American Journal of Experimental Agriculture
4(12): 1820-1831, 2014

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The Effect of Commercial Additive (Toxic-Chec) and Propionic Acid on the Fermentation and Aerobic Stability of Silage with Pig Excreta

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Authors' contributions

This work was carried out in collaboration between all authors. This work was carried out in collaboration between all authors. Author SJLG wrote the protocol and the first draft of the manuscript. Author MACP designed the study. Author GDMM managed the analysis of the study. Author MACE managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 14th June 2014
Accepted 3rd August 2014
Published 14th August 2014

ABSTRACT

The experiment was carried out to evaluate the effect of Toxic-chec commercial additive and propionic acid on the fermentation and aerobic Stability of silage made with pig excreta, corn stover, molasses and urea; during fermentation. The growth of microorganisms in the silage and aerobic stability of the silage were tested. Microsilos were conducted in the laboratory with the following treatments made in triplicate (T): T1 = silage without additive; T2 = silage + 0.05% commercial additive; T3 = silage + 0.05% propionic acid. Three microsilos were realized by treatment in a complete randomized design. The study was carried out in the laboratories of Rumen Microbiology and Animal Nutrition, Graduate College, Montecillo State Campus, Mexico, between June and November 2012. The results showed that the additives tested affected the pH in treatments, but did not affect the concentration of volatile fatty acids, acetic, propionic and

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butyric acid, and ammonia nitrogen. To evaluate the aerobic stability of the silages with treatments upon exposure to the presence of air for 16 days and the concentration of microorganisms in colony forming units per gram of silage was characterized. The additives tested did not avoid the decrease in the concentration of lactic acid bacteria and lactobacilli (P<0.05) between 8 and 16 days; in contrast, lactobacilli disappeared completely by 16 days in T1, maintaining a low concentration in T2 and T3. Enterobacteriae concentration was not affected by additives tested; yeast concentration decreased (P<0.05) between treatments, being more evident at day 16. Results suggest that the additives tested did not affect the fermentation to maintain viable populations of lactic acid bacteria and lactobacilli, and decreasing the concentration of yeast potentiate aerobic stability and preventing aerobic deterioration of silage pig excreta.

Keywords: Silage; pig excreta; fermentation; volatile fatty acids; microorganisms; aerobic spoilage; stability of anaerobic fermentation.

1. INTRODUCTION

A big pig farms challenge in Mexico is the excreta disposal; because they constitute an environmental and health problem. There is an inventory of 13 million head of pigs, which produce on average 1.0Kg of excreta animal^{-1}, day^{-1} [1] Pig excreta is a source of pollution to the environment, water and soil due to its high content of N, P, Cu, Zn and antibiotics [2]. It causes pollution of air due to the generation of toxic gases such as NH_3, H_2S; volatile fatty acids, indols and phenols; and during decomposition under anaerobic conditions it emits, greenhouse gases such as CH_4, CO_2, NO_2 and SO_2 are produced [3,4]. One solution to this problem is to use the pig excreta in animal feeds, such as silage and they form part of diet for ruminant animals. It can be mixed with other agricultural or industrial by-products. Pig excreta are a rich source of organic matter, and minerals, and its reuse in animal feeds can reduce feed costs and environmental pollution [5]. Silage is the method that has more advantages for excreta reuse in animal nutrition [6,7]. During silage, the excreta retain their nutrients, Castellanos et al. [1] determined the chemical composition of pig excreta silage, that it presented 36% of organic matter, 15% crude protein, 4.5% crude fat and 14% ash; however, pathogenic bacteria such as *Salmonella* spp., fungi, and viruses are destroyed, and reduces the viability of parasites in excreta [8,9]. Unpleasant odors are also eliminated, improves digestibility, palatability and increases the consumption of excreta of animals [10]. Fermentation of ensiled animal excreta differ from those made with forages because excreta contain a high amount of anaerobic and facultative bacteria from the digestive tract [11,12], which may competing during silage fermentation with lactic acid bacteria for substrates, which can affect the quality of silage [13]. By using pig excreta for silage, a gradual decrease in pH occurs because of slow population growth of lactic acid bacteria and lactobacilli, due to the buffering capacity of excreta and forced competition between fecal bacteria with lactic acid bacteria and lactobacilli. This causes a slow lowering of the pH of the silage and when exposed to air, the growth of undesirable microorganisms such as yeasts and enterobacteriae that cause aerobic deterioration, resulting in considerable losses of the silage [14]. Few studies have been done on the effect of propionic acid in the fermentation and aerobic stability of the silage of pig excreta when exposed to the presence of air; and in addition requires knowing the type of predominant microorganisms under such conditions. The objective of this study was to characterize the fermentation and development of microorganisms *in vitro*, in addition assess the aerobic stability of pig excreta silage through the development of desirable microorganisms (lactic acid bacteria and lactobacilli) and

undesirable (Yeast and enterobacteriae) with the addition two additives: one commercial and other propionic acid basis for 16 days.

2. MATERIALS AND METHODS

2.1 Production of Microsilos

This research was conducted in the laboratories of Rumen Microbiology and Animal Nutrition, Graduate College, Montecillo State Campus Mexico. Silage was made from corn stover, molasses, and pig manure; which came from a farm fattening pigs located in Chapingo, State of Mexico.

Silage had on a wet basis, 50% of fresh swine manure, 30% ground corn stover, 7.6% molasses, 1% urea and 11.4% water; according to the techniques reported by Cobos et al. [5]; the final mixture contained 40% moisture. To the mixtures were added a commercial additive (Toxic-chec ®, a commercial product made from base 63% aldehydes, 15.6% propionic acid and 5% acetic acid) or propionic acid according to the following treatments (T): T1=0% additives; T2 =0.05% commercial additive; T3 = 0.05% propionic acid. The aggregate amount of additives was wet basis. Treatments were packaged in PVC microsilos 1.4 kg capacity, then sealed and stored at room temperature for a period of 30 d.

2.2 Characterization of the Fermentation

Thirty days after the start of the process of silage fermentation was characterized by measuring pH values, which are determined according to Hardy and Elias [15], with a potentiometer Orion model 250A, calibrated at pH 4.0 and 7.0. To determine the concentration of volatile fatty acids (VFA) and ammonia nitrogen the samples were acidified with a 25% solution of metaphosphoric acid, then were centrifuged at 3500 rpm for 25min at 4 C; the supernatant was passed through an acetate filter and stored in 2mL of each sample into vials and refrigerated at 4ºC. To determine the concentration of VFA: acetic, propionic, and butyric, samples 3µL triplicate were measured and analyzed in a gas chromatograph (Model 5890 series II, Hewlett Packard®), with a column of superoxide formeril 1.2µm flow of 3 mL N_2 min^{-1} and a temperature of the injector and column detector 120, 150 and 130 C, respectively.

The concentration of ammoniacal nitrogen was determined according to the technique by McCullough [16], 20µL of supernatant from each sample were used, which were poured into tubes with capacity of 10mL, to which were added 1mL of phenol and 1mL of sodium hypochlorite basified with NaOH (5g of NaOH and 10 mL of sodium hypochlorite). The tubes were incubated at 37 C for 30 min, then was added 5mL of distilled water to dilute the samples, the absorbance was recorded on a UV visible spectrophotometer (Varian® CARY model 1 - E) at 630 nm. A reference blank was used which contained 1ml of phenol, 1ml of sodium hypochlorite and 5mL of distilled water. To determine the final concentration of the samples a standard curve was prepared, with aliquots of 2.5; 5.0; 15.0; 30.0; 45.0 and 60.0µL and brought to a volume of 100mL; these are equivalent to the NH_3-N concentration in mg/dL. Once determined concentrations values obtained were corrected considering the dilution factor by adding metaphosphoric acid and distilled water.

The development of lactic acid bacteria was determined, total lactobacilli, yeast, and enterobacteriae CFU g^{-1} during the fermentation [17]. The concentration of total lactic acid

bacteria was determined in the selective medium for lactic acid bacteria MRS (Man Rogosa and Sharpe, Merck®), according to the techniques described by Duffner et al. [18]. The concentration of total lactobacilli was determined in a selective medium Rogosa Agar (Merck®) according to the techniques reported by Fenton [19]) and Meeske et al. [20]. Yeasts were quantified in Malt Extract Medium (Merck®) according by Spoelstra et al. [21] and Sanderson [22]. Enterobacteriae concentration was determined according to the techniques of Difco Manual [23] and Lin et al. [24], the Violet Red Bile Agar (VRBA, Merck®) modified with the addition of 1% glucose was used, using the double-plate.

2.3 Evaluation of the Aerobic Stability

To assess the aerobic stability of silage of pig excreta with different treatments were exposed to the presence of air for a period of 16 days. Silage samples for analysis at 0, 8 and 16 days were taken a depth of 5 cm; the opening of the silos was considered as day zero. Subsequently, for each sampling the concentration of desirable microorganisms was measured: lactic acid bacteria, total lactobacilli; and concentration of undesirable microorganisms such as yeasts and enterobacteriae CFU g^{-1} by [17]. The techniques for determining the concentration of microorganisms during aerobic stability was done as described above.

2.4 Statistical Analysis

Data were analyzed by analysis of variance procedure appropriate for completely randomized design with three replicates per treatment, using the General Linear Models (GLM) procedure of SAS [25]. To determine if there was statistical difference between means, Turkey's test was used [26]. To evaluate the aerobic stability of silage during the 16 d exposure to the presence of air, in a completely randomized design three replicates per treatment was used, analyzing the concentration of desirable microorganisms like lactic acid bacteria and total Lactobacillus; and undesirable microorganisms: Yeast and enterobacteriae at 0, 8 and 16 d [25], by the method described Wilcox et al. [27] for repeated measurements.

3. RESULTS AND DISCUSSION

The pig excreta microsilos when uncovered after 30 days of fermentation showed slightly higher values of pH to 4.5 (Table 1). It was found that pH values were statistically different ($P<0.05$) between treatments, resulting in lower pH in T2 and T3, relative to T1. No significant differences ($P>0.05$) in the VFA concentration among treatments (Table 1) were found.

Acetic acid was the highest concentration, followed by propionic acid and finally, butyric acid. Acetic - butyric important one, since fermentation was submitted that butyric acid levels were low. In this study the concentration of lactic acid was not determined; however, considering the pH values obtained, it is estimated that their levels remained close to optimal for a proper process of lactic fermentation. In relation to the concentration of nitrogen, expressed as a percentage of total nitrogen (% N- Total), significant differences ($P<0.05$) among treatments (Table 1) were determined. The lowest ammonia production ($P<0.05$) the recorded T2 (6.01%) and, in general, the concentration of nitrogen in the different treatments was below 8%.

The addition of propionic acid to pig excreta silage (T3), provided that a suitable fermentation process is developed, although the pH did not reach values lower than 4.0, it was considered

the product to be acceptable because of the presence of a major fermentation lactic; these results are in accordance with studies Seale [28] and Filya et al. [29], who reported that at pH 4.5 silage usually has good aerobic stability and good concentration of organic acids as acetic, propionic and lactic. Apparently the low concentration of propionic acid produced during fermentation in T1, was not sufficient to ensure a reduction of the pH of the ensiled material.

Table 1. Fermentation properties pig excreta silage with their treatments

Properties	Treatments			
	T1	T2	T3	SE
pH	4.55[a]	4.29[b]	4.27[b]	0.04
Acetic acid (mmol L^{-1})	117.40[a]	115.42[a]	98.95[a]	20.21
Propionic acid (mmol L^{-1})	14.55[a]	21.61[a]	22.20[a]	1.98
Butiric acid (mmol L^{-1})	11.34[a]	14.34[a]	14.07[a]	1.67
Ammonia N (% N Total)	7.40[a]	6.01[b]	7.66[a]	0.16

[abc]Values having different superscripts in a row are significantly (P≤0.05) different
T1: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea (control)
*T2: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% of a commercial additive (Toxic-chec ®)***
T3: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% propionic acid (Merck ® trademark) SE = Standard Error
***(Anglo Corporation ®) Commercial Product. Made from 63% aldehydes, 15.6% propionic acid and 5% acetic acid*

The inclusion of additives in T2 and T3 did not affect the production of acetic, propionic and butyric acids during fermentation; therefore not provided an acetic - butyric fermentation, because the levels of butyric acid produced during fermentation were low. In this study the concentration of lactic acid was not determined; however, it was felt that their levels were maintained close to 3% of the dry matter in the different treatments. This can be confirmed by the pH values obtained in T1, T2 and T3, which on average remained less than 4.5; indicating that the material ensiled at these pH levels, usually presents aerobic stability [30]. In general the concentrations of ammonia nitrogen (NH_3-N) for the various treatments in this study were less than 8%. According to Umana et al. [31] in silage which has been made a suitable fermentation, the NH_3-N content is usually less than 8% of total nitrogen.

During the fermentation, the development of lactic acid bacteria showed statistical differences ($P<0.05$) among treatments, T2 presented the lowest concentration (4.6×10^5) bacteria per g^{-1} fresh silage. In contrast, the development of lactobacilli did not change significantly between treatments, thus an effect of the commercial additives and propionic acid were not detected in the development of lactobacilli in the fermentation process (Table 2).

With respect to the presence of undesirable microorganisms, the yeast concentration was not statistically different between the different treatments (Table 2). It is considered that propionic acid additive tended to inhibit the growth of yeasts in this type of silage. Enterobacteriaceae were the predominant microorganisms in T1 ($P<0.05$), showing a concentration of 7.6×10^4 $CFUg^{-1}$ of silage; while in T2 and T3 had lower counts of these microorganisms, with statistical difference ($P<0.05$) (Table 2).

Table 2. Development of microorganisms during fermentation in silage of pig excreta with the addition of chemical additives

Microorganisms	Treatments			
Concentración (UFC g-1) +	**T1**	**T2**	**T3**	**SE++**
Lactic acid bacterias	$2.6X10^{6\ a}$	$4.6X10^{5\ b}$	$2.6X10^{6\ a}$	$2.7X10^{5}$
Total Lactobacilli	$2.3X10^{4\ a}$	$3.3X10^{4\ a}$	$2.0X10^{4\ b}$	$4.3X10^{3}$
Enterobacteriaceae	$7.6X10^{4\ a}$	$2.6X10^{4\ b}$	$1.6X10^{4\ b}$	$4.7X10^{3}$
Yeast	$3.0x10^{6\ a}$	$1.6X10^{6\ a}$	$1.6X10^{6\ a}$	$4.3X10^{5}$

[abc]Values having different superscripts in a row are significantly (P≤0.05) different
T1: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea (control)
T2: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% of a commercial additive (Toxic-chec ®) + + +
T3: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% propionic acid (Merck ® trademark)
+ Colony-forming units per gram of silage
+ + SE = standard error
+ + +(Anglo Corporation ®) Commercial Product. Made from 63% aldehydes, 15.6% propionic acid and 5% acetic acid

The lack of development of lactic acid bacteria during fermentation in T2, was probably due to two situations; first, adding the commercial additive to the mixture, the pH tended to fall more quickly and therefore there was no proper development of lactic acid bacteria; and second, due to competition for nutrients from bacteria in the manure with lactic acid bacteria [32,33]. The development of lactobacilli during fermentation in silages pig excreta provided no difference between treatments. Evans and Smith [11] found that when pig manure and silage components are used, the gradual increase in lactobacilli is due to forced competition for nutrients between bacteria and lactobacilli excreta; under the conditions of this experiment, lactobacilli constituted only a small proportion of the total microbiota developed during ensiling. However, the growth of yeasts during fermentation was not affected in T2 and T3. Kung [34] have shown that yeasts are capable of tolerating low pH levels, although their growth can be inhibited by organic acids like acetic, propionic and lactic acid produced during fermentation. Moon [35] reported that propionic acid used as silage additive inhibits the growth of yeasts and filamentous fungi; meanwhile, Selwet [30] reports that a mixture of formic acid, propionic acid and ammonium salts used as an additive to silage, has proved a potent inhibitor of yeast, thus preventing aerobic deterioration. However in this study, the development of enterobacteriae during fermentation was inhibited by the additives added in T2 and T3; in contrast, the highest count of enterobacteriae occurred in the control treatment, which may be because in this treatment there was a limited production of lactic acid in the early stages of the fermentation process, which could enable the further development of enterobacteriae [36,37]. In general the lack of any of the two additives in T1, possibly avoided early acidification of the ensiled material during fermentation, which contributed to the development of enterobacteriae.

During the determination of the aerobic stability of silage of pig excreta, to be uncovered and exposed to air for the presence of 0, 8, 16 d; the development of lactic acid bacteria and lactobacilli showed statistical differences (P<0.05) (Table 3). Lactic acid bacteria had a linear cut with a very high statistical significance in the T1 (P<0.0001) during this period, while the lactobacilli showed a drastic decrease, disappearing completely at 16 d exposure to the presence of silage air, showing a linear effect (P<0.0009) and a quadratic (P<0.0001), both with very high statistical significance (Table 3). In T1 concentration of lactobacilli and lactic acid bacteria, similarly decreased during exposure to air for the same period of time; the

response was linear (P<0.0001) and quadratic (P<0.0002), respectively, in the lactic acid bacteria; whereas lactobacilli for this response was linear (P<0.0001). In all cases, statistical significance was very high (Table 3). During this time period, T3 concentration in lactic acid bacteria and lactobacilli decreased; lactic acid bacteria had a linear response (P<0.0001) and a quadratic (P<0.00021) of very high statistical significance, whereas lactobacilli response was linear (P<0.0165) were statistically significant (Table 3). The results did not show a clear advantage of the addition of additives in T2 and T3 with respect to the development of acid lactic bacteria. However, in T1 Lactobacilli disappeared completely at the end of the period evaluated (16 days). This may be because T2 contained low proportion of organic acids (15.6% of propionic acid and 5.0% acetic acid); on the other hand, contained a high proportion of aldehydes (63.0%); Shi et al. [38] mention that the silage treated with formaldehyde are more sensitive to aerobic deterioration, because they are inhibitors of the fermentation. Is also likely that the small amount of added molasses (less than 8%) to treatments, did not provide soluble sugars needed to maintain an adequate population of lactic acid bacteria and lactobacilli during the fermentation phase, which would allow increased production of organic acids and increase the period of aerobic stability of silage to the presence of air [39]. Lima et al. [40] have found that by adding levels of molasses between 9 and 10% in silage, the aerobic stability is improved because the molasses sugars they are promoters of acid lactic bacteria growth, moreover, prevents the increase of the temperature, which significantly reduces the loss of organic matter from the silage. In contrast, in T2 and T3 was presented a low concentration of lactobacilli. These results contrast with those obtained by Sanderson [22], who found that the concentration of lactobacilli increased in silage exposed to air. On the silage of pig excreta, by using additives to improve the aerobic stability, should be considered the buffer capacity of excreta due to its high content of non-protein nitrogen (NPN). Evans and Smith [11] reported that in silage of animal excreta, the development of lactic acid bacteria is slow due to a forced competition of bacteria of excreta for substrates with lactic acid bacteria.

The concentration of undesirable microorganisms such as yeast, for determining the aerobic stability of silage of pig excreta presented statistically significant differences (*P*=.05) between treatments; T1 in the trend in the concentration of the yeast according to a nonlinear effect with high statistical significance (P<0.0007) and quadratic with statistically significant difference (P<0.0105) with respect to time, with a decrease in the concentration (Table 4).

This indicates a tendency to decrease the concentration of yeast through periods analyzed 0, 8 and 16d. In T2 reducing yeast concentration was more marked, disappearing completely by 16 days, but the difference was not statistically (P<0.0578). Moreover, in T3 a less marked concentration of yeasts in the 0 and 16 d period decrease , a response of a linear type (P<0.0009) with a very high statistical difference and a quadratic response with a significant difference is present statistically (P<0.0124).

In general, the concentration of enterobacteriae remained statistically unchanged (P<0.05), indicating that the addition of the commercial additive and propionic acid treatments did not affect, in a statistically significant manner, the development of enterobacteriae in the exposure of silage pig excreta to the presence of air for 16d (Table 4).

Table 3. Concentration of lactic acid bacteria and lactobacilli in pig excreta silages exposed to the presence of air for a period of 16 days, with the use of chemical additives

Microorganism (CFU g^{-1}) [1]	Days	Treatment		
		T1	T2	T3
Lactic acid bacteria	0	$2.6X10^{6ad}$	$4.6X10^{5bd}$	$2.6X10^{6ad}$
	8	$8.6X10^{5ad}$	$5.3X10^{5bd}$	$1.6X10^{5cd}$
	16	$2.3X10^{4b\ e}$	$3.6X10^{4b\ e}$	$1.3x10^{5ae}$
Effect[2]				
Lineal		0.0001	0.0001	0.0001
Quadratic		0.0009	0.0009	0.0021
Lactobacílli	0	$2.3X10^{4ad}$	$3.3X10^{4ad}$	$2.0X10^{4ad}$
	8	$5.3\ X10^{4ad}$	$2.3X10^{4bd}$	$1.3X10^{4ad}$
	16	$0.0000^{c\ e}$	$5.0X10^{2be}$	$2.0X10^{3ae}$
Effect[2]				
Lineal		0.0009	0.001	0.0165
Quadratic		0.0001	0.1026	0.6394

[abc]Values having different superscripts in a row of the same microorganism are significantly (P≤0.05) different

[def]Values having different superscripts in a column of the same microorganism at 0, 8 and 16 d, are significantly (P≤0.05) different

T1: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea (control).

T2: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% of a commercial additive (Toxic-chec ®) 3

T3: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% propionic acid (Merck ® trademark)

[1]Colony forming 1Unidades per gram of silage

[2]Probability of type I error

[3]Commercial Product (Anglo Corporation ®) Made from 63% aldehydes, 15.6% propionic acid and 5% acetic acid)

The concentration of yeast, considered undesirable microorganisms in silage, decreased at the end of the exposure period (day 16) to the presence of air in the silage; apparently, organic acids such as the acetic and the propionic acid produced during fermentation inhibited the development of yeasts. Therefore, it is considered that the silage of pig excreta presented aerobic stability during the 16 d of exposure to air [41,30]. In particular, in T2 and T3 decreased significantly the concentration of yeast during the period of exposure to the presence of air, due to the addition of propionic acid in T3, and the small proportion of the propionic acid added T2.

In this sense, Moon [35] and Hashemzadeh-Cigari et al. [42] have determined that propionic acid is a very powerful antifungal that inhibits the development of yeasts and filamentous fungi during the exhibition of the silage to the presence of air, which significantly increases your aerobic stability. In general, the concentration of enterobacteriae remained unchanged during the period of exposure of the silos to the presence of air during 16d; therefore, the use of additives in T2 and T3 did not affect the development of enterobacteriae.

The results obtained in this study are in contrast with Östling and Lindgren [36], who found that Enterobacteriaceae are sensitive to organic acids, because these organisms were inhibited at levels 10 times lower than acetic, lactic and formic acid to the inhibiting yeast

used. Furthermore, Muck [43] found that the population of enterobacteriae increased in the first 2 d of exposure to air in forage silos and day 14 after exposure to air, the count turned out to be low (100 CFUg-^{1} of fresh silage) .

Although not show a great development of enterobacteriae in the present study , during the exposure of the silage to the presence of air for a period of 16d, it is determined that the enterobacteriae compete with lactic acid bacteria for the soluble sugars in the ensiled [44]. However, the results of this study suggest that enterobacteriae in pig excreta silage only partially competed with lactic acid bacteria by soluble sugars, which may have affected the development of the first.

Table 4. Concentration of undesirable microorganisms in pig excreta silages exposed to the presence of air for a period of 16 days, with the use of chemical additives

Microorganism (CFU g^{-1}) [1]	Days	Treatment		
		T1	T2	T3
Yeasts	0	3.0X10^{6ad}	1.6X10^{6ad}	1.6X10^{6ad}
	8	6.3X10^{3be}	1.6X10^{4ad}	7.6X10^{3be}
	16	4.0X10^{3be}	0.0000^{ce}	1.3x10^{4be}
Effect[2]				
Lineal		0.0007	0.0578	0.0009
Quadratic		0.0105	0.336	0.0124
Enterobacteriae	0	7.6X10^{4ad}	2.6X10^{4bd}	1.6X10^{4bd}
	8	1.3 X10^{4ad}	4.3X10^{3bd}	1.3X10^{4ae}
	16	1.6X10$^{4b\ e}$	1.3X10$^{4b\ e}$	4.3X10$^{4a\ e}$
Effect[2]				
Lineal		0.0001	0.0135	0.0013
Quadratic		0.0001	0.0135	0.0013

[abc]Values having different superscripts in a row of the same microorganism are significantly (P≤0.05) different

[def]Values having different superscripts in a column of the same microorganism at 0, 8 and 16 d, are significantly (P≤0.05) different

T1: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea (control).

T2: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% of a commercial additive (Toxi-chec ®) 3

T3: 50% pig excreta, 30% corn stover, molasses 7.6%, 11.4% water and 1% urea + 0.05% propionic acid (Merck ® trademark)

[1]Colony forming 1Unidades per gram of silage

[2]Probability of type I error

[3]Commercial Product (Anglo Corporation ®) Made from 63% aldehydes, 15.6% propionic acid and 5% acetic acid)

4. CONCLUSION

The additives evaluated were the commercial (Toxic- chec®) and propionic acid, added in pig excreta silage. The treatments did not affect the silage fermentation process as they had no effect on the concentration of volatile fatty acids or the content of ammoniacal nitrogen.

The additives tested inhibited the growth of yeasts in silage during exposure to air for 16d, which showed potential for aerobic stabilization of silage and prevented the aerobic deterioration of silage pig excreta. To stimulate lactic acid fermentation, and thus increasing

the concentration of lactic acid bacteria and lactobacilli in the silage, it is suggested to increase the concentration of molasses to 9% on a wet basis, which also improves the aerobic stability of pig excreta silage.

Before using the pig excreta silage in feeding of ruminants, we would suggest a study *in vitro* to evaluate the digestibility, fermentation ruminal; later to realize a test *in vivo* with sheep, to evaluate the consumption, the weight gains and the feed conversion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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