
	VTS (%)	79.00	
	Moisture (%)	12.30	
	COD (mg L <sup>-1</sup> )	7306.36	
	Ratio C/N	24	
		Bovine	Swine
Inoculum	pH	7.34	7.65
	TS (g L <sup>-1</sup> )	5.44	2.46
	FTS (g L <sup>-1</sup> )	2.65	1.78
	VTS (g L <sup>-1</sup> )	2.79	0.68

Source: authors

### 3.2 Anaerobic biodigester stability

Stability of the biodigesters was monitored based on the ratios VA/TA and IA/PA. As for Drogg (2017), alkalinity concentration cannot be generalized since stability limits are defined according to each biogas plant specificities. The maximum limits of VA/TA ratio for anaerobic process stability can vary from 0.3 to 0.8, with higher values indicating instability.

For Ripley et al. (1986), when the ratio IA/PA is above 0.3, disturbances may occur in an anaerobic digestion. Conversely, Fleck et al. (2017) stated that it is possible to



observe stability in IA/PA values higher than 0.3, as per the specific characteristics of each substrate.

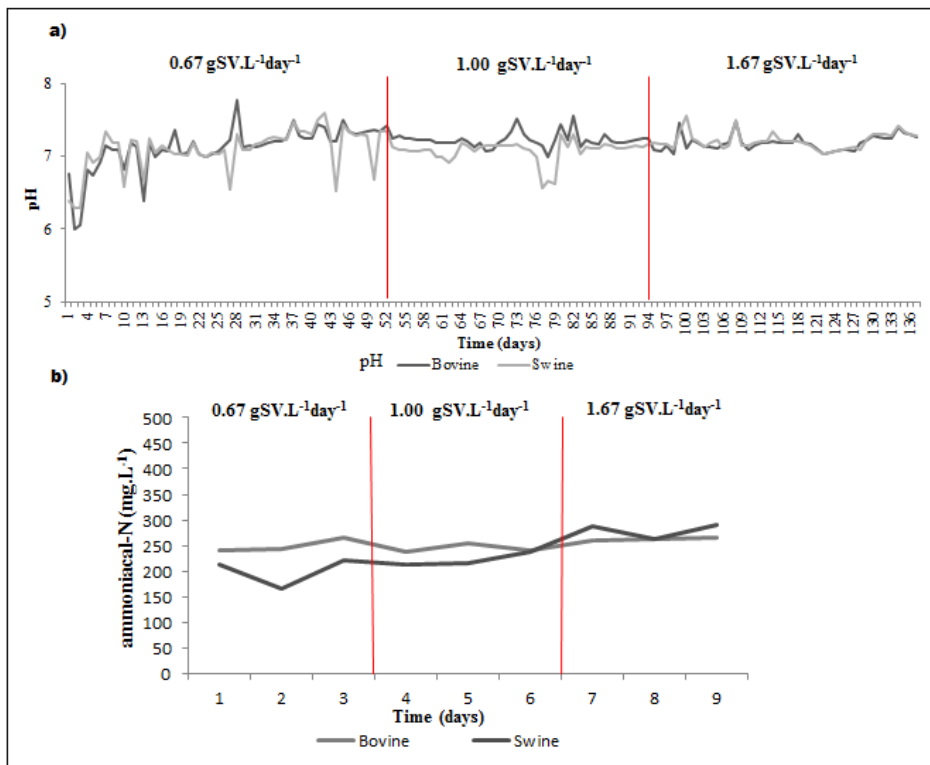
Based on this information, we considered the evaluated process as stable from the 16<sup>th</sup> operation day on, so data collection could be started, with the objective of monitoring the stabilization of the degradation process and the maximization of methane production, since it is still considered a great challenge to maintain a stable process with biogas production in association with a high percentage of biomass utilization (DI WU, et al., 2019).

### 3.3 Monitoring of pH and ammoniacal nitrogen

Figure 2 displays the variation of pH (2a) and ammoniacal nitrogen concentration -  $\text{NH}_4^+$  (2b) during the anaerobic digestion. As shown in Figure 2a, pH was stable, and loads of 1.00 (L2) and 1.67 (L3)  $\text{gVS L}^{-1} \text{ day}^{-1}$  had the highest values, being 7.23 and 7.22 for bovine and swine inoculum, respectively. Chernicharo (1997) evidenced these values when reporting that methane-forming microorganisms have optimum growth at pH values between 6.6 and 7.4.

According to Dębowski et al. (2018), the decrease in pH indicates anaerobic digestion instability due to the presence of ammoniacal nitrogen, resulting in volatile fatty acids (VFAs) accumulation. Even though no variations were observed in relation to pH, the presence of ammoniacal-N was monitored throughout the process. As shown in Figure 2b, the highest ammoniacal-N concentration ( $280.51 \text{ mg L}^{-1}$ ) occurred with a load of  $1.67 \text{ gVS L}^{-1} \text{ day}^{-1}$  of swine inoculum.

Figure 2 - Experimental monitoring measuring pH (a) and Ammoniacal N (b)



Source: authors

For Parra-Orobio, Torres-Lozada and Marmolejo-Rebellón (2017), concentrations of ammoniacal-N up to 200 mg L<sup>-1</sup> are beneficial for the anaerobic digestion, presenting harmful effects only at concentrations higher than 1000 mg L<sup>-1</sup>. Thus, we concluded that there was no deleterious effect of ammoniacal-N on the assessed anaerobic digestion.

Orrico Junior et al. (2016) investigated the anaerobic digestion of swine manure with the addition of 434.5, 614.6, and 757.1 mg L<sup>-1</sup> ammoniacal-N and observed the absence of process inhibition by these ammonia concentrations, as the calculated doses were below the reported limits in literature.

### 3.4 Biogas production

For biogas production, feeding load and inoculum factors had a significant effect on the anaerobic digestion process, as the p-value obtained was lower than the 5% significance level adopted here.

The test of means for biogas production as a function of inoculum and feeding load factors is displayed in Table 3. The highest average biogas yield (0.511 Lbiogas day<sup>-1</sup>) was reached using bovine inoculum, being statistically different from the swine one. Yet for feeding load (L1, L2, and L3), the highest average biogas production (0.627 Lbiogas day<sup>-1</sup>) was attained by loading 1.67 gVS L<sup>-1</sup> day<sup>-1</sup> (L3). The average productions of biogas as a function of loads differed statistically from one another at a 95% confidence interval.

Thus, the results, together with Mohammad Roman Miah et al. (2016) who observed that a bed of chicken mixed with a proportion of cow manure presented a high potential of biogas generation by anaerobic digestion.

In addition, it should be noted that increased loads increased biogas production, where the highest production was 1.67 gVS L<sup>-1</sup> day<sup>-1</sup>. This result corroborates with Yadvika et al. (2004), who reported results from a similar study in Pennsylvania, in which the organic load ranged from 346 kgVS day<sup>-1</sup> to 1030 kgVS day<sup>-1</sup>, they noticed an increase in gas production from 67 to 202m<sup>3</sup> day<sup>-1</sup>, respectively.

Table 3 - Tukey's test for the comparison of means

Factor	Production
Inoculum	L <sub>biogas</sub> day <sup>-1</sup>
Bovine	0.511 A
Swine	0.264 B
Feeding loads (gVS L <sup>-1</sup> day <sup>-1</sup> )	
L1 - 0.67	0.168 C
L2 - 1.00	0.412 B
L3 - 1.67	0.627 A

Note: Equal letters in the column do not differ by Tukey's test at 5% level of significance.

Source: authors

Numerous studies have evaluated biogas production from anaerobic digestion with poultry litter as a substrate. Silveira et al. (2014) evaluated biogas production in mini-biodigesters made from polyethylene terephthalate (PET) bottles and supplied with poultry litter. These authors assessed the use of three water proportions (90, 80, and 70%) and concluded that a 70:30 blend ratio (water: substrate) obtained the largest yield of biogas (6353.3 mL).

Suzuki et al. (2012) evaluated different ratios of mixtures between poultry litter and manipueira (cassava wastewater) with the objective of producing renewable energy. Although they concluded that poultry litter dilution in manipueira has unsatisfactory results, single-phase digesters fed only poultry litter reached a good methane yield, they also found that 100% poultry litter was the most successful in biogas production (573.1 cm<sup>3</sup>), serving as a comparison for the other treatments.

### 3.5 Specific biogas production per volatile solids

Regarding specific biogas production per added volatile solids (VS), both feeding load and inoculum were statistically significant at 95% confidence interval. Table 4 presents the Tukey's test for specific production of biogas as a function of added VS for each inoculum and feeding load used. The highest production occurred with the use of bovine inoculum (0.786 L<sub>biogas</sub> gVS<sup>-1</sup>), being significantly different from the swine one.

Table 4. Tukey's test for the specific biogas production per volatile solids

Factor	Specific production
Inoculum	L <sub>biogas</sub> gVS <sup>-1</sup>
Bovine	0.786 A
Swine	0.328 B
Feeding loads (gVS L <sup>-1</sup> day <sup>-1</sup> )	
L1 - 0.67	0.588 A
L2 - 1.00	0.696 A
L3 - 1.67	0.388 B

Note: Equal letters in the column do not differ by Tukey's test at 5% level of significance.

Source: authors

When comparing the feeding loads, the highest average production was recorded for 1.00 gVS L<sup>-1</sup> day<sup>-1</sup> (0.696 L<sub>biogas</sub> g Vs<sup>-1</sup>). In addition, the average productions for the loads of 0.67 and 1.00 gVA L<sup>-1</sup> day<sup>-1</sup> presented no statistical difference from one another, at a 95% confidence interval.

If compared to swine inoculum, the biogas production using bovine inoculum was about 2.4 times higher. Moreover, the production of biogas per added VS

complies with the reference value obtained by the Biochemical Methane Potential Methane (BMP) test for the poultry litter under study, i.e. 0.5 Lbiogas gVS-1.

Several studies have reported biogas production as a function of VS added to biodigesters. Bouallagui et al., (2009) obtained biogas production ranging from 0.44 to 0.61 Lbiogas gVS-1, with a feeding load of 2.5 gSV L-1 day-1, and using slaughterhouse effluent as substrate.

Fukayama et al. (2009) evaluated the production of biogas with poultry litter reused at different times. They observed that the average productions of biogas were of 0.401, 0.410, 0.400, and 0.520 m3biogas gVS-1 for the first, second, third, and fourth lots, respectively. The main conclusion of the study refers to an increase in biogas production with the reuse of broiler litter.

### 3.6 Specific biogas production as a function of COD

Concerning specific production per COD, the ANOVA showed a significant interaction at 5% significance, thus, a statistical breakdown was required. Table 5 shows the breakdown of means for specific biogas production as a function of COD. When fixing the feeding load parameter, we observed no significant differences in the inoculum levels at 5% significance. Unlike, as the inoculum factor was fixed, we noted a statistically significant difference between the loads of 0.67 and 1.00 gVS L-1 day-1. In addition, the highest specific biogas yields as a function of COD were obtained with bovine inoculum at the feeding loads of 1.325 and 1.211 Lbiogas gCOD-1, respectively. The feeding load of 1.67 gVS L-1 day-1 presented no significant statistical difference at 5% level of significance.

Table 5 - Tukey's test for the comparison of means for specific production of biogas as a function of COD

Inoculum	Specific Production (L <sub>biogas</sub> g COD <sup>-1</sup> )		
	0.67 gVS L <sup>-1</sup> day <sup>-1</sup>	1.00 gVS L <sup>-1</sup> day <sup>-1</sup>	1.67 gVS L <sup>-1</sup> day <sup>-1</sup>

Bovine	1.32 5	A a	1.21 1	A a	1.12 1	B a
Swine	0.39 4	B a	0.51 6	B a	0.84 7	B a

Note: Means followed by the same uppercase letters in the columns (inoculum) and lowercase in the lines (feeding loads) do not differ by the Tukey's test at 5% significance.

Source: authors

The average specific production of biogas as a function of COD was higher with the use of bovine inoculum. The largest production was  $1.325 \text{ L}_{\text{biogas}} \text{ gCOD}^{-1}$ , being obtained with a feeding load of  $0.67 \text{ gVS L}^{-1} \text{ day}^{-1}$ . These results corroborate those by Tessaro et al., (2015), who claimed the use of poultry litter associated with bovine biofertilizer as the best for biogas production, with or without water addition.

We should highlight the use of bovine inoculum as more feasible for biogas production, not only for production quantity compared to the swine biofertilizer, but also for its fastness since the beginning of the first feeding load. The reverse is also true, which may be related to the first stage of anaerobic digestion (hydrolysis) when the high contents of cellulose in poultry litter were degraded more slowly.

### 3.7 Methane production

For methane production, the feeding load and inoculum factors were statistically significant at 95% according to the ANOVA. Table 6 shows the Tukey's test for methane production. For inoculum, the highest average methane production ( $0.311 \text{ L}_{\text{methane}} \text{ day}^{-1}$ ) occurred using bovine biofertilizer, with a significant statistical difference.

The highest methane production using bovine inoculum is related to a greater easiness of microorganisms to degrade the poultry-litter constituents. According to Shah et al., (2014), cellulose is the most significant biopolymer in solid waste and can be digested anaerobically. However, a large variety of enzymes is required for this extensive enzymatic hydrolysis. These enzymes are found in large amounts in the

rumen, mainly due to three bacterial species: *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* (IZUDDIN et al., 2018).

According to Li et al. (2019), any enhancement of the stages of the biodigestion process (hydrolysis, acidification, acetogenesis and methanogenesis), inoculation, will lead to an increase in methane generation, which is in agreement with the presented results, since the inoculation with the biofertilizer was more favorable to the production of methane.

Still in relation to the methane production, it was observed that the loads of 0.67 and 1.67 gVS L<sup>-1</sup> day<sup>-1</sup> did not present statistical difference among themselves at 5% of significance. However, the load of 1.00 gVS L<sup>-1</sup> day<sup>-1</sup> differs statistically from the others, with the highest average methane production (0.392 Lmethane day<sup>-1</sup>). A decrease in methane production was observed by increasing the load from L2 to L3, which was also reported by Yadvika et al. (2004). Salminen & Rintala (2002) reported methane yields around 0.55 m<sup>3</sup> methane kgVS<sup>-1</sup>.

Table 6 - Tukey's test for methane production

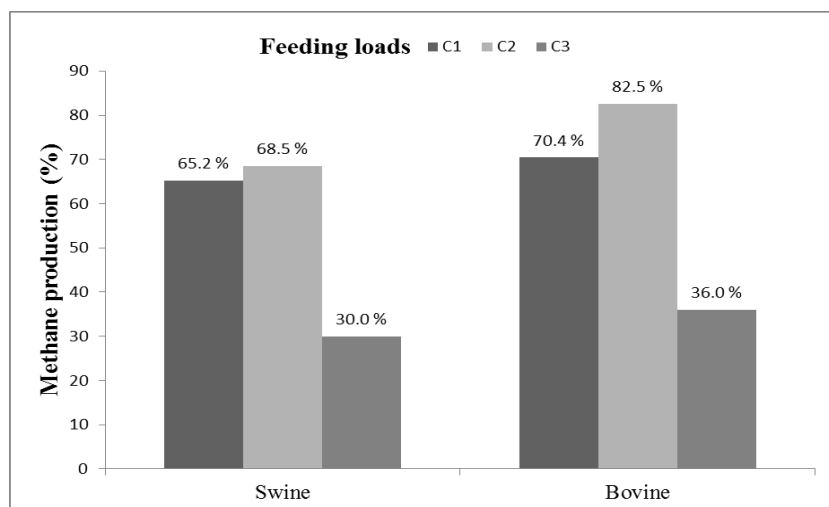
<b>Factor</b>	<b>Methane production</b>
Inoculum	L <sub>methane</sub> day <sup>-1</sup>
Bovine	0.311 A
Swine	0.083 B
Feeding loads (gVS L <sup>-1</sup> day <sup>-1</sup> )	
L1 - 0.67	0.094 B
L2 - 1.00	0.392 A
L3 - 1.67	0.105 B

Note: Equal letters in the column do not differ by Tukey's test at 5% level of significance.

Source: authors

Fukayama (2008) obtained on average 82.5% methane in an anaerobic digestion using first-batch poultry litter constituted by peanut shell as a substrate. Similarly, we obtained a close result using bovine waste as inoculum, at a feeding load of 1.00 gVS L<sup>-1</sup> day<sup>-1</sup>, as shown in Figure 3.

Figure 3 - Methane production



Source: authors

During the last loading, a reduction of methane production was observed for both inocula. Moreover, when comparing biogas and methane productions, we can observe a similarity between L1 and L3 for biogas, among which L3 reached higher amounts (Table 7). Thus, we can conclude that the percentage of methane, in relation to the total volume of biogas produced, was higher in conditions of lower biogas production.

Table 7. Average productions of biogas and methane

Inoculum	Feeding loads (gVS L <sup>-1</sup> day <sup>-1</sup> )	Biogas (L day <sup>-1</sup> )	Methane (L day <sup>-1</sup> )
Bovine	L1 0.67	0.1831	0.1290
	L2 1.00	0.7618	0.6288
	L3 1.67	0.4864	0.1752
Swine	L1 0.67	0.0906	0.0590
	L2 1.00	0.2269	0.1555
	L3 1.67	0.1162	0.0344

Source: authors



The total average percentage of methane production showed values of 63.0% and 54.5% for bovine and swine inoculum, respectively. Kelleher et al. (2002) reported that anaerobic digestion using poultry litter as substrate generates biogas with a methane content of 60%. According to Ribeiro et al., (2016) the authors analyzed the viability for the production of biomes from chicken manure, and the best biogas production verified in the experiments was  $0,36 \text{ m}^3_{\text{biogás}} \cdot \text{kg ST}^{-1}$  with a methane content of 63%.

#### 4 CONCLUSIONS

It is observed a viability of the biogas and methane production associated to the inoculum use possible to conclude the biofertilizers are feasible as inoculum in a poultry-litter anaerobic digestion. Based on our results, both bovine and swine inocula are indicated for this process, presenting stability and pH close to neutrality, without any inhibition by ammoniacal nitrogen.

Particularly, the use of bovine inoculum could be most beneficial since it shows higher values of total and specific biogas yields as a function of VS, specific biogas production as a function of COD, and methane production if compared to those with swine inoculum.

#### 5 ACKNOWLEDGMENTS

The authors would like to thank the Graduate Program in Agricultural Engineering of the State University of Western Paraná, to the Coordination for Higher Education Staff Development (CAPES) and to the Araucaria Foundation for granting Master's, Scientific Initiation, and Research Productivity scholarships.

#### REFERENCES

APHA. **Standard Methods for the Examination of Water and Wastewater**. 21th ed. Washinton, DC – EUA: Ed. American Public Health Association, 2005. 1600 p.

AQUINO SF, CHERNICHARO CAL, FORESTI E. **Metodologias para determinação da Atividade Metanogênica Específica (AME) em lodos anaeróbios.** Engenharia Sanitária e Ambiental, v. 12, n. 2, 2007, p. 192-201.

AQUINO SF, CHERNICHARO CAL. **Acúmulo de ácidos graxos voláteis (AGVs) em reatores anaeróbios sob estresse: causas e estratégias de controle.** Engenharia Sanitária e Ambiental, v 10, n 2, 2005, p.152-161.

AVILA VS, ABREU VMN, FIGUEIREDO EAP, BRUM PAR, OLIVEIRA U. **Valor Agrônômico da Cama de Frangos após Reutilização por Vários Lotes Consecutivos.** Comunicado Técnico 466 – Embrapa, 2007, 4p.

BARCELOS BR. **Avaliação de diferentes inóculos na digestão anaeróbia da fração orgânica de resíduos sólidos domésticos.** 2009. 75f. Dissertação Mestrado (Tecnologia Ambiental e Recursos Hídricos). Departamento de engenharia civil e ambiental da faculdade de tecnologia da universidade de Brasília, BRASÍLIA –DF,2009.

BOLAN NS, SZOGI AA, CHUASAVATHI T, SESHADRI B, ROTHROCK JRMJ, PANNEERSELVAM P. **Uses and management of poultry litter.** World's Poultry Science Journal, v. 66, 2010, p. 673-698.

BOUALLAGUI H, LAHDHEB H, BEN ROMDAN E, RACHDI B, HAMDY M. **Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition.** Journal of Environmental Management. Tunis, n.90, 2009, p. 1844-1849.

BRASIL. **Instrução Normativa nº 8,** de 25 de março de 2004. Ministério da Agricultura, Pecuária e Abastecimento.

CARREAS N. **O biogás.** Programa de capacitação em energias renováveis – ONUDI. 2013, p.151.

CHERNICHARO CAL. **Reatores anaeróbios: princípios do tratamento biológico de águas residuárias.** 2 ed. Belo Horizonte: UFMG, 1997.

CHOI IH, MOORE JRPA. **Effect of Various Litter Amendments on Ammonia Volatilization and Nitrogen Content of Poultry Litter.** J. Appl. Poult. Res. v.17, 2008, p.454–462.

COSTA JC, BARBOSA SG, ALVES MM, SOUSA DZ. **Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter.** Bioresource Technology, 2012, p. 141–147.

DĘBOWSKI M, ZIELIŃSKI M, KISIELEWSKA M, KRZEMIENIEWSKI M, MAKOWSKA M, GRĄDKOWSKI M, TOR-ŚWIĄTEK A. **Simulated dairy wastewater treatment in a pilot**

**plant scale magneto-active hybrid anaerobic biofilm reactor (MA-HABR).** Brazilian Journal of Chemical Engineering, 35(2), 2018, p. 553–561.

DI WU, LEI LI, XIAOFEI Z, YUN P, PINGJIN Y, XUYA P. **Anaerobic digestion: A review on process monitoring.** Renewable and Sustainable Energy Reviews. v. 103, 2019, p. 1-12.

DROSG, B. **Process monitoring in biogas plants.** IEA Bioenergy, ISBN 978-1-910154-03-8. 2017.

EUROSTAT. **Agriculture, forestry and fishery statistics 2015 edition.** European Statistics. Luxembourg: Publications Office of the European Union, 2016.

FERNANDEZ CG, ENCINA PAG. **Impacto of substrate to inoculum ratio in anaerobic digestion of swine slurry.** Biomass And Bioenergy, v. 33, 2009, p. 1065-1069.

FERREIRA DF. **SISVAR - Sistema de análise de variância.** Versão 5.3. UFLA, 2010.

FLECK L, TAVARES MHF, EYNG E, ANDRADE MAM, FRARE LM. **Optimization of anaerobic treatment of cassava processing wastewater.** Engenharia Agrícola, vol.37 no.3 Jaboticabal May/June 2017.

FUKAYAMA EH. **Características quantitativas e qualitativas da cama de frango sob diferentes reutilizações: efeitos na produção de biogás e biofertilizante.** 2008.99f. Tese Doutorado (Ciências agrárias e Veterinárias). Universidade Estadual Paulista, Jaboticabal, São Paulo, 2008.

FUKAYAMA EH, LUCAS JUNIOR J, AIRES AM, SILVA AA, OLIVEIRA, R. A. **Produção de biogás utilizando cama de frangos de corte.** I Simpósio Internacional sobre Gerenciamento de Resíduos de Animais Geração de Energia a partir de Resíduos de Animais. Florianópolis – SC, 2009.

GUERI MD, DE SOUZA, SNM, KUCZMAN O. **Parâmetros operacionais do processo de digestão anaeróbia de resíduos alimentares: Uma revisão.** BIOFIX Scientific Journal, 3(1), 17, 2017.

IZUDDIN WI, LOH TC, SAMSUDIN AA, FOO, HL. **In vitro study of postbiotics from Lactobacillus plantarum RG14 on rumen fermentation and microbial population.** Revista Brasileira de Zootecnia, 47. 2018.

JANG HM, KIM KH, HÁ JH, PARK JM. **Bacterial and methanogenic archaeal communities during the single-stage anaerobic digestion of high-strength food wastewater.** Bioresource Technology, v. 165, 2014, p. 174-182.

KELLEHER BP, LEAHY JJ, HENIHAN AM, O'DWYER TF, SUTTON D, LEAHY MJ. **Advances in poultry litter disposal technology - a review.** Bioresource Technology. v. 83, 2002, p. 27–36.

KONRAD O, HASAN C, HICKMANN EV, MARDES M, FILHO MG, SILVA MC. A. **Produção de biogás a partir de biomassas residuais provenientes do setor agroindustrial.** Boletim Técnico - Universidade do Vale do Taquari - Univates, Dez. 2018.

KUNZ A, OLIVEIRA PAV. **Aproveitamento de dejetos de animais para geração de biogás.** Revista de Política Agrícola. Ano XV, n.3, 2006.

LI Y, CHEN Y, WU J. **Enhancement of methane production in anaerobic digestion process: A review.** *Applied Energy.* v.240, 2019, p. 120 – 137.

MIAH MR, RAHMAN AKML, AKANDA MR, PULAK A, ROUF MD. A. **Production of biogas from poultry litter mixed with the co-substrate cow dung.** Journal of Taibah University for Science. V. 10, 2016, p. 497-504.

MILANEZ AY, GUIMARÃES DD, MAIA GBS, SOUZA JAP, LEMOS MLF. **Biogás de resíduos agroindustriais: panorama e perspectivas.** Caderno Biogás, Banco Nacional de Desenvolvimento Econômico e Social - BNDES Setorial, 47, 2018, p. 221-276.

MINITAB. **Minitab for Windows Release 18.** State College: Minitab Inc., 2018.

ORRICO JUNIOR MAP, SCHWINGEL AW, CENTURION SR, ORRICO ACA. **Co-digestion of swine excreta associated with increasing levels of crude glycerin.** Revista Brasileira de Zootecnia, 45(3), 2016, p. 101–106.

ORRICO ACA, LOPES WRT, ORRICO M, SUNADA NS. **Codigestão anaeróbia dos dejetos de bovinos leiteiros e óleo de descarte.** Engenharia Agrícola, Jaboticabal, v.36, n.3, maio./jun. 2016, p. 537-545.

PARRA-OROBIO BA, TORRES-LOZADA P, MARMOLEJO-REBELLÓN LF. **Anaerobic digestion of municipal biowaste for the production of renewable energy: Effect of particle size.** Brazilian Journal of Chemical Engineering, 34(2), 2017, p. 481–491.

PAULINO MTF, OLIVEIRA EM, GRIESER DO, TOLEDO JB. **Criação de frangos de corte e acondicionamento térmico em suas instalações: Revisão.** PUBVET, v.13, n.2, a280, Fev. 2019, p. 1-14.

PERES S, MONTEIRO MR, FERREIRA ML, NASCIMENTO JUNIOR AF, PALHA MLAPF. **Anaerobic Digestion Process for the Production of Biogas from Cassava and Sewage Treatment Plant Sludge in Brazil.** BioEnergy Research, Volume 12, Issue 1, March, 2019, p. 150–157.

RIBEIRO EM, BARROS RM, FILHO GLT, SANTOS IFdos, SAMPAIO LC, SANTOS TVdos, SILVA FGB, SILVA APM, FREITAS JVR. **GHG avoided emissions and economic analysis by power generation potential in posture aviaries in Brazil.** Renewable Energy. v. 120, 2018.

RIBEIRO EM, BARROS RM, FILHO GLT, SANTOS IFdos, SAMPAIO LC, SANTOS TVdos, SILVA FGB, SILVA APM, FREITAS JVR. **Power generation potential in posture aviaries in Brazil in the context of a circular economy.** Technologies and Assessments. v. 18, 2016, p. 153-163.

RIPLEY LE, BOYLE WC, CONVERSE JC. **Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes.** Journal of Water Pollution Control Federation, Alexandria, v. 58, n. 5, p. 406-411, 1986.

SAGULA AL, DA COSTA LVC, DE LUCAS JUNIOR J. **Diferentes diluições e uso de reciclo na biodigestão anaeróbia de cama de frango triturada e peneirada: ensaio batelada / different dilutions and use of recycle in the anaerobic biodigestion of crushed and sieved poultry litter: batch test.** Revista Brasileira de Engenharia de Biosistemas, 11(4), 2017, p. 373.

SALMINEN E, RINTALA J. **Anaerobic digestion of organic solid poultry slaughterhouse waste - a review.** Bioresource Technology. v. 83, 2002, p. 13-26.

SANCHEZ E, BORJA R, TRAVIESO L, MARTIN A, COLMENAREJO MF. **Effect of organic loading rate on the stability, operational parameters and performance of a secondary up flow anaerobic sludge bed reactor treating piggery waste.** Bioresource Tecnology, v. 96, 2005, p. 335-344.

SANTOS NM, MALHEIROS R, TAVEIRA RZ. **Disposição adequada de resíduos orgânicos geradas no setor de avicultura de produção de frango de corte por meio da compostagem.** Anais. VIII Congresso Brasileiro de Gestão Ambiental. Campo Grande/MS, 2017.

SANTOS TMB, LUCAS JUNIOR J, SAKOMURA NK. **Efeitos de densidade populacional e da reutilização da cama sobre o desempenho de frangos de corte e produção de cama.** Revista Portuguesa de Ciências Veterinárias, 2005.

SHAH FA, MAHMOOD Q, SHAH MM, PERVEZ A, ASAD SA. **Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis.** International Journal of Chemical Engineering, 2014, p. 21.

SILVA HW. **Produção de biogás utilizando dejetos de vacas leiteiras - Uma alternativa viável para redução de impactos ambientais.** Revista Técnico-Científica do CREA-PR, 13ª edição, Set. 2018.

SILVA MOS. **Análises físico-químicas para o controle das estações de tratamento de esgoto.** São Paulo: Cetesb, 1977.

SILVEIRA MA, KRETZER SG, NAGAOKA AK, ARROYO NAR, BAUER FC. **Produção de biogás em biodigestores de tamanho reduzido abastecido com cama de aviário.** Acta Tecnológica.v.9, n.2, 2014, p. 9-15.

SINDIVIAPAR. **Anuário Paranaense da Avicultura 2018**. Revista Avicultura do Paraná. Edição 2018.

SPERLING MV. **Princípios básicos do tratamento de esgotos**. Departamento de Engenharia Sanitária e Ambiental, UFMG, v.2. 1996, p. 211.

SUN Y, CHENG J. **Hydrolysis of lignocellulosic materials for ethanol production: a review**. Bioresource Technolog. v. 83, n. 1, maio 2002, p. 1-11.

SUZUKI ABP, FEIDEN A, FERNANDES DM, MARTINS GL, FARIA RAP. **Utilização de manipueira juntamente com sólidos da cama de aviário em biodigestores para geração de biogás**. Ambiência Revista do Setor de Ciências Agrárias e Ambientais. Guarapuava, v.8 n.3, 2012, p. 809 - 820.

TAVARES SG, FEIDEN A, CORREIA AF, SOARES CMT, GREGOLIN MRP. **Influência das Variações Térmicas e Climáticas na Produção de Biogás**. Nativa, 4(5), 2016, p. 287-295.

TESSARO AB, TESSARO AA, CANTÃO MP, MENDES MA. **Potencial energético da cama de aviário produzida na região sudoeste do paraná e utilizada como substrato para a produção de biogás**. Revista em Agronegócio e Meio Ambiente, Maringá. v.8, n.2, 2015, p. 357-377.

UNITED STATES DEPARTMENT OF AGRICULTURE - USDA. **This report contains assessments of commodity and trade issues made by USDA staff and not necessarily statements of official U.S. government policy**. 2019 Poultry and Poultry Products Semi-Annual Report. Number 1901, 2019.

YADVIKA, SANTOSH, SREEKRISHNAN TR, KOHLI S, RANA V. **Enhancement of biogas production from solid substrates using different techniques - a review**. Bioresource Technology. v. 95, 2004, p. 1-10.

YETILMEZSOY K. **Integration of kinetic modeling and desirability function approach for multi-objective optimization of UASB reactor treating poultry manure wastewater**. Bioresource Technology, v. 118, 2012, p. 89-101.

ZIGANSHIN AM, LIEBETRAU J, PRÖTER J, KLEINSTEUBER S. **Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials**. Bioenergy and Biofuels, v. 97, 2013, p. 5161-5174.