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Effect of Forage Species and Additives on Quality of Tropical Forage Silage

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Abstract: The current experiments was carried out to investigate the chemical composition and fermentation of silage for 2 species of tropical forage (Mulato II grass; *Bracharia ruziziensis x B. brizantha x B. decumbens* and Verano stylo; *Stylosanthes hamata*), havested at 45 days after regrowth. There were combined with 4 additive treatments (cassava meal 5%, molasses 2% and the Fermented Juice of epiphytic Lactic acid Bacteria (FJLB) 1% in a factorial arrangement in Completely Randomized Design (CRD). Sealed plastic bag and each treatment were replicated five times. Silage quality was affected by both species and additive treatments. The DM content of Verano stylo silage (31.26-33.60%) had higher ($p \leq 0.001$) than Mulato II grass silage (20.77-22.23%). The Mulato II grass added with molasses and cassava meal resulted in lower ($p \leq 0.05$) pH (3.30 and 4.01) and higher ($p \leq 0.001$) lactic acid content (81.12 and 102.03 g kg^{-1} DM), compared to the control and FJLB treatments (4.22 and 4.28, 48.34 and 52.36 g kg^{-1} DM, respectively). For Verano stylo silage, the control had higher pH value than cassava, molasses and FJLB additive (5.06, 4.81, 4.46 and 4.49, respectively) while there was no significant ($p > 0.05$) difference for lactic acid content (34.33, 41.08, 48.89 and 48.04, respectively). There was highest acetic and propionic acid contents for the Mulato II grass added with FJLB whereas acetic and propionic acid content of the Verano stylo added with FJLB had highest among other two additives but lower than the control group. For the practical relevance of this research, it can be concluded that addition of FJLB would improve silages quality of Mulato II by increase preserving properties, although a little effect found for lactic acid content.

Key words: Tropical forage, quality of silage, chemical compositions, grass, legume

INTRODUCTION

Forages such as grass and legume are preserved as silage, especially during dry season. The silage can be provided as feed for ruminant production throughout the year or in periods of restricted seasonal availability of pasture by supplementing the diet with a valuable source of energy and protein (Heinritz *et al*., 2012). In principle, forage silage was made by controlled anaerobic fermentation. An important technique to make a good silage is using the external weight to squeeze out all of the air from contained bag and arresting the natural process of oxidation and decay of harvested forages. Silage is produced successfully when bacteria producing lactic acid dominate fermentation and restrict the activity of clostridia (Bureenok *et al*., 2006). There are quite difficult to make silage and to control fermentation quality for tropical forage silage because tropical forage are low in Water Soluble Carbohydrates (WSC), high buffering capacity and low Lactic Acid Bacteria (LAB) (Niimi and Kawamura, 1998). Most of grasses have high moisture content and low water soluble carbohydrate levels (Nussio, 2005) whereas most of legumes have low sugar content and high buffering capacity (McDonald *et al*., 1991). Many researchers have attempted to devise for improving the quality of tropical forage silage. Supplementation of additive for ensiling is usually way for the improvement. Increasing supply of WSC for ensiling

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resulted in producing sufficient lactic acid for rapid pH reduction and improving the fermentative quality of silage made from tropical forage as the reports eariler (Bureenok *et al*., 2005a; Yahaya *et al*., 2004; Tamada *et al*., 1999; Sibanda *et al*., 1997). The additives commonly used for tropical forage ensiling are cassava meal, molasses and Lactic Acid Bacteria (LAB).

It is a well-known fact that LAB plays a crucial role in silage fermentation (Bureenok *et al*., 2005b). Lactic acid bacteria are generally added into forage to enhance the nutritional value of silage and prevent the growth of fungi or yeast that could cause aerobic spoilage (Amado *et al*., 2012; Flythe and Russell, 2004; Woolford, 1990). In studies earlier, inoculation of LAB at silage has been completed by many groups with inconsistent results. Somtime it was effective (Kumai *et al*., 1990; Tengerdy *et al*., 1991; Masuko *et al*., 1992; Rooke and Kafilzadeh, 1994) whilst sometime it was not (Lindgren *et al*., 1983). An important factor affecting the success of ensiling is a number of species and strains of LAB applied which is related to adaptation to the specific environment and enhancing the lactic acid production (Ohshima *et al*., 1997).

The natural microorganisms presented in forage crops are responsible for fermentation of silage and influence quality of silage. In addition, the proportional population of LAB is usually low and variable with standing crops. There are many reports showing that Fermented Juice of epiphytic Lactic acid Bacteria (FJLB), a culture solution produced by LAB have been used successfully to improve the nutritive value of various silage preparations (Ohshima *et al*., 1997; Masuko *et al*., 2002; Bureenok *et al*., 2005a, b, Takahashi *et al*., 2005; Horiguchi and Takahashi, 2007), for alfalfa, timothy and orchardgrass, guineagrass, rice and green soybean stover. However, there is no information available for ensiling of Mulato II grass and a little information for Verano stylo silage. Both Mulato II grass and Verano stylo are tropical forage found in Thailand and are used for ruminants. Ensiling for both forage species would be an alternative preservation during dry season of shortage of forage.

The aim of the present study was to determine the effect of FJLB, cassava meal and molasses as the additive treatment in Mulato II grass and Verano stylo harvested at 45 days after regrowth on the nutritive value and quality of tropical forage silage.

MATERIALS AND METHODS

Plant materials: The grasses and legumes were used and evaluated in this study as the followings: Mulato II grass (*Brachiaria ruziziensis x B. brizantha x B. decumbens*) and Verano stylo (*Stylosanthes hamata*). A series of 10 plots (each 3×3 m) was sowed without fertilizer on February 2008 at Faculty of Natural Resources, Rajamangala University of Technology-Isan, Sakon Nakhon Campus (located in North-Eastern part of Thailand). Forage samples were taken in July 2008 at 45 days after regrowth.

Silage making: After forage harvesting, the experimental forage were immediately chopped into 1-2 cm length pieces. Then, molasses, cassava meal and Fermented Juice of epiphytic Lactic acid Bacteria (FJLB) were added at 5, 2 and 1% of fresh matter as a silage additive, respectively while no additive added for the control grass and legume silages. Five replicated plastic bags per each treatment were prepared and allowed to be fermented for 80 days at room temperature.

Fermented Juice of epiphytic Lactic acid Bacteria (FJLB) preparation: The FJLB was prepared from Mulato II grass and Verano stylo before harvesting; 200 g of fresh grass was macerated with 600 mL of distilled water using a blender. The macerate was filtered and 50 mL of the filtrate was put into each flask. These filtrates in the flask were treated with glucose at the rate of 2% of volume and incubated at 30°C for 2 days. Chemical composition of the materials prior to ensiling is shown in Table 1.

Fermentation quality evaluation: After each bag of Mulato II grass and Verano stylo silage was opened, the silage contents were mixed thoroughly. Then, 20 g of the contents was sampled from each bag. Each sample was followed by adding about 70 g of distilled water and then macerating at 4°C for 24 h. This sample solution was filtered through two layers of cheesecloth and a filter paper No. 1 and then the filtrate was stored at -20°C prior to chemical analysis. The filtrate was used for determining pH, ammonia Nitrogen (NH_3-N), Lactic Acid (LA), Acetic Acid (AA), Propionic Acid (PA) and Butyric Acid (BA). Lactic acid and Volatile Fatty Acids (VFAs) were determined by High Performance Liquid Chromatography (HPLC). Silage pH was measured using a glass electrode pH meter.

Chemical analysis: DM content of the fresh materials and silages were determined by drying in a hot-air oven

Table 1: Chemical composition of the Mulato II grass and Verano stylo at 45 days after regrowth prior to ensiling

	Chemical composition (%)					
Forage species	DM	CP	EE	NDF	ADF	Ash
Mulato II grass	22.96	5.6	1.8	71.9	32.6	14.3
Verano stylo	30.62	18.0	1.9	52.0	38.1	16.3

at 60°C for 72 h then ground to pass through a 1 mm mesh screen and subsequently analyzed for chemical composition. Total N was determined using the Kjeldahl Method (Bremner, 1965) and Crude Protein (CP) was calculated by multiplying the N content by 6.25. Ether Extract (EE) and ash contents were quantified by AOAC (1985). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) estimated by the methods described by Goering and Van Soest (1970).

Statistical analysis: The data were statistically analyzed according to a 2×4 factorial in Completely Random Design (CRD) using the PROC GLM procedure (SAS, 1990). Significant differences among treatments were determined using Duncan's news multiple range test according to Steel and Torrie (1980). The following model were used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Where:

- Y_{ijk} = The observation made on kth experimental unit of jth species with the ith additive
- μ = Overall mean
- α_i = The effect of additive (i = 4)
- β_j = The effect of species (j = 2)
- $\alpha\beta_{ij}$ = The interaction effect of $\alpha_i \times \beta_j$
- ε_{ijk} = The residual error

RESULTS AND DISCUSSION

Chemical composition: Chemical composition of fresh Mulato II grass and Verano stylo at 45 days regrowth, prior ensilage are shown in Table 1. The value of CP contents in fresh Mulato II grass was rather low when compared with the reports earlier in other grasses (Demirkus and Budag, 2010; Herrero *et al*., 2001). This would be that no use of fertilizer applied for the forage during planting as the report of Peyraud and Astigarraga (1998) who have reviewed that nitrogen fertilization increased nitrogen contents in forages. This would explain the low CP contents of fresh Verano stylo as the rather higher CP contents (21-22%) in *Stylosanthes guianensis* cvv. Tha Phra and Ubon stylo reported by Hare *et al*. (2007). However, variation of environment may partly result in varied CP concentration in Verano stylo such as about 12% CP found in Verano stylo sowed in Nigeria (Bamikole *et al*., 2001). For other chemical composition (DM, NDF, ADF, EE and ash), there was closed to other grasses (Demirkus and Budag, 2010).

After ensiling, chemical composition of the silages has shown in Table 2. Both experimental silages had a little change of values for DM, CP, ADF and ash except for ash contents in Mulato II silage and Verano stylo silage supplemented with molasses. In the meantime, the values of EE and NDF of both studied silages were increased and decreased, respectively. These large decreases of NDF values would be that fermentation process of ensiling attribute to the hydrolysis of the cell wall of plant materials providing monosaccharides as addition sugars for lactic acid production during fermentation (Huisden *et al*., 2009). For the large increases of EE contents after ensiling, bacteria in silage would play a crucial role in biosynthesis of fatty acids (Fujita *et al*., 2007) and result in higher content of fat in silage.

DM content of the Verano stylo silage was higher than the Mulato II grass silage. The DM content of Mulato II grass silage achieved range from 20.76-22.22% which were lower ($p \le 0.001$) than that of

Table 2: Chemical compositions (%), pH, NH_3-N (g kg^{-1} of total N) and VFA content (g kg^{-1} DM) of legume and grass silage in control and treated additives

	Mulato II grass silage				Verano stylo silage					Significant		
Items	Control	Cassava	Molasses	FJLB	Control	Cassava	Molasses	FJLB	SEM	S	Ad	S×Ad
Chemical compositions												
DM	21.55^{B}	21.85^{B}	22.23^{B}	20.77^{B}	30.26^{A}	32.59^{A}	32.26^{A}	30.56^{A}	0.756	***	NS	NS
CP	5.27^{D}	6.62^{D}	6.27^{D}	6.72^{D}	19.03^{B}	22.93^{A}	16.19^{C}	16.92^{C}	0.237	***	**	***
EE	5.08^{B}	3.96^{C}	4.18^{C}	5.48^{A}	3.42^{D}	2.22^{F}	2.66^{E}	3.06^{D}	0.040	***	***	*
NDF	58.06^{A}	50.73^{BC}	55.17^{AB}	57.98^{A}	47.81^{C}	46.79^{C}	47.47^{C}	46.28^{C}	0.747	***	NS	NS
ADF	37.12^{A}	31.55^{B}	32.11^{B}	34.38^{AB}	34.21^{AB}	34.70^{AB}	33.99^{AB}	37.01^{A}	0.478	NS	NS	NS
Ash	22.45^{B}	21.25^{B}	23.32^{B}	22.92^{B}	15.01^{C}	14.67^{C}	27.89^{A}	12.59^{C}	0.464	***	***	***
Fermentation quality of silage												
pH	4.22^{CD}	4.01^{D}	3.30^{E}	4.28^{CD}	5.06^{A}	4.81^{AB}	4.46^{BC}	4.49^{BC}	0.044	***	**	**
NH_3-N	46.33^{A}	36.50^{B}	35.57^{B}	48.28^{A}	28.42^{C}	25.34^{C}	26.57^{D}	21.46^{D}	0.638	***	**	**
LA	48.34^{B}	81.12^{A}	102.03^{A}	52.36^{B}	34.33^{B}	41.08^{B}	48.89^{B}	48.04^{B}	2.372	***	***	*
AA	15.67^{DE}	10.66^{EF}	7.76^{F}	24.53^{B}	31.22^{A}	18.51^{CD}	16.06^{DE}	23.68^{BC}	0.591	***	***	**
PA	15.67^{CD}	17.90^{C}	8.34^{E}	24.53^{B}	31.22^{A}	12.74^{CDE}	12.46^{DE}	23.48^{B}	0.528	*	***	***
BA	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	54.75^{A}	11.36^{B}	0.00^{C}	10.99^{B}	0.205	***	***	***

$^{A-F}$Means followed by a different letter within the same row are significant different ($p \le 0.05$); NS: Not Significant different ($p > 0.05$); *$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$; SEM = Standard Error of Mean; FJLB = Fermented Juice of epiphytic Lactic acid Bacteria; S = Species, Ad = Additive; LA = Lactic Acid; AA = Acetic Acid; PA = Propionic Acid and BA = Butyric Acid

Verano stylo silage containing DM ranged from 30.26-32.59% (Table 2). During ensilage moisture contents of material may affect the silage fermentation quality because low DM content, extensively fermented silages are characterized by low concentrations of WSC (McDonald *et al*., 1991) and DM content below 25% may be marked loss in effluent during ensiling (Van Vuuren *et al*., 1995). In addition, Conaghant reported perennial ryegrass were of a potentially difficult to preserve nature with low DM (10.7-13.2%) concentration.

The NDF content of Mulato II grass silage had higher ($p\leq 0.001$) than Verano stylo silage while there were no differences for ADF content between both species silages and among additives. For the CP, EE and ash content of the silage were detected significant species ($p\leq 0.001$) and additive treatments ($p\leq 0.01$ and $p\leq 0.001$) effect. Obviously, the average CP levels were higher ($p\leq 0.001$) for the Verano stylo silage, compared with the CP content of the Mulato II grass silage. The Verano stylo added with cassava meal had the highest ($p\leq 0.001$) CP content followed by control and FJLB and molasses. However, there were no differences for CP levels among additive treatments in Mulato II silage. The higher CP contents in the Verano stylo silage reflected the CP contents in forage prior to ensiling. This would also imply that no influence of the experimental additives on CP concentration in the silages. When compared the average EE and ash content in the Mulato II grass silage and the Verano stylo silage, there was higher EE and ash content in the Mulato II grass silage. Obviously, adding FJLB to the Mulato II grass increased and given highest EE content and the Verano stylo added with FJLB had higher EE content than that added with cassava meal and molasses. This trend also found in the Verano stylo. Thus, it would be imply that the increase of fat contents in the silages was the influence of lactic acid bacteria, mainly contained in the control and FJLB groups. The ash content of molasses treated Verano stylo silage was highest ($p\leq 0.001$), compared to other additive treatments and the second high of ash contents in all additives of Mulato II grass silage. The explanation of these results would be the fact that ensiling resulted in reduced NDF, hemicelluloses and cellulose content compared with fresh forage (Hunt *et al*., 1993), consequently higher proportion of inorganic or ash contents remained in the silages. However, the interaction between species and additives would result in highest ash content in the Verano stylo treated with molasses containing ash about 8-10% (Olbrich, 1963). On overall, the three additives in the current study would preserve chemical composition as well as ensiling without additive and chemical composition of silages was mainly influenced by the composition in fresh forage before ensiling. However, ensiling decrease NDF and increase EE resulting in possibilities for the increase of digestibility and availability as earlier reports (Bureenok *et al*., 2011; Lima *et al*., 2011) and the higher proportion of energy supply from fat contents.

Fermentation quality: For the fermentation quality of silage (Table 2), the forage was detected significant species ($p\leq 0.05$ and $p\leq 0.001$) and additive treatments ($p\leq 0.001$) effect. The interaction of species and additive was found for all parameters of the fermentation quality of silage.

The pH value of the Mulato II grass silage was lowered than ($p\leq 0.001$) the Verano stylo. However, the NH_3-N content of the Mulato II grass silage was higher ($p\leq 0.001$) than the Verano stylo. The Mulato II grass treated with cassava meal and molasses had higher ($p\leq 0.05$) lactic acid content than control and FJLB while there was no significant ($p>0.05$) difference for lactic acid content among additive treatments in the Verano stylo. The Mulato II grass silage and the Verano stylo silage contained higher ($p\leq 0.001$) acetic and propionic acid content in FJLB than other additive except for the Verano stylo untreated (control) had the highest acetic and propionic acid content. The butyric acid content of the Verano stylo higher ($p\leq 0.001$) than the Mulato II grass. For Verano stylo, the butyric acid content was higher ($p\leq 0.001$) for control than cassava meal and FJLB, indicating poor silage quality (Woolford, 1984). However, butyric acid content was not detected in Mulato II grass silage. The fermentation development at of silage was reflected in pH value. Molasses is rich in Water Soluble Carbohydrate (WSC), particularly sucrose (Olbrich, 1963; Topps and Oliver, 1993), easier for producing lactic acid by bacteria. Higher proportion lactic acid contents in silage resulted in lower pH then stabilize fermentation and finally lower risk of spoilage from harmful microorganisms, especially Clostridium bacteria, mold and yeast. For non-sugar carbohydrate, Castle and Watson (1985) reported the fermentation of the carbohydrates was dominated by lactic acid bacteria, cause of reduce pH of silage. In addition, legumes normally contain high CP and high buffering capacity (Phiri *et al*., 2007) resulting in slowly dropping of ensiling pH during fermentation. The concentration of NH_3-N reflects part of the proteolysis occurring during ensiling and is explained by plant enzymatic (Heron *et al*., 1986). Well-preserved silage should have NH_3-N that is no more than 110 g kg^{-1} of total nitrogen (Umana *et al*., 1991). This would imply that the ensiling with and without additives in this study could preserve quality of silage. Acetic acid also obviously acts as an inhibitor of the growth of spoilage organisms, resulting in increases of the aerobic stability exponentially (Danner *et al*., 2003). The report of Sebastian *et al*. (1996) who found that propionic acid treatment was effective in preventing secondary fermentation, in limiting the growth of yeast and molds throughout fermentation and in controlling proliferation of yeast and molds in aerated silage. So, both acetic and propionic acids would be beneficial for silage preserving which is synergistic effect with lactic acid bacteria. However, the increase of butyric acid content in silage indicates more spoiling processes occurred.

Well preserved silage types could be characterized as low pH (<5), low ammonia N (<9% of total N) and low concentration of butyric acid (<5.5 g kg^{-1} DM) as concluded by Phiri *et al*. (2007). From the results of the present study, fermentation quality of the Mulato II grass silage with and without additives meets the criteria of well preserved silage as mention earlier, especially not detectable butyric acid with obvious high lactic acid contents in the Mulato II grass silage treated with cassava meal and molasses and high acetic and propionic acid contents in the Mulato II treated with FJLB. This indicates that sufficient amounts of sugar substrates for producing lactic acid at the initial phase of ensiling fermentation, simultaneously low buffering capacity resulting in lowering pH rapidly and stabilizing the low pH value. Only Verano stylo silage treated with molasses, fermentation quality had met the well preserved criteria above. This would be the results of more sugar content in molasses served for sufficient substrates at the initial phase of the fermentation compared to the other additives. Clearly, the Verano stylo silage without additive and with Cassava meal and FJLB had rather high butyric acid contents which would be the resulted of low sugar available for lactic acid bacteria and high buffering capacity in Verano stylo rendering rather difficulty to reduce pH. Therefore, adding with FJLB in Mulato II grass and Verano stylo would beneficial for storing silage during supply for ruminants, although not much increase of lactic acid contents. The FJLB would be an alternative additive as the low cost and easy preparing for farmers.

CONCLUSION

For the practical relevance of this research it can be concluded that all additives improved silage quality and increased lactic acid content for both Mulato II grass and Verano stylo silages. However, ensiling of Verano stylo should be aware for risk of spoiling. Fermented Juice of epiphytic Lactic acid Bacteria (FJLB) additive gave higher acetic and propionic acids, improving silage preservation. Supplementation of the FJLB for Mulato II ensiling would be an alternative additive for preserving silage.

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