EVALUATION OF DIFFERENT PRESERVATIVES FOR WET DIETS OF CATTLE

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ABSTRACT

We tested the effectiveness of different preservatives for wet diets of cattle. We evaluated the growth of lactobacilli, clostridia, enterobacteria, molds, and yeasts in three experiments using: 1) different concentrations of citric acid (0, 1%, 2%, 3%, and 4%); 2) different preservatives (Benzoate, Propionate, Myco Curb, Sodium, Protonic Acid and AS4) diluted at 1%; and 3) preservatives Benzoate, Propionate, Myco Curb®, Sodium Acid, and Propionic Acid. The results were summarized using descriptive statistics. In experiment 1 there was a large amount of lactobacilli both in the control and in the treated groups. In experiment 2, the amount of colonies was higher than expected. In experiment 3, concentration of lactobacilli colonies increased throughout the time. Our results demonstrate that preservatives should be tested using a higher inclusion level for wet diets to reduce the number of colonies. Propionate, propionic acid, and myco-curb were efficient in controlling molds and yeasts from day 7 to 15.
KEYWORDS: Citric Acid, Propionic Acid, Sodium Acid, bovines, nutrition.

AVALIAÇÃO DE DIFERENTES CONSERVANTES PARA DIETAS MOLHADAS DE GADO

RESUMO

Testou-se a eficiência de diferentes conservantes em preservar dietas úmidas para bovinos. Avaliou-se o crescimento de lactobacilos, clostrídios, enterobactérias, bolores e leveduras em três experimentos manipulando: 1) diferentes concentrações de Ácido Cítrico (0, 1%, 2%, 3%, e 4%); 2) diferentes conservantes (Benzoato, Propionato, Myco Curb®, Ácido Sórbico, Ácido Propiônico e AS4) diluídos a 1%; 3) conservantes Benzoato, Propionato, Myco Curb®, Ácido Sórbico e Ácido Propiônico. Os resultados foram resumidos usando estatística descritiva. No experimento 1 houve grande quantidade de lactobacilos tanto no controle, quanto nos grupos tratados. No experimento 2, a quantidade de colônias foi acima do esperado. No

experimento 3, concentração de colônias de lactobacilos aumentou ao longo do tempo. Nossos resultados demonstram que conservantes devem ser testados utilizando maior nível de inclusão para dietas úmidas para reduzir o número de colônias. O propionato, ácido propiônico, e myco-curb foram eficientes no controle de bolores e leveduras do dia sete ao 15.
PALAVRAS-CHAVE: Ácido Cítrico, Ácido Propiônico, Ácido Sórbico, bovinos, nutrição.

INTRODUCTION

Animal nutrition is the most important factor determining the economic viability of dairy and beef cattle. Agro-industrial by-products usually reduce losses and promote early pasture rotation, which favors crop and livestock production in the same area. The early pasture rotation is also beneficial for the installation of pasture itself, reducing the drying costs. The limiting factors for using industrial by-products are their seasonal availability and differences in the chemical composition of each batch. These differences influence supply rate and require frequent laboratory analysis to provide different formulations to meet nutritional requirements of animals (CHAVES et al., 2014).

Wet diet is a by-product of the storage of seeds and cereal grains when preserved in anaerobic media, shortly after seed maturation. At this phase, seeds have the highest content of starch, proteins, and lipids, which make them retain great amount of humidity (approximately 30%), because the transference of nutrients from the plant to the seed ceases (NFT ALLIANCE, 2011). Wet feeding provides the animal with a high energy diet and digestible food that can be stored. Thus, wet diets should be used whenever possible, since they reduce costs while maintaining high productivity (GUIMARÃES et al., 2013).

Wet diets are produced by agro-industries and made of co-products from the processing of corn, sugarcane, and citric acid. However, wet diets have also some drawbacks, mainly associated with their storage. For example, the high moisture usually causes qualitative losses associated with open-air exposure. In addition, this co-product is produced in large amounts to reduce costs. Also, its storage does not involve any specific technique and is usually made by covering tall piles with plastic (FRANÇA et al., 2015).

GoldenMill is the commercial name for Wet Corn Gluten Meal (WCGM), a co-product from the manufacture of syrup and starch. Shortly, the milling begins with the separation of corn kernels, followed by the removal of undesirable material. Then, corn kernels are swelled by soaking in water and sulfur dioxide. Nutrients migrate to aqueous solution in the immersion. When the maceration is complete, the solution is drained to become the WCGM. WCGM is a highly nutritive food, since it has moderate levels of protein (20-25%), low content of starch (about 20%) and oil, and high content of digestible fiber (SANTOS et al., 2013).

The glucose syrup is filtrated in rotary filters using diatomaceous earth precoat as filter aid. The filtrate is composed of 90% silica. This filtrate is used to clarify glucose (GONÇALVES et al., 2014). The chemical composition of the precoat has on average 73% of humidity, 12.8% protein, 17.5% of ether extract, 50.2% of NDF, 40.8% of FDA, 0.13% of lignin, and 41.6% of mineral matter (WATANABE, 2013). Raffinate is a syrup produced by the sugar fermentation during the citric acid purification. It has on average 39% humidity, 9.9% PB, 3.7% ether extract, and 16.5% mineral matter (WATANABE, 2013). Mycelium is produced by the fungus

Aspergillus niger in the sugar fermentation. It has 60 to 65% of humidity, 7.15% of PB, 0.04% of ether extract, 23.09% of NDF, 31.03 % of FDA, 0.13% of lignin, and 0.14% of mineral matter (WATANABE, 2013).

Chemical preservatives prevent or delay the effects of microorganisms on wet diets while maintaining food quality. The effectiveness of a preservative is influenced by other substances that inhibit the growth of microorganisms (e.g., salt, vinegar, and sugar), pH, product composition, water content of the food, and the initial level of contamination of the food or environment. Chemical preservatives are mainly used in wet and warm regions, where the deterioration of food is higher. Preservatives are also necessary when storage facilities are precarious or when transport is deficient by long distances between production and consumption centers (ALIMENTOS & INGREDIENTES, 2013).

Several substances can be used as food preservatives, such as citric acid, propionate, sorbate, propionic acid, and benzoic acid (ANVISA, 2011). The choice of preservative should consider the types of microorganisms to be inhibited, handling safety, impact on human or animal consumption, cost, and effectiveness (FOOD INGREDIENTS, 2013). Laboratory tests can help inform the best preservative and its concentration that suppress microorganism growth. Here, we tested the efficiency of different preservatives and their concentrations in inhibiting microorganism growth of wet diets for cattle.

MATERIAL AND METHODS

Microbiological analyzes were performed at the Laboratory of Quality Control and Food Safety, School of Veterinary Medicine, Federal University of Uberlândia, Minas Gerais, Brazil. We placed 3 Kg of each sample of wet diet (Table 1) in buckets at 25 ºC covered with polyethylene lids.

TABLE 1. Percentage and raw weight of each material composing wet diets analyzed.

	Material (%)	Raw weight (Kg)
Sugar cane bagasse	6.69	0.201
GoldenMill®	42.35	1.271
Mycelium	11.55	0.347
Pre-coat	3.45	0.104
Raffinate	9.96	0.299
Broken corn	24.86	0.746
Nucleus	1.14	0.034
Total	100.00	3.000

We assessed the growth of lactobacilli, clostridia, enterobacteria, molds, and yeasts. We first diluted 25 g of each sample into 225 mL of sterile saline solution for sowing in specific culture media for each microorganism. Plates containing Lactobacilli MRS Dehydrated Agar (Difco™) culture medium for lactobacilli were incubated at 35 ºC for three days. For yeasts and molds, we used Potato Dextrose Agar (PDA) culture medium, incubated between 25 and 30 ºC for 3 to 5 days. We differentiated yeasts from molds by the physical structure of colonies. Molds form filamentous multicellular colonies, whereas yeasts are unicellular. For sulphite-reducing clostridia, we used the SPS Agar (Difco™) as culture medium. We performed serial dilutions and incubated the plates in anaerobic conditions at 46 ºC

for 24 to 48 h (ANVISA, 2001). Enterobacteria were cultured in MacConckey Agar medium.

We conducted three experiments to assess the growth rate of microorganisms. In the first experiment, we used different concentrations of citric acid (0%, 1%, 2%, 3%, and 4%). We counted Colony Forming Units (CFU) on the first, fourth, and eighth day of incubation. We tested the dilutions: 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. In the second experiment, we used different preservatives at 1% concentration: Benzoate, Propionate, Myco Curb, Sodium, Protonic Acid, and AS4, plus a control (without preservative). We counted CFUs at the start of incubation and 15 days after. The dilutions on day one were 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. For day 15, we used dilutions 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. In the third experiment, we used Benzoate, Propionate, Myco Curb®, Sodium Acid, and Propionic Acid, plus a control (without preservative). We counted CFUs on day one, seven, and 15 after the start of the incubation. Dilutions for day one were 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}; and 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} for day 7 and 15. Results were summarized using descriptive statistics for the duration of the experiments. Data on CFUs are presented in log scale.

RESULTS AND DISCUSSION

Experiment 1

We found a large amount of lactobacilli in both the control and samples treated with citric acid (Table 2). Lactobacilli rapidly decreased the pH of the stored material. The CFU/g of silage between 6 and 10 rapidly reduced pH, allowing the conservation of silage in an acidic environment due to the production of lactic acid (JOBIM et al., 1997). Here, the CFU/g of lactobacilli was within the range reported by JOBIM et al. (1997), except for citric acid at 4%. High values of lactobacilli are desirable because they favor silage conservation.

TABLE 2. Lactobacillus count on samples of wet diets treated with citric acid at 1%, 2%, 3%, and 4%, plus control (0%) on days one, four, and eight.

	Storage Days		
	1	**4**	**8**
	UFC/g		
0%	8.22	8.09	9.04
1%	8.55	8.25	8.93
2%	-	8.67	8.05
3%	NA	8.60	8.70
4%	1.65	8.31	8.06

-: non countable; NA: missing value

Clostridia growth decreased 10% in the first day of incubation (Table 3) in groups treated with up to 3% citric acid. Conversely, clostridia colonies increased in groups treated with 3% and 4% of citric acid on day four. On day eight, colony count reduced by 12.5% with the increase in citric acid concentration (Table 3). Even though the clostridia growth was halted with 4% citric acid on day eight, demonstrating an improve compared to previous days, it did not show the desired effect. The clostridia count found here are well above those reported by COAN et al (2007) between 1 to 3 CFU/g.

TABLE 3. Clostridia count (CFU/g) on wet diet samples treated with citric acid at 1%, 2%, 3%, and 4%, plus a control on first, forth, and eighth day of incubation.

	Incubation time		
	1	**4**	**8**
	UFC/g		
0%	8.23	8.27	8.33
1%	9.20	8.47	8.75
2%	8.20	8.38	8.91
3%	7.38	8.49	8.42
4%	7.49	8.28	7.29

Citric acid reduced the growth of enterobacteria at 4% concentration in the first and eighth day of incubation, but their count is still high (Table 4). Low concentrations of citric acid may have stimulated enterobacteria growth, which is undesirable. This pattern was found for clostridia treated with formaldehyde (LALA et al., 2010). The colony counts for enterobacteria we found (Table 4) are above those reported by Jobim et al. (1997, 1999), who found a variation of 1 to 6 CFU/g in wet corn silage, and ZAMBOM et al. (2014), whose highest value found was 6.17 CFU/g in silage. Citric acid at 4% heavily reduced colonies count on day eight. However, the values are still higher than the desired ones.

TABLE 4. Enterobacteria count (CFU/g) on wet diet samples treated with citric acid at 1%, 2%, 3%, and 4%, plus a control on first, forth, and eighth day of incubation.

	Incubation time		
	1	**4**	**8**
	UFC/g		
0%	4	7.07	9.19
1%	6.81	7.96	8.41
2%	7.73	8.87	7.87
3%	7.73	8.13	7.83
4%	7.03	8.28	6.83

On day one, citric acid increased the counts of mold and yeast colonies from 6 to 8% compared to the control. On day four, the control had 8.32 CFU/g, but reduced by 4.4% in 2% citric acid. However, the counts of molds and yeasts increased 4% with 4% citric acid. At day eight, 4% of citric acid decrease 41.8% colony counts, ranging from 4.51 to 7.13 CFU/g (Table 5).

TABLE 5. Count of molds and yeasts (CFU/g) on wet diet samples treated with citric acid at 1%, 2%, 3%, and 4%, plus a control on first, forth, and eighth day of incubation.

	Incubation time		
	1	**4**	**8**
	UFC/g		
0%	7.81	8.32	7.13
1%	8.41	8.18	8.30
2%	8.15	7.95	8.04
3%	8.30	8.13	8.32
4%	8.44	8.65	4.51

JOBIM et al. (1997, 1999) found 7 to 13 CFU/g of mold and yeast. Our results were within that range, except for day eight with 4% citric acid. The 4% concentration

on day 8 decreased colony count compared to the control and other concentrations. However, colony count remained high in the remaining incubation times, suggesting that higher nitric acid concentrations should be used.

Experiment 2

On day one, there was a large number of colonies of lactobacilli, clostridia, enterobacteria, and molds in the control, making the counting on day 15 unnecessary (Table 6).

TABLE 6. Counts of lactobacilli, clostridia, enterobacteria, and molds (CFU/mL) at different dilutions on days one and fifteen of incubation.

	Lactobacilli		Clostridia		Enterobacteria		Molds	
	UFC/g							
	1	**15**	**1**	**15**	**1**	**15**	**1**	**15**
Control	7.84	**	7.50	**	7.61	**	8.23	**
Propionic acid	0	7.13	0	7.69	0	7.59	0	9
Sorbic acid	0	7.98	0	7.61	0	7.69	0	0
Benzoate	0	7.03	0	7	0	7.41	0	0
Myco Curb®	0	10.28	0	8.19	0	9.46	0	0
Propionate	6.46	6.02	0	5.36	0	8.73	0	6.02
SA4	0	10.33	0	9.21	0	9.43	0	0

**did not included in assay

On day one, colony count was close to 0 in almost all samples, except for Propionate, which had 6.46 CFU/g of lactobacilli. At day 15, there were lactobacilli, clostridia, and enterobacteria even with the addition of preservatives. Mold occurred only in samples with propionic acid and propionate (Table 6). The amount of microorganisms we found on the 15th day was higher than those reported by JOBIM et al. (1997, 1999) (maximum 6 CFU/g), suggesting that these preservatives were ineffective to halt the growth of clostridia and enterobacteria. Preservative were not effective to preserve samples up to 15 days, since colony count was higher than expected. Therefore, higher concentrations of these preservatives should be tested.

Experiment 3

The group treated with Myco-Curb had 4.27 CFU/g at day zero, 4 CFU/g day seven, and 4.14 CFU/g on day 15, showing a decrease in 4.6 and 3%, respectively (figure 1).JOBIM et al. (1997) found that lactobacilli varied from 6 to 10 CFU/g of silage and rapid decreased pH. JOBIM et al. (1999) found 8.3 to 8.9 CFU/g in silages of corn cobs and kernels, respectively. COAN et al. (2007) found none lactobacillus colonies on day zero, 6 CFU/g on day four, and 11 CFU/g on day seven in Tanzania grass silages (COAN et al, 2007). Here, propionic acid and myco-curb rapidly decreased pH at day zero. However, we did not obtain counts above 4.5 CFU/g for other preservatives.

Clostridial concentration of the control on days zero and seven was 4.99 CFU/g, and 4.89 CFU/g on day 15. For propionate, it was 3.92 CFU/g on day zero, 4.61 CFU/g on day seven, and 4.32 CFU/g on day 15. For propionic acid, it was 4.34 CFU/g at day zero, 3.94 CFU/g on day seven, and 4.11 CFU/g on day 15. For Myco-Curb, counts were 4.79 CFU/g at day zero, 4.2 CFU/g day seven, and 4.38 CFU/g on day 15. A previous study (Coan et al., 2007) found less than 1 CFU/g of clostridia

on day zero, 1 CFU/g on day four, and about 3 UFC/g on day seven in Tanzania grass silage. These values are smaller than the ones found here (see Figure 1).

The control had 5.04 CFU/g of enterobacteria at day zero, 6.52 CFU/g on day seven, and 6.52 CFU/g on day 15. For the propionate, it was 4 CFU/g on day zero, 4.04 CFU/g on day seven, and 4.07 CFU/g on day 15. Samples treated with propionic acid had 4.44 CFU/g at day zero, 4.04 CFU/g on day seven, and 3.83 CFU/g on day 15. For those treated with myco-curb, the count was 4.62 CFU/g at day zero, 4.14 CFU/g day seven, and 3.85 CFU/g on day 15. JOBIM et al. (1997, 1999) found 1 to 3.5 CFU/g of enterobacteria on wet corn grains. Conversely, COAN et al. (2007), found about 4.3 CFU/g of enterobacteria at day zero in Tanzania grass silage, but colony count was 0 on days four and seven. The preservatives tested here were efficient after day seven, when compared to the control, but colony count was higher than those found in previous studies.

The control had 5.80 CFU/g of molds and yeasts at day zero, 6.51 CFU/g on day seven, and 6.52 CFU/g on day 15. For propionate, colony count was 4 CFU/g on day zero, 2.23 CFU/g on day seven, and 3.39 CFU/g on day 15. Propionic acid had 4.49 CFU/g on day zero, 1.77 CFU/g on day seven, and 1.0 CFU/g on day 15. For Myco-Curb, count was 4.73 CFU/g at day zero, 2.32 CFU/g day seven, and 2.41 CFU/g on day 15. Yeasts in wet corn kernel and corn cod silages varied between 6.4 and 8.6 CFU/g, while counts for fungi varied from 0.6 to 3.8 CFU/g (JOBIM et al., 1997). Therefore, the preservatives tested were efficient between day seven and 15 when compared to the control (see Figure 1).

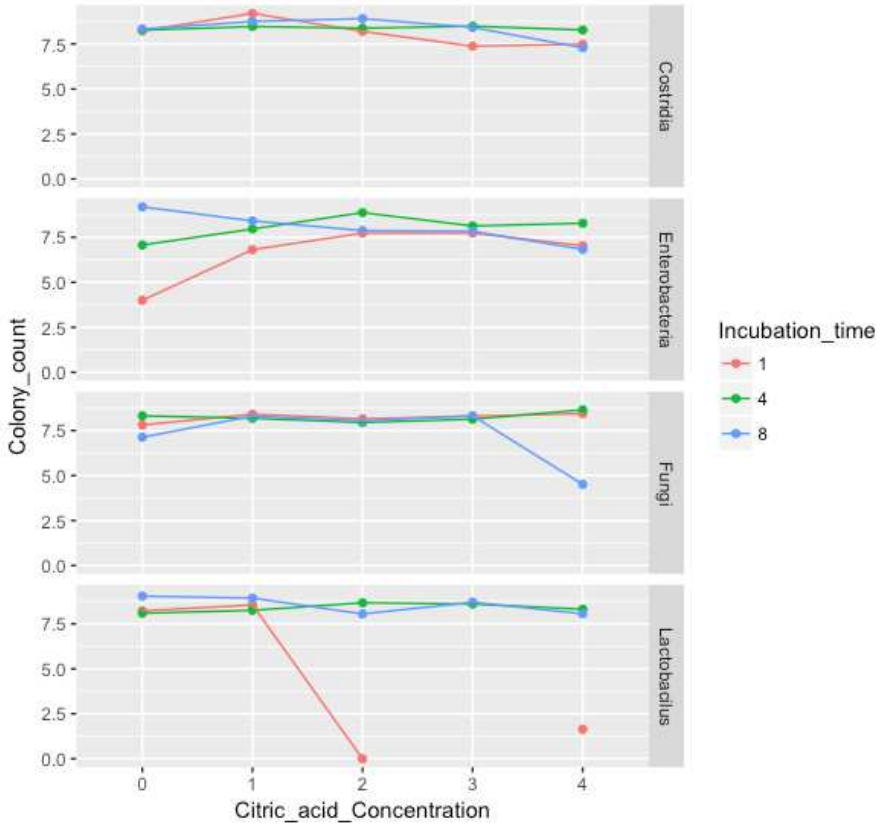

FIGURE 1 Count (CFU/g) of clostridia, enterobacteria, fungi and lactobacilus in wet diets for cattle using different preservatives and incubation times.

CONCLUSION

Propriontin, propionic acid, and myco-curb were effective to control molds and yeasts from day 7 to day 15. However, future studies should test the efficiency of higher concentrations of preservatives in reducing colony counts of wet diets.

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