Effect of effective microorganism (EM) and cassava starch on the physical quality and organic acid compositions of native grasses silage in central region of Lao PDR

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ABSTRACT: This study evaluated the physical quality and organic acid composition of grass silages prepared with and without effective microorganism (EM) and cassava starch at the time of ensiling. The native grasses in central region of Lao PDR were cut into pieces as long as pieces 2-3 cm and randomly allocated in a randomized complete block design with six treatments: T1= 10 ml EM + 0 g starch, T2= 7.5 ml EM + 2.5 g starch, T3= 5 ml EM + 5 g starch, T4= 2.5 ml EM + 7.5 g starch, T5 = 0 ml EM + 10 g starch, T6 = silage without additive (control). Three of which were sampled on days 0 and 30 after ensiling. The color of silage was measured by the CIE L*a*b* system using Hunter Labmini scan EZ. The results showed that the silage treated with 10 g of starch had a lowest pH (pH = 3.82). Silage with 2.5 ml of EM with 7.5 g starch had higher greenness more than control. Organic acids and short chain fatty acids of silage such as succinic acid, formic acid, acetic acid, propionic acid and butyric acid at 0 day of ensiling were not significantly different. However, the highest level of lactic acid at 30 days of ensiling was *Hymenachne* sp. grass silage treated with 5 ml EM + 5 g starch (T3). In conclusion, effective microorganism and starch used in this study improved color characteristics, reduced pH content of silage and increased lactic acid concentration of grass silage.

Keywords: Effeective microorganism, cassava starch, grass silage

Introduction

Ensiling is a forage preservation method based on lactic acid bacteria converting water-soluble carbohydrates into lactic acid. Based on combination of acidity and anaerobic condition protects the forage from the proliferation of deleterious bacteria and fungi, and it also increases the palatability of the forage due to lactic acid production (Filya et al, 2000; Yang et al., 2006). Therefore, the instability of fermentation quality is mainly attributed to the ability of lactic acid bacteria and water soluble carbohydrate in substrate (Ohmomo et al., 2007). To obtain a high-quality fermented product, bacterial inoculants are added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH, and thus improving silage preservation. Moreover, many additives have been used during silage fermentation to promote the growth of lactic acid bacteria populations as a way to increase the decomposition of lactic acids during ensiling.

The objectives of this study were to evaluate the physical quality and organic acid composition of Lao-native grasses silage with the addition of effective microorganism (EM) and cassava starch.

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Materials and methods

Silage Preparation

The native grasses in central region of Lao PDR were cut into pieces as long as pieces 2-3 cm and dried up to obtain moisture content 60-70% and randomly allocated in a randomized complete block design (RCBD) with six treatments: T1= 10 ml EM + 0 g starch, T2= 7.5 ml EM + 2.5 g starch, T3= 5 ml EM + 5 g starch, T4= 2.5 ml EM + 7.5 g starch, T5 = 0 ml EM + 10 g starch, T6 = silage without additive (control). These materials were prepared by using a small-scale system of silage fermentation (Cai et al. 1999). Silages were preserved into plastic film bags and ensilaged at room temperature (25-32°C) for 0 and 30 days.

Chemical and Statistical Analysis

Dry matter (DM) and organic matter (OM) were analyzed according to AOAC (1990). The acid detergent fiber (ADF), acid detergent lignin (ADL) and neutral detergent fiber (NDF) were analyzed by the methods of Van Soest et al. (1991). Fermentation products of the silages were determined from cold water extracts. Wet silage (10 g) was homogenized with 90 ml of sterilized distilled water (Cai et al., 1999). The pH was measured with a glass electrode pH meter (MP230; Mettler Toledo, Greifensee, Switzerland). The organic acid contents were measured by HPLC methods as described by Cai et al. (1999). Data on the chemical composition of the 15 and 30 d silages were analyzed by general linear model, and the significance of differences among means was tested by duncan's new multiple range test (Steel and Torrie, 1980).

Results and Discussions

Chemical composition and color characteristics

The effect of applying effective microorganism and cassava starch at ensiling on the fermentation of Lao-native grass silage was studied under laboratory conditions. The chemical composition and color characteristics of the *Hymenachne* sp. and *Echinochloa* sp. silage are presented in **Table 1**. The results showed that the dry matter of silage ranges from 20.44- 27.13% after 30 days of ensiling. The treatment also improved the nutritive values of the *Hymenachne* sp. silages by decreasing the levels of NDF contents. Moreover, *Hymenachne* sp. treated with 7.5 ml EM and 2.5 g starch had a lower NDF content than control.

Color characteristics ranged from 27.98-37.22 for L*, 4.66-6.11 for a* and 26.12-43.88 for b*. The *Hymenachne* sp. silage treated with 2.5 ml EM with 7.5 g of cassava starch had higher greenness more than control. Fermentation of the Control treatments in both forages showed a darkness with detection of L* in silages.

Silage pH and organic acid composition

Changes in pH and organic acids of grass silages without or with EM and cassava starch are shown in **Table 2**. The pH values of all silages decreased sharply after 30 days of fermentation. All pH values were within the range of 3.82 to 4.75 (**Table 2**), which reflects adequate fermentation for restricting the growth of undesirable microorganisms like Clostridia (Tang et al., 1989).

Adding effective microorganism and cassava starch to fresh Lao-native grass at the time of ensiling has resulted in increased lactic acid production variation ranging from 0.75 to 7.85% (**Table 2**). The significantly highest value was recorded in the *Echinochloa* sp. with 7.5 ml EM + 2.5 g starch while lowest in silage without additive (control). Moreover, the results showed that *Hymenachne* sp. silage was effective in elevating butyric acid than *Echinochloa* sp. silage. In addition, the minimum butyric acid recorded was around 1.42 for *Echinochloa* sp. silage with 10 g starch. However, inoculating the native grass non- significant decreased the butyric acid content of the silage (P>0.05). The results which are in agreement with previous research in other researchers had found that microbial inoculants did not affect acetic or butyric acid contents of silage (McAllister et al., 1995; Moshtaghi Nia and Wittenberg, 1999). On the other hand, the overall fermentation quality of the two forages the *Echinochloa* sp. silage treated 10 g starch revealed a better fermentation because silage pH is lower than 4.0 and without traces of acetic acid and butyric acids in silage compared to *Hymenachne* sp. silage. This indicates good quality silage and within the suggested range of 4.0-4.2, as has been reported by several studies (Ryser et al., 1997; Kuoppala et al., 2007)

Table 1 Effect of EM and cassava starch on the color characteristics (L*, a* and b*) of silage

	Hymenachne sp.						*Echinochloa* sp.						SEM	*P*-value		
	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6		Grass (G)	Trt (T)	G*T
Chemical composition, % of dry matter basis																
Dry matter																
0	23.37	26.29	27.30	28.26	28.17	24.29	23.93	23.42	24.85	27.03	28.68	26.12	0.29	***	***	***
30	20.44	22.62	25.09	26.83	24.58	21.94	22.49	23.64	25.34	25.44	27.13	24.06	0.43	***	***	***
Organic matter																
0	97.93	98.01	98.06	98.15	98.09	97.89	98.47	98.54	98.62	98.47	98.66	98.28	0.05	***	***	***
30	97.97	98.08	98.08	97.99	98.09	97.85	98.40	98.33	98.41	98.50	98.66	98.25	0.04	***	***	NS
Neutral detergent fiber																
0	74.78	74.10	69.77	64.24	61.56	73.99	58.32	60.34	59.08	55.26	58.87	55.48	1.49	***	***	***
30	59.03	57.68	68.93	57.11	66.28	76.45	76.69	73.61	72.84	67.96	63.34	71.86	1.42	***	***	***
acid detergent fiber																
0	40.86	39.00	37.37	34.91	32.10	41.34	41.90	42.24	37.69	35.10	33.02	41.72	0.73	***	***	*
30	40.17	43.14	39.78	33.74	32.69	42.65	47.65	43.09	42.96	37.84	35.51	46.17	0.95	***	***	***
acid detergent lignin																
0	10.81	10.57	8.93	6.16	6.04	7.76	7.59	7.59	6.21	6.44	5.76	7.55	0.35	***	***	***
30	8.57	8.32	7.32	7.05	6.24	7.48	7.95	8.24	7.22	6.90	5.69	8.14	0.18	NS	***	***
Color characteristics																
L*																
0	26.39	32.25	32.62	32.17	34.44	27.69	22.60	26.59	30.77	29.38	33.45	23.95	0.87	***	***	NS
30	31.15	32.72	30.93	35.21	33.09	27.98	29.68	30.60	34.13	31.22	37.22	28.59	0.87	NS	***	***
a*																
0	-0.55	-0.58	0.01	-0.48	-0.49	-0.88	-0.64	-3.30	-3.25	-4.09	-4.26	-2.71	0.32	***	***	***
30	5.14	5.32	5.52	4.66	4.78	6.11	5.65	5.12	4.78	5.06	4.75	5.08	0.23	NS	**	**
b*																
0	32.95	29.27	27.97	26.73	24.36	35.12	36.68	40.11	37.21	35.22	34.42	34.50	1.09	***	***	***
30	40.27	38.51	38.00	29.48	26.12	31.30	43.88	40.95	39.89	36.37	36.61	39.37	1.21	***	***	***

* = P<0.05, ** = P<0.01, *** = P<0.001, NS = Non significant

T1= 10 ml EM + 0 g starch, T2= 7.5 ml EM + 2.5 g starch, T3= 5 ml EM + 5 g starch, T4= 2.5 ml EM + 7.5 g starch, T5 = 0 ml EM + 10 g starch, T6 = silage without additive (control)

L* represents lightness from black (0) to white (100); a* (+ redness to - greenness); b* (+ yellowness to - blueness)

Table 2 Effect of EM and cassava starch on the pH and organic acid compositions (% of Fresh matter) of silage

	Hymenachne sp.						*Echinochloa* sp.						SEM	P-value		
	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6		Grass (G)	Trt (T)	G*T
Chemical composition, % of dry matter basis																
pH																
0	5.66	5.59	5.80	5.73	5.67	5.83	4.66	4.77	4.60	4.54	4.43	4.32	0.16	***	NS	NS
30	4.65	4.30	4.15	4.21	4.37	4.93	4.58	4.01	4.28	4.20	3.82	4.75	0.03	***	***	***
Acetic acid																
0	5.42	6.27	5.87	6.32	4.32	5.78	8.40	6.60	5.22	5.72	5.42	7.96	0.33	NS	NS	NS
30	9.10	6.72	6.91	6.28	7.92	10.16	5.72	5.11	6.04	5.11	2.20	7.79	0.46	***	NS	NS
Propionic acid																
0	0.11	0.17	0.11	0.11	0.15	0.15	0.12	0.15	0.07	0.12	0.12	0.13	0.02	NS	NS	NS
30	1.29	0.80	0.27	0.28	0.28	0.40	0.37	0.30	0.76	0.37	0.13	0.35	0.06	*	***	***
Butyric acid																
0	0.61	0.94	0.55	0.64	0.87	0.96	0.66	0.75	0.33	0.69	0.68	0.67	0.07	NS	NS	NS
30	4.38	4.72	3.66	2.64	3.50	1.72	2.40	2.36	2.85	2.41	1.42	2.43	0.22	***	NS	NS
Lactic acid																
0	2.56	1.52	2.45	2.43	3.18	1.17	0.37	0.88	1.00	1.01	0.77	1.18	0.17	***	*	NS
30	1.67	3.36	5.54	3.75	2.50	1.42	1.01	7.85	1.92	2.61	7.71	0.75	0.43	NS	***	***
Formic acid																
0	0.12	0.14	0.14	0.15	0.09	0.11	0.14	0.13	0.15	0.14	0.13	0.18	0.01	NS	NS	NS
30	0.09	0.07	0.05	0.07	0.13	0.11	0.13	0.10	0.07	0.06	0.05	0.06	0.01	NS	NS	*
Succinic acid																
0	0.25	0.36	0.39	0.40	0.43	0.60	0.29	0.34	0.34	0.24	0.34	0.46	0.03	NS	NS	NS
30	0.98	1.61	2.31	1.98	0.64	1.13	0.67	0.77	1.93	1.53	0.41	0.62	0.13	*	***	NS

* = P<0.05, ** = P<0.01, *** = P<0.001, NS = Non significant

T1= 10 ml EM + 0 g starch, T2= 7.5 ml EM + 2.5 g starch, T3= 5 ml EM + 5 g starch, T4= 2.5 ml EM + 7.5 g starch, T5 = 0 ml EM + 10 g starch, T6 = silage without additive (control)

Conclusions

Adding effective microorganism and cassava starch to fresh Lao-native grass at the time of ensiling has resulted in decreased NDF content and pH value. As expected, all treatments resulted in silage with a lower pH compared with the untreated silage. In addition, *Echinochloa* sp. silage treated with 10 g starch had the lowest pH (3.82). Inoculating the native grass increased the lactic acid content of the silage. The significantly highest value was recorded in the *Echinochloa* sp. with 7.5 ml EM + 2.5 g starch while lowest in silage without additive (control). In addition, the minimum butyric acid recorded was around 1.42 for *Echinochloa* sp. silage with 10 g starch. The overall fermentation quality of the two forages the *Echinochloa* sp. silage treated 10 g starch revealed a better fermentation because silage pH is lower than 4.0 and without traces of acetic acid and butyric acids in silage compared to *Hymenachne* sp. silage.

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