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The Effect of Molasses and Probiotic as Additive on the Physical Quality, Chemical and Microbial Composition of Napier Silages

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Abstract

The effect of molasses and probiotic on physical quality, chemical and microbial composition of 40 days ensilage of napier variety, Pakchong 1 was evaluated using wilted napier grass with 10% molasses as silage additive and combination of 5%, 10%, and 15% molasses with 1% probiotic/kg DM. Physical quality of silages showed similarities in color, smell texture, and acidity while viable concentrations of lactic acid bacteria did not vary significantly ($p>0.05$) among treatments. The concentrations of lactic acid and acetic acid were significantly higher ($p<0.05$) in silage using molasses alone as additive compared to different levels of additive molasses with probiotics. On the other hand, proximate chemical composition of dry matter, crude protein, crude fat, crude ash did not differ significantly ($P>0.05$) among treatments. Species of Lactobacillus and Streptococcus were isolated from Pakchong 1 and from the probiotic used in this study. In conclusion, physical, chemical and microbial composition of Napier silages can be influenced by the type of additives used. In particular, molasses was observe to be a potential source of soluble carbohydrates needed by natural lactic acid bacteria in napier. Likewise, probiotic produces high concentrations of lactic acids with subsequent preservation of the silage at a given duration of ensiling.

Key Words: Napier variety Pakchong 1, *Lactobacillus*, *Streptococcus,* low water soluble carbohydrates, silages

Introduction

Drying has always been the most common method of preserving grain. Grains should be dried to a certain moisture content (MC) depending to the purpose such as milling and storage to avoid potential problems. Many mechanical dryers were developed to address the drying problems especially during rainy season and sun drying is not applicable.

Fluidized bed dryer has been introduced to address the problems in drying large volume of grains especially during wet season. Fluidized bed dryers provide faster moisture reduction and uniformity of drying. This dryer was designed to handle large volume of grains with high levels of moisture content and impurities. In the fluidized bed dryer, grains are entrained in very high airflow, causing vigorous mixing of grain and air, and resulting to fluid-like behavior of the grains. These also result to rapid and uniform drying of grains. Moreover, the clumping and clogging of grains are minimized and the impurities are blown away into the cyclones.

However, improper drying using these machines may affect the quality of head rice after milling. Hence, many simulation analyses were developed to provide prediction on drying operation. A computer-based simulation model Recent development related to forage production for dairy buffalo production in the Philippines was the introduction of a hybrid Napier "Pakchong 1" from Thailand. The hybrid forage crop agri-management, high dry matter yield and crude protein content contribute to its potential for ensilage. The technology of feed preservation is well adapted by small scale dairy farmers who profit from milk and dairy products (Wadi *et al.,* 2004). Silage additive is important in forages with low water soluble carbohydrates in order to improve process efficiency, silage quality and animal performance in silages using forages (Yahaya *et al.* 2004; Ohmomo *et al.* 2002). Among the commercially available feed ingredient, molasses is commonly used silage additives because of the high content of water soluble carbohydrates and stimulating effect on growth of LAB (Van Niekerk *et al.* 2007; Yunus *et al.* 2000; Yokota *et al.* 1991). In fact, according to Bureenok *et al.* (2012) molasses improves fermentative quality, including animal feed intake and digestibility of Napier silage better than silages treated with lactic acid bacteria (LAB).

Microbial inoculants serve different purposes in silage production in forages such that preliminary evaluations are empirical before its adaption. Being an extrinsic factor, the composition of lactic acid bacteria prior to ensiling plants may influence conditions like pH and lactic acid concentrations in silages. According to Cai, (1999), not all lactic acid forming bacteria from forage crops can improve fermentation just like *Lactobacillus* species. Species of *Enterococcus* and *Clostridia* from forage crops produce fermentation acids that did not improve ensiling. Thus, microbial composition in the plant is as important a factor as composition of LAB in microbial additives (Jarc *et al*. 2009; Weinberg *et al.,* 2006; Holzer *et al.,* 2003; Harrison *et al.,* 1989 and Ely *et al.,* 1981). Preliminary information on the composition and concentration of lactic acid bacteria in Philippine forages are limited, hence, the need for a database and development criteria for improving silage production using different grass species and newly introduced varieties like Pakchong 1 hybrids of napier. This study determined the composition and concentration of lactic acid bacteria in napier and the effect of molasses and probiotic on physical, chemical and microbial composition of ensilage with the goal of improving silage quality and silage production efficiency. Also, the study determined the morphological characteristics of lactic acid bacteria in wilted napier, probiotic, and products of silages with molasses and probiotic after 40 days ensilage and the production cost due to silages additives, molasses and probiotic.

Materials and Methods

Experimental Lay-out

Napier grass with a growth stage of 50 to 60 days was harvested and ensiled with molasses without probiotic, combined 5%, 10%, and 15% molasses with 1% probiotic. The experiment was laid out in Complete Randomized Design with molasses and probiotic as main treatments.

Treatment 1 (Control)	= Napier + 10% Molasses
Treatment 2	= Napier + 5% Molasses + 1% Probiotic
Treatment 3	= Napier + 10% Molasses + 1% Probiotic
Treatment 4	= Napier + 15% Molasses + 1% Probiotic

Ensiling Procedure

Pakchong 1 was harvested 10 cm above the ground, chopped to about 1 cm using laboratory chopper, then wilted under shade for 6 hours. One (1) kg sample of grass was mixed with molasses at 10% of the dry weight of sample and placed in airtight polypropylene bag. Another one (1) kg sample each was mixed with 5, 10, 15% molasses plus 1% probiotic, then placed in polypropylene bag and sealed tight using rubber band. Each treatment was prepared in three silo bags, each bag served as replicate. The duration of ensiling was 40 days.

Figure 1. Napier grass

After ensiling, silages were evaluated for fermentation characteristics such as physical, chemical components and microbiological composition. The ensilages were poured in stainless pan, homogenously mixed then sub-samples were collected by quartering methods. Sub-samples of one quarter were used for sap microbial analysis, another quarter sample for chemical analysis (AOAC, 1980).

Isolation and morphological characterization of isolated microbes in napier, probiotic and silages

For the microbiological assay of lactic acid bacteria, one (1) ml of serial dilution sap samples was inoculated in MRS Agar (MRS, 1960). Sample plates were placed in an anaerobic jar, and then incubated at 37^{o}C for 3 to 5 days. The cultured bacteria in MRSA were swabbed using sterilized cotton buds, then stained in vials with Erythrosine B. Thereafter, 0.1 ml of stained sample was placed in hemocytometer and viewed under the microscope for direct bacterial population counting and morphological evaluation.

For lactic acid bacteria, samples of sap from 6 hr. wilted napier, probiotic and treatments were cultured in MRSA (MRS, 1960) under anaerobic condition for 24 hours at 37°C. After incubation fresh culture of lactic acid bacteria in MRSA plates were evaluated for concentration of lactic acid bacteria by direct count using a New Bauer hemocytometer. The growth was sub-cultured in the test tube with MRS Agar and incubated at 37ºC for 3 days.

The fresh culture was used in the morphological characterization of isolate following standard procedure for catalase test, spore formation, and reaction to gram staining technique following standard procedures on microbiological assay on feeds.

For the analysis of mold in silages, 1 ml of silage sap was pour plated in plates with PDA cultured medium. The plates were incubated in anaerobic jar, and then gassed with CO_2 for 5 minutes. The anaerobic jar was incubated at 30^{o}C for 5 days or until the growth is visible. Molds were characterized according to their colonial features in PDA culture medium.

Characterization of Silage Fermentation

Ensilage pH was determined from sap sample of treatments silages using portable pH meter. The concentrations of lactic acid and acetic acid were determined by titration acidity using 0.1N NaOH solution. The acidity of the silage as percent lactic acid and percent acetic acid was calculated using the following formulas:

$$\% \text{ Titratable Acidity } (\% \text{ lactic acid}) = \frac{\text{ml NaOH} \times 0.009 \times N \times 500}{W} \times 100$$

$$\% \text{ Titratable Acidity } (\% \text{ acetic acid}) = \frac{\text{ml NaOH} \times 0.006 \times N \times 500}{W} \times 100$$

Where:
1 ml of 0.1 N alkali is equivalent to 0.009 g Lactic Acid
1 ml of 0.1 N alkali is equivalent to 0.006 g Acetic Acid
N = normality of standardized NaOH
W = sample weight

Proximate Chemical Composition Analysis of Silages

Samples of silages from each treatment were analyzed for moisture content, dry matter, crude protein, crude fat, crude fiber, crude ash using the proximate Wendee method (AOAC, 1980).

Statistical Method

Data gathered on lactic acid concentrations, pH, lactic acid, acetic acid, and composition analysis were analyzed using analysis of variance (ANOVA) in Complete Randomized Design (CRD) using statistical software SAS, 1984. Significant means were compared using Duncan's Multiple Range Test at 5% level of significance.

Results and Discussion

Fermentation characteristics of Pakchong 1 silages. The concentrations of lactic acid bacteria in all treatments with probiotic did not vary with those using molasses alone after 40 days. All treatments showed no significant ($P>0.05$) effect on the concentrations of lactic acid bacteria (Table 1). Pakchong 1 silages had concentrations of LAB (10^8) that was higher than the recommended concentration of LAB (10^5) for efficient ensiling (Harrison *et al.* 1989; Ely *et al.* 1981). In terms of pH, all treatments had low pH silages, indicating of high concentrations of lactic acid and other naturally produced acids during ensilage (Table 1). Silages showed physical characteristics of mild, pleasantly acidic smell and leaf colors that vary from green to greenish tints and green to light yellowish and soft texture and intact stems.

Silages with 10% molasses without probiotic had the highest lactic acid (P<0.05) concentrations compared with different levels of molasses with 1% probiotic (Table 1). This findings showed that the difference in lactic acid concentration was attributed to composition of indigenous LAB in treatment 1 compared to treatment 2, 3 and 4. Cai, 1999 had indicated that some isolated *Enterococcus* in raw plant material did not improve fermentation compared to *Lactobacillus* species. Studies showed also that *Clostridia* in silages fermented soluble carbohydrates into volatile fatty acids at the earlier stages of ensiling.

Table 1. Fermentation characteristic of Napier ensiled with molasses and probiotic

Particular	10% Molasses	5%Molasses +1% Pro	10% Molasses + 1% Pro	15% Molasses + 1% Pro
Lactic acid bacteria ($x10^8$) cfu/ml	7.2	7.4	6.7	7.3
pH	4.75	4.80	4.58	4.78
Lactic acid,%	16.67*a*	13.07*b*	13.67*b*	14.33*b*
Acetic acid,%	0.10 *a*	0.07*b*	0.08*b*	0.09*b*

Means with same letter superscript did not indicate significant difference at 5% DMRT

Napier silage with molasses had the highest acetic acid production at 0.10% compared to different percentage of molasses with 1% probiotic (Table 1). The trend on lactic acid and acetic acid production in the Pakchong 1 silages was similar with other species such as the *Pennesitum* and other grass silages. According to Danner *et al.* (2003) and Lima *et al.* (2010), a good quality of silage is associated with at least 70% but not more than 90% lactic acid and not more than 20% acetic acid.

Table 2. Chemical composition of silages of Napier from different treatment

Composition	10% Molasses	5% Molasses +1% Pro	10% Molasses +1% Pro	15% Molasses + 1% Pro
Moisture Content	77.53	76.31	77.53	75.64
Dry matter,%	20.09	20.54	20.05	20.25
Crude Protein,%	4.85	5.10	5.56	5.45
Crude Fiber,%	35.06	35.2	35.22	37.08
Crude Fat,%	4.35	3.95	4.50	5.70
Crude Ash,%	8.61	8.43	8.89	9.68

Means with common letter are not significant at 5% Duncan's Multiple Range Test

Napier ensiled with molasses had the highest percentage of moisture compared to other treatments (Table 2). Dry matter contents ranged from 20.05% to 20.54% after 40 days. Treatments had insignificant effect on the moisture content of silages (P<0.05). Increasing the molasses to 15% with the same level of probiotic did improved protein at 5.45%. Pakchong grass contain 16 to 15% crude protein (Wadi *etal*.,2004) so the low crude protein of the Pakchong silages was attributed to the high proportions of stems in material harvested at 50 to 60 days of growth (Eslabra *et al.*, 2016).

For the silage crude fiber content, Napier silage treated with molasses had 35.06% crude fiber had slight variations with different amount of molasses and 1% probiotic. Crude fiber content of Pakchong silages had values within the crude fiber content of aerial part of Napier grass harvested at 40 to 70 days (Kearl, 1982).

For crude fat content**,** Pakchong 1 silages after 40 days revealed highest crude fat content being 5.70% in treatment 1, 4.5% crude fat with treatment 2, 4.35% crude fat in treatment 3, and 3.95% crude fat in treatment 4. Statistically, treatments had insignificant effect on crude fat content of silages (P<0.05). Pakchong 1 silages content of crude fat was within the fat content of Napier grass (Kearl, 1982) while increases due to molasses and probiotic was in agreement with Fujita *et al.* (2007 on the effect of silage additives on nutritive content of silages. The silages in treatment 4 had the highest crude ash content of 9.68% compared to other treatment silages. Statistically, treatments did not have significant effect on crude ash content of silages (P<0.05). Difference may be attributed to macro minerals like calcium, phosphorus, sulfur, and potassium in molasses (Kearl, 1982).

Morphological Characteristics of LAB in Napier, probiotic and silages

Table 3 showed the lactic acid forming bacteria (LAB) isolated from treatments silages collected at 40 days. The isolates of LAB were long, slender, all non-spore forming, gram-positive rods shaped typical of *Lactobacilli* species while the gram-negative cocci were typical of *Lactobacilli* and *Streptoccci* spp. Study showed the prominence of long, and robust rods to short coccobacilli rods typical to *Lactobacillus plantarum* (Hammes and Hertel (2009). Other companion species of lactic acid forming bacteria with cocco- ovoidal shapes were specie from genus *Streptococcus,* and *Pediococcus*, *Enterococcus*, and Leuconostoc (Pahlow *et al*. (2003). The prominence of the LAB species in the different treatments were attributed to the LAB found in Pakchong grass harvested at 50 to 60 days and the probiotic introduced with molasses use in the study.

Table 3. Morphological characteristic of lactic acid bacteria isolated from Napier, probiotic and silages treated with molasses and probiotic

Treatment	Morphological Features				
	Catalase	Spore	Shape	Gram Positive	LAB
10% Molasses	- +	-	Rods Cocci	+ -	*Lactobacillus* sp *Streptococcus* sp
5% Molasses + 1% Probiotic	- +	-	Rod Cocci	+ -	*Lactobacillus* sp
10% Molasses+ 1% Probiotic	- +	-	Rod Cocci	+ -	*Streptococcus* sp *Lactobacillus* sp
15% Molasses +1% Probiotic	-	-	Rod	+	*Lactobacillus* sp

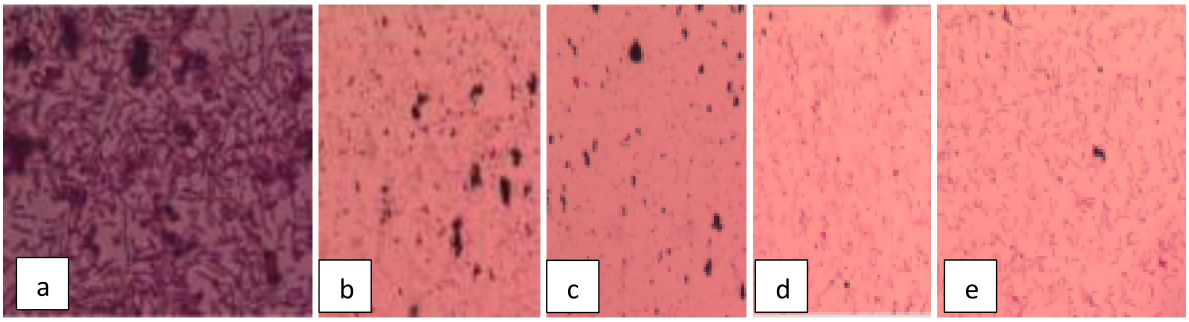

Figure 1a-e. Composition of microbes showed filamentous rods and cocci a) probiotics, b) molasses as additive, c) 5% molasses +1% probiotic d) 10% molasses plus 1% probiotic and e) 15% molasses and 1% probiotic. Cells were stained with erythrosine B and observed under low power objective microscope and viewed under high density screen.

Characteristics of molds in probiotic and silages

Study had shown that wilted Napier and molasses had no molds isolated but the probiotic solution indicated the presence of white and black molds colonies when cultured in PDA medium. Treatment 1 had no growth compared to treatments 2, 3 and 4 respectively. According to Storm *et al*., (2008), molds of several species can cause spoilage of silages due to micro-aerobic condition and acidic condition in the early stage of the ensiling process but since no mycelia was visible in the silages harvested at 40 days, the high concentration of lactic acid by LAB due to molasses and probiotic had resulted to dormancy of active spores. Spores of molds were implicated in poor aerial stability at feedout (Stefanie *et al*. 2016; Wadi *et al*., 2004)

According to Wadi *et al*., (2004), silage technology was well adapted by dairy farmers in advanced countries like Malaysia because of the profitability gained from dairy products. The introduction of Pakchong 1 as forages in the Philippine dairy production in the countryside had provided farmers the wide opportunity to increase their stocks and mitigate problems associated with lack of quality forages. The study showed that good physical, chemical and microbial composition in low water soluble carbohydrates Napier ensiled with molasses additives attributable to molasses effectiveness as source of soluble carbohydrates that stimulate the lactic acid bacteria into concentrations that rapidly reduce pH to 4 with subsequent preservation of the silage at a given duration of ensiling. Based on the significant results, this study has considered the following protocol for ensiling Napier with silages additives:

1) The need to evaluate the composition and concentration of lactic acid bacteria in forage material in relation to plant concentration of water soluble carbohydrates and fiber content.
2) When cost of molasses is high, the combination of molasses and probiotic is recommended.
3) Needs to periodically check the purity of the silage probiotic since the presence of molds can affect quality during feedout.
4) It is also a recommendation to further study the composition of the isolated LAB from plant material using physiochemical and molecular methods for future studies on plant probiotic as silage additives.

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