

SUGARCANE MOLASSE AND WHEY AS ADDITIVES IN THE SILAGE OF LEMONGRASS (*Cymbopogon citratus* [DC.] Stapf) LEAVES

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After extraction of essential oil from lemongrass (*Cymbopogon citratus* [DC.] Stapf) leaves by steam distillation (heat treatment), large amounts of organic wastes are obtained. This by-product could easily be used as animal feed. The potential of sugarcane molasse and whey as additives to ensile lemongrass leaves was investigated. The vegetative material was obtained after essential oils were extracted from lemongrass leaves with steam distillation. Lemongrass leaves were chopped, mixed with sugarcane molasse and whey, placed in cylindrical plastic containers, hermetically closed. The experimental design for pH optimization was a response surface 3-level factorial design 3² with three replicates. Sugarcane molasse at 5, 10 and 15% (w/w) and whey at 20, 25 and 30% (w/w) were used. The pH of the silage decreased significantly in each treatment, but it was faster when sugarcane molasse was added. The lactic acid concentration was 2.8 g kg⁻¹ DM in silage amended with 15% molasse plus 25% whey and was higher than in silage supplemented with commercial additive after 15 d. In conclusion, it was shown that lemongrass leaves obtained as by product after extracting essential oils can be effectively ensiled with whey and sugarcane molasse as additives inducing a faster production of lactic acid.

Key words: Silage quality, response surface methodology, pH, lactic acid.

Ensiling plants often gives silage suitable for feeding (Weinberg and Muck, 1996). Water-soluble carbohydrates (WSC) are fermented to lactic acid by lactic acid bacteria (LAB), which decreases pH, inhibits the activity of plant enzymes and reduces pathogenic or spoilage bacteria that can decrease the nutritive value of the silage (Davies *et al.*, 2000). The main objective to apply additives for ensiling is to reduce pH more rapidly so as to preserve carbohydrates and proteins and inhibit the growth of microorganisms that might deteriorate the silage (Zhang *et al.*, 2000). Silage additives can be divided into two major groups: fermentation inhibitors (e.g. organic acids) and fermentation stimulators (e.g. strains of lactobacilli or readily degradable sugars) (Hetta *et al.*, 2003). The cost and availability of commercial silage additives are often a limiting factor and by-products can then serve as an

alternative. Use of by-products depends on availability and their possible nutritive value for cattle. In developed countries, the main treatment for silage is primarily sugarcane molasse, a by-product in sugar production (Kahi and Rewe, 2008). It has been used as a supplement for cattle manure silage (Martínez-Avalos *et al.*, 1998). Sugarcane molasse also has been used as a supplement for groundnut (*Arachis hypogaea* L.), sweet potato (*Ipomoea batatas* [L.] Lam.) and Jerusalem artichoke tops (*Helianthus tuberosus* L.) silages (Kaya and Caliskan, 2010).

Whey is a cheap residue of cheese production and it has been added to wheat straw and rice bran silage (Daniels *et al.*, 1983). A combination of molasse and dehydrated whey has been used as supplements to ensile fish by-products (De Lurdes *et al.*, 1998) and as additives for the ensiling of maize (Bautista-Trujillo *et al.*, 2009), but it is important to find the optimal concentration for each of them. Response surface methodology (RSM) is a set of mathematical and statistical techniques used widely to determine the effects of the studied variables and to optimize different biotechnological process (Cao *et al.*, 2008).

Lemongrass (*Cymbopogon citratus* [DC.] Stapf) are cultivated in Chiapas and extracted for their essential oils. The extracted plant material is nutrient rich, but little is known about its potential to ensile. The objective of the present work was to optimize cane molasse and whey

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as silage additives to lemongrass leaves applying the response surface methodology.

MATERIALS AND METHODS

Lemongrass leaves were provided by 'Unión de Pequeños Productores de las Zonas Marginadas del Sureste de Chiapas S.P.R de R.I.' and harvested at Cintalapa (16°40' N 93°43' W), Chiapas, México. The vegetative material was obtained after essential oils were extracted from lemongrass leaves with steam distillation at 120 °C for 90 min.

Optimization of sugarcane molasse and whey as silage additives to lemongrass leaves

The experimental design for pH optimization was a response surface 3-level factorial design 3² with three replicates. Sugarcane molasse at 5, 10 and 15% (w/w) and whey at 20, 25 and 30% (w/w) were used (Table 1). From each mixture, 1 kg was placed in a cylindrical plastic container (15 cm height × 12 cm diameter) and closed airtight with a plastic lid, i.e. mini silos. Each mini silo was an experimental unit and applied in triplicate. Mini silos were kept at 30 °C for 15 d. A 10 g sub-sample was taken after 0, 3, 7 and 15 d, weighted and characterized (Megías *et al.*, 1999).

Chemical analysis

Samples of pre- and post-ensiled mixtures were analyzed for pH and dry matter, organic matter, total N and ash content (AOAC, 1980). The neutral detergent fiber content was analyzed as described by Goering and Van Soest (1970). Lactic acid was determined colorimetrically using standard solutions (0-30 µg mL⁻¹ in 5-µg increments) (Taylor, 1996). Briefly, 1 mL standard solution was added to 10 mL borosilicate tubes containing 6 mL concentrated H₂SO₄ and mixed on a vortex mixer. The mixed solutions were incubated in a water bath at 95-100 °C for 10 min. The tubes were cooled to room temperature, added with 100 µL CuSO₄ and 200 µL para-phenyl phenol reagent and mixed on a vortex mixer. The tubes were kept at room temperature (> 20 °C) for 30 min and the absorbance measured at 570 nm (Madrid *et al.*, 1999). All analyses were done five times.

Table 1. Experimental design to investigate the effect of sugarcane molasse and whey as additives in the silage of lemongrass leaves (*Cymbopogon citratus*) by-product and pH determined after 5 d.

Treatment	Sugarcane molasse		pH after 5 d
	Whey	%	
1	20	5	5.7 ± 15
2	20	10	5.1 ± 14
3	20	15	4.7 ± 0.5
4	25	5	5.5 ± 23
5	25	10	5.0 ± 20
6	25	15	4.6 ± 0.5
7	30	5	5.4 ± 0.8
8	30	10	4.9 ± 14
9	30	15	4.6 ± 20

Determination of silage quality

After 15 d, the fermentation was terminated, the mini silos opened and the silage characterized. The measured characteristics were color, taste, structure, and pH. Sub-samples from different points and depths were taken, pooled and the water content determined by oven drying at 65 °C until a constant weight was achieved. The samples were hammer-milled and characterized chemically. The pH was measured by heating a 100 g sub-sample in a beaker containing 100 mL distilled water at 60 °C for 5 min. The supernatant was decanted, cooled at room temperature and the pH measured. Color was determined using color charts. The taste of the silage, which was defined as being nice, pleasant or fruity, was determined as described by Babayemi (2009). Briefly, a panel of seven people was trained to use their tongue to detect a taste by comparing it with known ones. A variety of taste-like substances, such as wine, vinegar, fruits, were provided for comparison. The structure of the silage was also determined and was found to be separable or firm.

Comparison with a commercial product

Once the optimal concentrations of sugarcane molasse and whey for ensilage were determined, lemongrass leaves were ensiled under these conditions and compared with lemongrass leaves ensiled with a commercial product Pro B&W® (B&W Feeds (Mobile) Ltd. Bussey Stool Farm Tarrant Gunville Blandford, England), i.e. wetttable powder for silage, which contains bacteria, such as *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Bacillus* and *Lactobacillus casei*, and enzymes, such as amylase, beta-gluconase, and hemicellulase. Lactic acid production and pH were determined after 0, 3, 5, 7, 15 d.

Statistical analysis

All results were subjected to a one-way ANOVA to test for significant differences between treatments using PROC GLM (SAS statistical package) with the Tukey's Student Range test (P < 0.05) (SAS Institute, 1989).

In the optimization process, all data were analyzed by multiple regressions with the least square method:

$$Y = \beta_0 + \beta_1 SM + \beta_2 W + \beta_3 SM * W + \beta_4 SM^2 + \beta_5 W^2 \quad [1]$$

where β are coefficients to identify, SC is sugarcane molasse (%) and W is whey (%). The R² were calculated for equation.

RESULTS AND DISCUSSION

Optimization of sugarcane molasse and whey as silage additives to lemongrass leaves

The concentration of sugarcane molasse (SM) and whey and the interaction between the two factors significantly affected the pH in silage of lemongrass leaves. Both sugarcane molasse and whey individually induced a

decrease in pH while the interaction between them increased it (Figure 1a). The effect of SM on the pH decrease was more accentuated than that of whey (Figure 1b). The response surface curve indicated that a lower pH was obtained using 13-15% sugarcane molasses combined with any of the whey concentration used (Figure 1c). A maximum pH decrease was obtained with 25% whey and 15% SM.

After lineal regression Equation [1] could be written as:

$$Y = 8.36 - 0.21SM - 0.13W + 0.003SM * W + 0.002SM^2 + 0.001W^2 \quad [2]$$

with a R^2 of 90%. p-Values indicate that only SM and W factors of the model were significant ($p < 0.05$). The model could be written as:

$$Y = 6.36 - 0.09SM - 0.16W \quad [3]$$

with a R^2 of 87.27%.

Minimal values for pH after ensiling were used to optimize the ensiling process. A minimum pH of 4.54 was thus obtained using 25% of whey and 15% of SM.

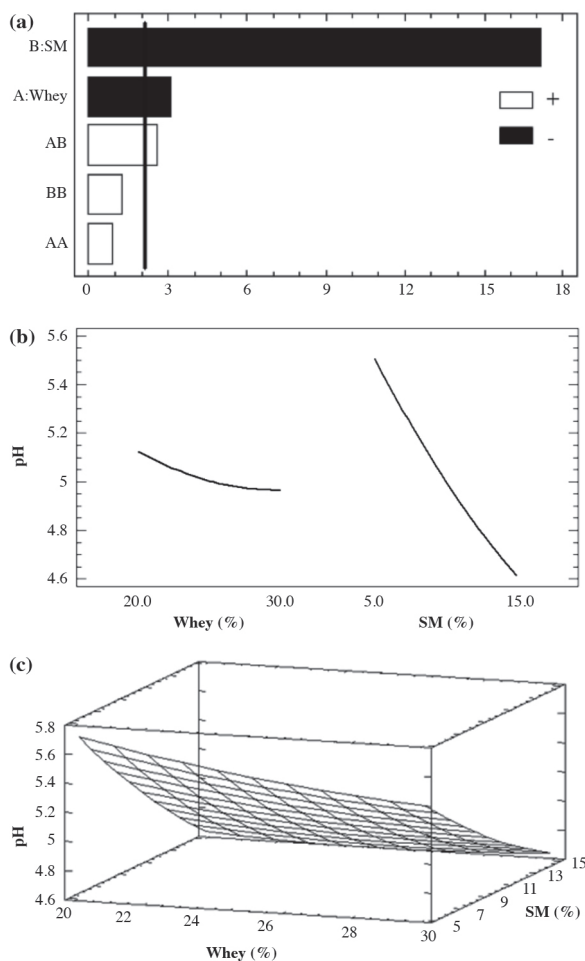


Figure 1. Standardized Pareto chart (a), main effects (b) and response surface plot (c) of the effect of sugarcane molasse (SM) and whey on pH in the ensiling of lemongrass (*Cymbopogon citratus*) leaves.

The model explained 87.3% of the variation found for pH in the silage. The pH of the silage varied from 5.7 to 4.6 in each treatment (Table 1). The pH decrease at the onset of the ensiling was presumably related to lactic acid production. The pH reduction could be due to the rapid degradation of the carbohydrates added with the SM (McKendrick *et al.*, 2003). These carbohydrates were used immediately by lactic acid forming bacteria resulting in a reduced pH (Weinberg and Muck, 1996). The decrease in pH aided to hydrolyze the plant polysaccharides (Zhang *et al.*, 2000). Whey increased the acidity and components, such as albumin and lactoglobulin rich in sulfur amino acids (cysteine and methionine) and minerals (Ha and Zemel, 2003), generated better conditions for bacterial growth by increasing the fermentative lactic acid production (Zhang *et al.*, 2000). Whey also contained lactic acid bacteria (LAB), such as *Lactobacillus delbreueckii*, *L. helveticus* and *Streptomyces termophilus*. Lactic acid will reduce pH inhibiting the growth of undesirable bacteria (Mannu *et al.*, 2002).

The chemical characteristics of the silage derived from lemongrass leaves with addition of SM (15%) and whey (25%) are given in Table 2. The ash content was higher in lemongrass after silage than raw lemongrass leaves. This increase in ash content may be due to the SM and whey added. Shafaghat *et al.* (2010) reported 0.12 mg L^{-1} ash content of SM, while for whey, Outinen *et al.* (2010) reported an average of 0.44 g $100 g^{-1}$. The main minerals in whey are Na, K, Ca, Mg, Cl, and P (Outinen *et al.*, 2010). These minerals probably favor the growth of LAB's. The neutral detergent fiber content was higher in lemongrass leaves before ensiling than at end of ensiling. On the contrary, N free extract was higher in lemongrass leaves further ensiling. The fiber content decreased as cellulolytic microorganisms degraded (hemi)cellulose (Lin *et al.*, 1992).

The organic matter and total protein content and the ether extractable matter of the lemongrass leaves were not significantly affected by ensiling with 15% SM and 25% whey. This is important, as a key factor in ensiling

Table 2. Chemical composition before and after ensiling (15 d) of lemongrass leaves (*Cymbopogon citratus*) amended with sugarcane molasse (15%) and whey (25%).

Characteristics	Before ensiling	After ensiling	MSD ^a (0.05)
	g 100 g ⁻¹ dry weight		
Ash	11.7 (0.2)b	12.3 (0.2)a	0.2
Organic matter	88.3 (0.5)a	87.7 (0.2)a	0.1
Crude protein	6.2 (0.1)a	6.6 (0.3)a	0.1
Ether extract	7.9 (0.8)a	9.0 (0.5)a	0.5
Neutral detergent fiber	29.7 (0.4)a	24.6 (0.31)b	1.2
Nitrogen free extract	44.5 (0.4)b	47.5 (0.86)a	1.5
Silage quality			
Color		Olive-green	
Taste		Pleasant	
Structure		Firm	
pH		4.1	

^aMSD: Minimum significant difference ($P < 0.05$); values between parenthesis are standard deviations of the mean ($n = 5$); values within the row with a different letter denote significant differences between treatments ($P < 0.05$).

is to maintain the DM content while reducing effluent production (Haigh, 1999). A negative relationship has often been found between the DM content of the ensiled crop and the amount of effluent produced and silage effluent is a major source of agricultural pollution (Megías *et al.*, 1999). Losses of nitrogenous compounds were negligible and indicative of a suitable fermentation, although changes in their distribution might have occurred during the ensilage (Driehuis and van Wikselaar, 2001).

Silage quality

The silage color was olive-green and similar to the color of the lemongrass leaves before ensiling. The taste of the ensiled lemongrass leaves was characterized as pleasant; the texture was firm, but not compact while the pH was 4.1 after 15 d (Table 2). These are characteristics of good quality silage. Omokanye *et al.* (2001) reported that a pleasant taste is common for well made silage and a good silage product usually preserves the original color of the raw plant material. The texture of the silage was as reported for good silage (Kung and Shaver, 2002). Generally, the pH is one of the simplest and quickest ways of evaluating silage quality (Babayemi, 2009). Silage of good quality should have a much lower pH than the original product (Meneses *et al.*, 2007). Lemongrass silage was a pH 9.2 at the beginning of the experiment and 4.1 after 5 d (Table 3).

Comparison with a commercial product

Changes in pH and lactic acid were registered in lemongrass silage supplemented with 15% SM and 25% whey and were compared with lemongrass silage with commercial additive (Pro B&W®). Addition of SM and whey resulted in a higher lactic acid production and a lower pH than the lemon leaves ensiled with the commercial product (Table 3). A rapid production of lactic acid is important to obtain high quality silage (Rong *et al.*, 2010). Lactic acid is responsible for the inactivation of plant enzymes and undesirable microorganism that might inhibit fermentation or lead to silage deterioration even after the ensiling was over, i.e. silage with a low stability (Opitz von Boberfeld, 2001; Kondo *et al.*, 2004).

Table 3. Changes in pH and lactic acid production in the silage of lemongrass leaves by-product (*Cymbopogon citratus*) with addition of sugarcane molasse (SM) and whey and commercial additive (Pro B&W®).

Treatment	Silage time				
	0 d	3 d	5 d	7 d	15 d
	pH				
SM + whey	9.2b	5.2b	4.1b	4.1b	4.1b
Commercial	10.0a	6.8a	6.2a	6.1a	5.4a
MSD (P < 0.05)	0.6	1.2	1.3	1.4	1.2
	Lactic acid (g kg ⁻¹ DM)				
SM + whey	0.09a	1.5a	2.4a	2.6a	2.8a
Commercial	0.09a	0.3b	0.6b	0.9b	1.1b
MSD (P < 0.05)	0.03	0.9	1.0	1.2	1.2

Values are the mean of five replicates (*n* = 5); values within the column with a different letter denote significant differences between treatments (P < 0.05); MSD: Minimum significant difference (P < 0.05).

CONCLUSIONS

It was shown that lemongrass leaves can be effectively ensiled with whey and sugarcane molasse. A maximum pH decrease was found with 25% whey and 15% sugarcane molasses and lemongrass silage suitable for ruminants was obtained within 15 d. The pH decrease at the onset of the ensiling was presumably related to lactic acid production. The silage was of good quality based on color, taste, structure and pH. Further experiments will be required to determine whether similar results can be achieved in commercial silos.

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Melaza de caña de azúcar y suero de leche como aditivos en el ensilaje de hojas de zacate de limón (*Cymbopogon citratus* [DC.] Stapf). Después de la extracción del aceite esencial de zacate limón (*Cymbopogon citratus* [DC.] Stapf) por destilación con vapor (tratamiento térmico) se obtiene un residuo. Este residuo podría ser utilizado para alimentación animal. El potencial de la melaza de caña y suero de leche como aditivos para ensilar hojas de zacate limón fue investigado. El material vegetal se obtuvo después que los aceites esenciales se extrajeron de las hojas de zacate limón usando destilación con vapor. Las hojas de zacate limón se picaron, mezclaron con melaza de caña y suero de leche y se colocaron en recipientes cilíndricos de plástico y se cerraron herméticamente. El diseño experimental para la optimización del pH fue uno de superficie de respuesta factorial 3² con tres repeticiones. Se utilizó melaza de caña de azúcar 5, 10 y 15% (p/p) y suero de leche 20, 25 y 30% (p/p). Las diferentes mezclas se caracterizaron químicamente y se colocaron en recipientes de plástico herméticamente cerrados. El pH del ensilaje se redujo significativamente en cada uno de los tratamientos, pero la disminución del pH fue más rápida cuando se añadió melaza de caña. La concentración de ácido láctico fue de 2,8 g kg⁻¹ MS en el ensilaje adicionado con 15% de melaza más 25% de suero de leche. La concentración de ácido láctico fue mayor que en el ensilaje complementado con aditivos comerciales después de 15 d. En conclusión, se demostró que las hojas de zacate limón que se obtienen como desecho después de extraerles los aceites esenciales pueden ser efectivamente ensiladas con melaza de caña de azúcar y suero de leche como aditivos para inducir una producción más rápida de ácido láctico.

Palabras clave: calidad del ensilado, metodología de superficie de respuesta, pH, ácido láctico.

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