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EVALUATION OF DRIED MAO POMACE (*ANTIDESMA BUNIUS* LINN.) AND LACTIC ACID BACTERIA AS ADDITIVES TO ENSILE STYLO LEGUME (*STYLOSANTHES GUIANENSIS* CIAT184)

S. Bureenok[1], N. Pitiwittayakul[1], C. Yuangklang[1], K. Vasupen[1], B. Saenmahayak[1], Y. Kawamoto[2], and J. T. Schonewille[1,3]

[1]Department of Agricultural Technology and Environment, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand; [2]Faculty of Agriculture, University of the Ryukyus, Nishihara-cho, Okinawa, 903-0213, Japan; [3]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
Correspondence Author Email: asmerjai@hotmail.com

ABSTRACT

The effect of application of dried mao pomace (DMP), fermented juice of epiphytic LAB (FJLB), and *Lactobacillus paraplantarum* (LPP) on the ensiling process of Stylo (*Stylosanthes guianensis* CIAT184) was evaluated. Stylo silage was treated with 10% DMP alone or in combination with either FJL Bor LPP. The addition of DMP to Stylo reduced the mean pH by at least 0.9 units while the use of LAB was found to maximally reduce pH by 0.21 units. Irrespective of the source of LAB, the pH reducing effect of LAB was only observed in the silages without DMP. NH_3-N contents were found to be significantly affected by DMP × LAB. The use of DMP reduced the NH_3-N contents by at least 50%, irrespective of the addition of LAB. Furthermore, HBut levels were below detection limits when DMP was used as a silage additive and in case the silages were not treated with DMP, the addition of either FJLB or LPP also resulted in lower HBut fractions. In view of the high NH_3-N and HBut levels, it was therefore concluded that ensiling of Stylo without DMP or LAB results in an inferior silage quality. The use of DMP as a silage additive resulted in good quality silage, irrespective of the use of additional LAB.

Keywords: Fermentation quality, *Lactobacillus paraplantarum*, Phenolics, Silage.

INTRODUCTION

It is generally accepted that, amongst others (McDonald *et al.,* 1991), the water soluble carbohydrate (WSC) content of forages is important to ensure the rapid production of lactic acid (Zhang *et al.,* 1997; Tamada *et al.,* 1999) in order to ensure a good quality silage. Stylo (*Stylosanthes guianensis* CIAT184) is a legume of practical interest in tropical countries (Phaikaew and Hare, 2005). However, Stylo contains low amounts of WSC (Bureenok *et al.,* 2016) which hinders the process of ensiling (Liu *et al.,* 2011, 2012). Therefore, addition of WSC to fresh Stylo prior to ensiling would likely be required to facilitate the growth of lactic acid bacteria (LAB) and ensure the rapid production of lactic acid (LA) to obtain a stable, nutritious silage. Dried mao (*Antidesma bunius* Linn.) pomace is a by-product of mao wine or juice processing from Mao Luang seeds and widely available in the North-East of Thailand, i.e. around 75 tons/year (Samappito and Butkhup, 2008). Dried mao pomace (DMP) may be a good source of WSC (Table 1) to facilitate the ensiling of Stylo. However, DMP also has a high content of total phenolics which have the capacity to inhibit the growth of various bacteria including gram positive bacteria (Butkhup and Samappito, 2011), thereby, potentially hindering the establishment of a stable silage.

In addition to the WSC content, the number of epiphytic LAB on forages also are important to ensure the rapid establishment of a stable silage. It has been shown that the addition of fermented juice of epiphytic LAB (FJLB) from tropical forages such as Napier grass (Bureenok *et al.,* 2006) and Guinea grass (Bureenok *et al.,* 2005) improved the quality of the respective silages. The latter two studies indicate that the number of epiphytic LAB on Napier and Guinea grass may be sub-optimal for ensiling purposes. Recently, Bureenok *et al.* (2016) demonstrated that the treatment of fresh Stylo with FJLB resulted in palatable, stable silage (pH<4.3) but the ensiling process of Stylo not treated with FJLB, was not evaluated. The presented study aimed to evaluate the efficacy of DMP, either or not in combination with additional LAB in the form of either FJLB or *Lactobacillus paraplantarum* (LPP). The latter was selected because in a wide variety of forages it appears to be the most dominant epiphytic LAB (Liu *et al.,* 2012; Pang *et al.,* 2011, 2012; Wang *et al.,* 2017; Zhang *et al.,* 2017).

MATERIALS AND METHODS

Experimental forages: The experiment was conducted at the Rajamangala University of Technology-Isan Sakon Nakhon Campus, Thailand in 2014**.** Stylo was grown with no fertilizer and harvested 60 days after regrowth and chopped to approximately 2-4 cm with a forage cutter and subsequently sampled to determine its nutrient composition (Table 1). Then, the six experimental silages were prepared immediately after cutting with the treatments arranged in a 2 x 3 factorial fashion, i.e. forage with or without additional DMP and with or without additional LAB from either FJLB or the isolated strain. The applied doses of forage specific FJLB and the isolated strain (*Lactobacillus paraplantarum,* LPP) were log 5.89 and 5.56 colony forming units (cfu) g^{-1} fresh forage, respectively. DMP was applied at a level of 100 g kg^{-1} fresh forage. Thereafter, ~100 g fresh material of each silage treatment, was packed in plastic pouches (20.32 x 33 cm, 120 µ thickness; M-PLASPACK, Bangkok, Thailand) and sealed with a vacuum sealer. For each sampling day and each experimental forage, three replicate pouches (n=3) were prepared and all pouches were kept at ambient temperature (27-30°C).

Preparation of FJLB and LPP: Fermented juice of LAB (FJLB) was made from fresh Stylo as described by Bureenok *et al.* (2011). Then, MRS agar plates were inoculated with samples from FJLB and subsequently incubated anaerobically at 35°C for 2 days. Thereafter, LPP was isolated and subsequently cultured on MRS broth at 37°C for 24 h. The 16S rDNA sequence of the type strains in the database of EzTaxon-eserver (http://eztaxon-e.ezbiocloud.net/) was used to compare with the 16S rRNA gene sequence of the isolated strain from FJLB (Kim *et al.,* 2012).

Collection of samples: Upon arrival from the local processing plant, mao pomace was dried at 60˚C in hot air oven and stored at ambient temperature (27–30°C) until the analysis of its nutrient composition and polyphenol content. Silage samples were taken at 1, 3, 5, 7, 14, 21 and 30 days to assess the ensiling characteristics of the forages. Subsamples (~20 g fresh material) of silage were mixed with distilled water (70 mL) and placed in a refrigerator at 4°C for 12 h. Then, the extract was filtered and the pH of the filtrate was recorded. The filtrate was stored at -20°C until the analysis of LA, volatile fatty acids (VFAs) and ammonia-N (NH_3-N). Subsamples of ~ 10 g were taken to assess LAB counts. Directly after sampling, 90 mL of a sterilized 0.85% NaCl was added and the samples were thoroughly mixed. Thereafter, serial dilutions (10^{-1}–10^{-8}) were made and LAB were counted on MRS agar and incubated at 35°C for 2 days (Kozaki *et al.,* 1992). The remaining part of the silage in each pouch was dried at 60°C (48 h) and subsequently ground (1-mm screen) and kept until the analysis of nutrient composition and total polyphenol content.

Chemical analysis: Lactic acid and VFA were measured using HPLC. The steam distillation technique was used to determine NH_3-N content (Cai, 2004). Total nitrogen (N) of silage was determined according to procedures set by the AOAC (method 981.10, 1995) and converted to crude protein (CP) by multiply 6.25 as a factor. The ash contents were determined according procedures set by the AOAC (method 938.08, 1995). Neutral detergent fiber (NDF) was determined by the method of Van Soest *et al.* (1991) and expressed exclusive of residual ash. The WSC content was determined as described by Dubois *et al.* (1956). The total phenolics content was determined by the method of Waterhouse (2005).

Statistical analysis: All data of Stylo legume after 30 days of ensiling were subjected to ANOVA using SAS (1994), based on the model:

$Y_{ij} = \mu + DMP_i + LAB_j + (DMP \times LAB)_{ij} + e_{ij}$

where Y_{ij} = response variable, μ = overall mean, DMP_i = Dried mao pomace (i = yes or no), LAB_j = Lactic acid bacteria (j = no or FJLB or LPP), $(DMP \times LAB)_{ij}$ = interaction term between DMP and LAB and e_{ij}= residual error. Furthermore, LAB counts and selected fermentation characteristics (LA, pH and NH_3-N) at the different time points were subjected to ANOVA with repeated measures using the model already described. Tukey's test was used to test the different effects of the treatment.

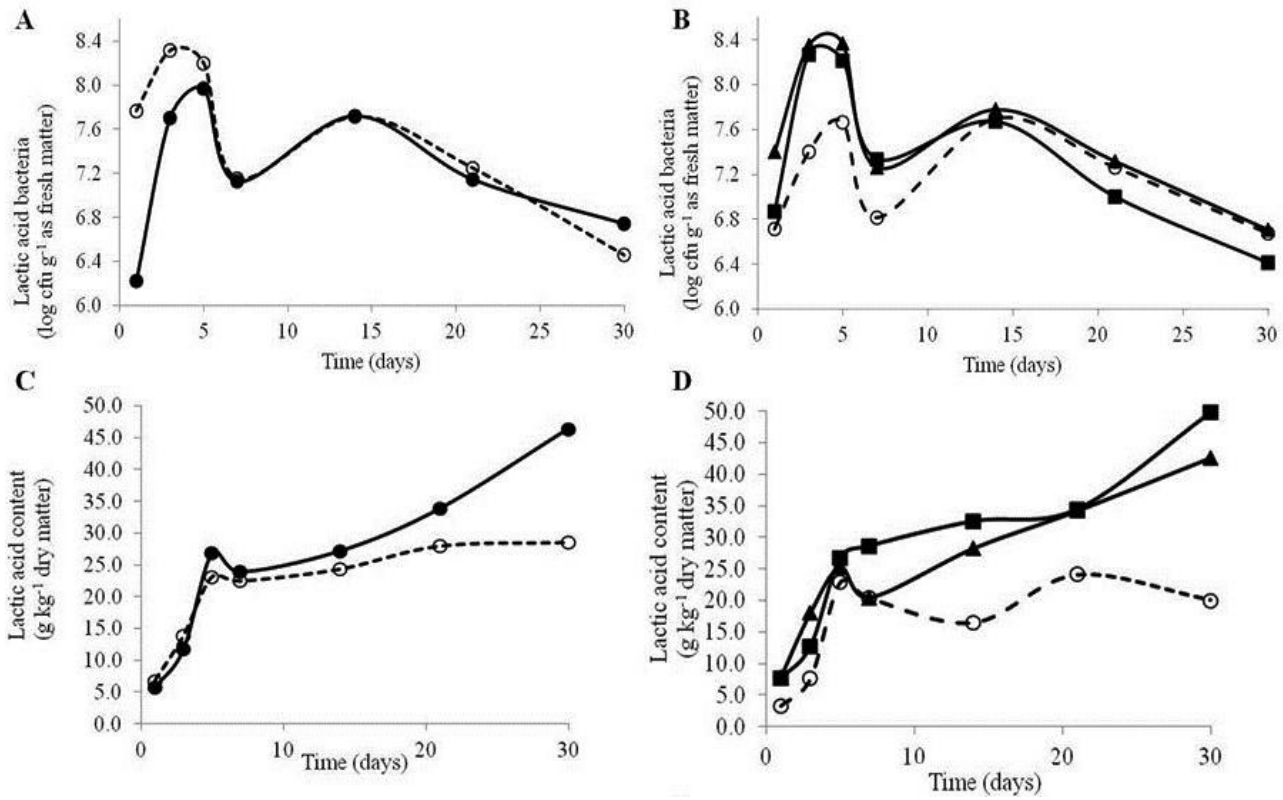
RESULTS

Fermentation characteristics and chemical composition: After 30 days of ensiling, all treatments had no effect on the number of LAB in the silages (Table 2). On average, the addition of DMP caused a 1.6 times increase ($P < 0.001$) in the LA content of the silages while the use of either FJLB or LPP caused on average a 2.3 times increase of the LA content ($P < 0.001$). The total content of fermentation acids (TFa) was not affected ($P = 0.809$) by DMP × LAB (Table 2). The addition of both DMP and LAB increased TFa ($P \leq 0.008$) by 47.3 and 68.5%, respectively. The LA fraction of TFa was influenced by DMP × LAB due to the low values found in the silage that was neither treated with DMP nor LAB ($P = 0.012$). The acetic acid (HAc) fraction of TFa was similar for all treatments ($P \geq 0.132$) and for all treatments combined, a mean value of 0.37 was found. The use of DMP depressed the fractions of both propionic and butyric acid (HBut) of the silages and the fraction of HBut was depressed below the detection limit. In case silages were not treated with DMP, HBut fractions were lower ($P < 0.001$) when LAB were added, irrespective of the source of LAB. The NH_3-N contents were not affected by DMP × LAB ($P = 0.128$). The use of

DMP caused a 54% decrease in NH_3-N values ($P < 0.001$) but addition of LPP only tended to decrease the NH_3-N content ($P = 0.059$). The silage pH was affected by a significant interaction between DMP and LAB. The lowest pH's ($P < 0.001$) were found in the DMP treated silages and the use of FJLB or LPP did not affect the pH in those silages ($P = 0.018$). In contrast, the use of either FJLB or LPP resulted in almost 5% lower pH values ($P = 0.008$) in the silages without DMP.

Due to its high DM content, the addition of DMP increased ($P < 0.001$) the DM contents of the silages (Table 2) while LAB reduced ($P = 0.020$) the DM contents. However, the reduction of DM contents only occurred when LAB were added to the silages without DMP ($P = 0.018$). The use of FJLB, but not LPP, caused higher ($P = 0.009$) CP contents in silages. This effect of FJLB, however, was only observed in silages without DMP ($P = 0.026$). The NDF contents were found to be similar between the silages ($P \geq 0.106$). Addition of LAB did not affect ($P = 0.477$) the total phenolic content of the silages. Across the LAB treatments, the use of DMP caused a 1.47 fold increase ($P < 0.001$) of the content of total phenolics but the use of both DMP and LPP decreased the content of total phenolics of the silages ($P = 0.004$).

Time courses on selected indices of fermentation: Besides the NH_3-N content (Fig.1, panels G and H), LAB counts, LA concentrations and pH of the experimental silages were not affected by the three-way interactions between DMP, LAB and time ($P \geq 0.122$) and are therefore not reported. The LAB counts were affected by time ($P < 0.001$) and the use of DMP caused a minor, but significant ($P < 0.001$), difference in LAB counts in the course of time (Fig. 1, panel A). Likewise, time patterns of LAB counts were found to be significantly ($P < 0.001$) affected by the addition of LAB (Fig. 1, panel B). The LA concentrations also were found to be affected by time ($P < 0.001$). However, the LA concentrations continued to rise after 15 days of ensiling when DMP was used while LA concentrations remained almost similar in case Stylo was ensiled without DMP (Fig. 1, panel C, $P = 0.008$). Likewise, LA concentrations increased with time after 10 days of fermentation in the case LAB was used (Fig. 1, panel D, $P = 0.011$). Silage pH was affected by time ($P< 0.001$) but not by DMP × time (Fig. 1, panel E, $P \geq 0.463$) while a trend ($P = 0.070$) was observed for LAB × time (Fig. 1, panel F). A three-way interaction between DMP, LAB and time was observed for silage NH_3-N contents (data not shown, $P < 0.001$). Both the addition of DMP and LAB caused different ($P < 0.001$) time courses of NH_3-N (Fig. 1, panels G and H).



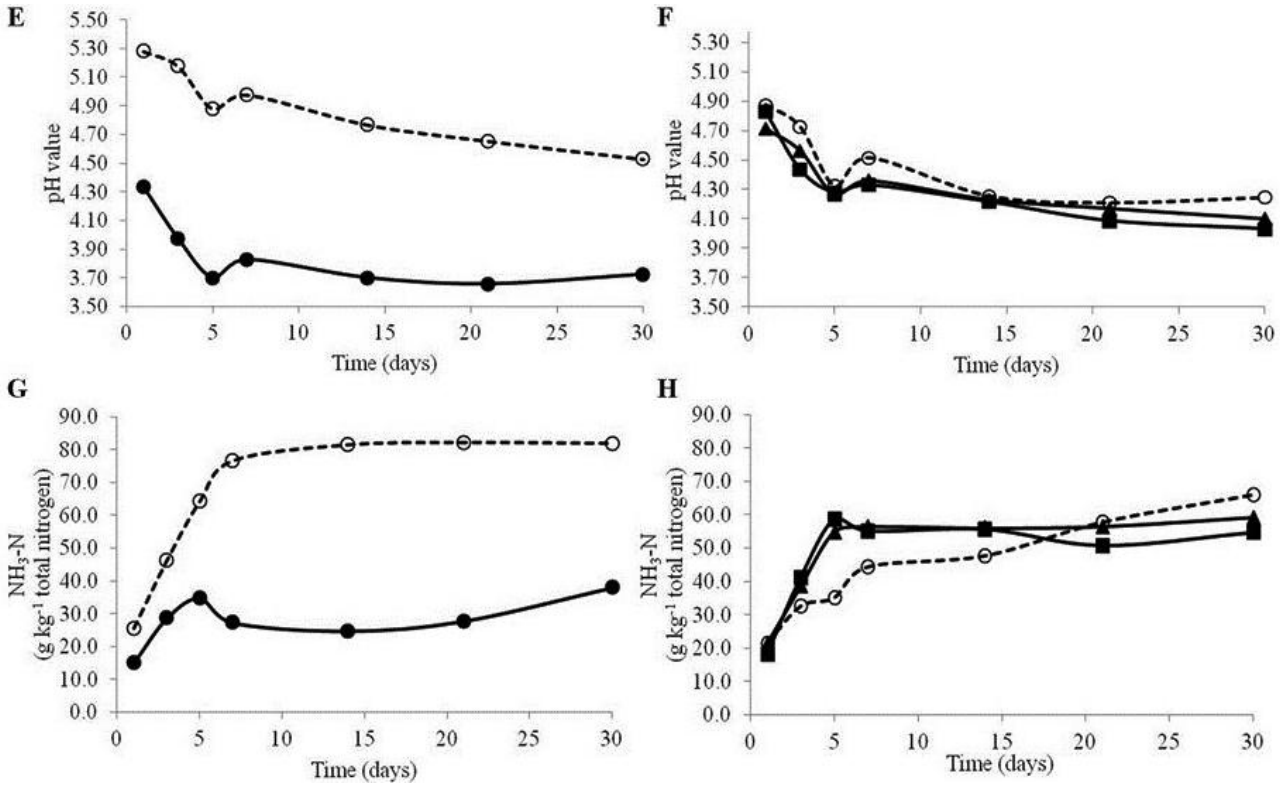


Figure 1. Time courses on LAB counts, (panel A, B), LA contents (panel C, D), pH values (panel E, F) and NH_3-N contents (panel G, H) during the ensiling of Stylo. To enhance readability, only the main effects of dried mao pomace (DMP; panels A, C, E and G) and lactic acid bacteria (LAB; panels B, D, F and H) are presented. Symbols: dotted line, o: untreated Stylo (no DMP or LAB), solid line, ●: Stylo treated with DMP; solid line, ▲: Stylo treated with fermented juice of epiphytic lactic acid bacteria; solid line, ■: Stylo treated with *Lactobacillus paraplantarum*.

Table 1. Chemical composition and pH of fresh Stylo and dried mao pomace (DMP).

	Stylo	DMP
Dry matter(DM, g kg^{-1})	284	883
Crude protein (g kg^{-1} DM)	122	70
Neutral detergentfibre (g kg^{-1} DM)[1]	496	402
Acid detergent fiber(g kg^{-1} DM)[1]	304	235
Hemicellulose (g kg^{-1} DM)	193	167
Water soluble carbohydrates (g kg^{-1} DM)	45	188
Total phenolic content (g kg^{-1} DM)[2]	nd	110
pH	5.56	3.70

[1] Expressed exclusive of residual ash. [2] Expressed as gallic acid equivalents. nd, not determined.

Table 2. Selected indices of fermentation and chemical composition in Stylo legume after 30 days of ensiling with (yes) or without (no) dried mao pomace (DMP) and either without (no) or with fermented juice of epiphytic lactic acid bacteria (FJLB) or *Lactobacillus paraplantarum* (LPP).

Added DMP	No			Yes			SEM	P-values		
Added LAB	No	FJLB	LPP	No	FJLB	LPP		DMP	LAB	DMPxLAB
Fermentation Characteristics										
LAB (log cfu g^{-1} FM)	6.39	6.76	6.22	6.96	6.66	6.60	0.02	0.075	0.221	0.184
Lactic acid (g kg^{-1} DM)	11.0^{d}	34.5^{bc}	40.2^{abc}	29.1^{c}	50.8^{ab}	59.3^{a}	3.45	<0.001	<0.001	0.850
TFa (g kg^{-1} DM)	29.6^{c}	50.2^{b}	56.8^{b}	47.7^{b}	76.0^{a}	77.6^{a}	2.39	0.007	0.008	0.809

LA/Tfa	0.38[b]	0.69[a]	0.71[a]	0.62[a]	0.67[a]	0.76[a]	0.02	0.052	0.001	0.012
HAc/Tfa	0.37	0.25	0.24	0.35	0.32	0.22	0.02	0.480	0.132	0.631
HBut/TFa	0.21[a]	0.03[b]	0.02[b]	bdl	bdl	bdl	0.01	<0.001	<0.001	<0.001
HProp/TFa	0.04	0.03	0.03	0.03	0.01	0.02	0.04	0.035	0.165	>0.999
NH_3-N (g kg^{-1} total N)	89.0[a]	81.7[a]	74.8[a]	36.5[b]	42.7[b]	34.4[b]	3.43	<0.001	0.059	0.128
pH	4.76[a]	4.44[b]	4.38[b]	3.73[c]	3.76[c]	3.68[c]	0.06	<0.001	0.008	0.018
Chemical composition										
DM (g kg^{-1})	269[b]	240[c]	236[c]	337[a]	329[a]	343[a]	2.35	<0.001	0.020	0.018
CP (g kg^{-1} DM)	116[c]	121[ab]	117[c]	122[ab]	123[a]	118[bc]	0.37	0.090	0.009	0.026
NDF (g kg^{-1} DM)	573	572	564	549	544	560	5.22	0.106	0.954	0.610
Total phenolics (mg gallic acid g^{-1} DM)	4.41[c]	4.20[c]	5.93[bc]	7.11[ab]	8.55[a]	5.76[bc]	0.22	<0.001	0.477	0.004

Mean values in a row with different superscript letters were significantly different ($P < 0.05$).
LAB = Lactic acid bacteria, FM = Fresh matter, DM = Dry matter, TFa = total fermentation acids (i.e. lactic acid (LA) + acetic acid (HAc) + propionic acid (HProp) + butyric acid (HBut)), CP = Crude protein, NDF = Neutral detergent fibre, bdl = below detection limit, zero value was used to statistically analyze the data. SEM = Standard error of mean.

DISCUSSION

During the first week of ensiling, the addition of DMP caused a reduction in LAB counts. This observation is most likely explained by the fact that DMP has a high content of total phenolics because Butkhup and Samappito (2011) have demonstrated that such compounds inhibit bacterial growth including gram positive bacteria. However, the depressant effect of DMP on LAB counts was found to be relatively small and did not affect LA contents. Numerically, LA contents were even higher when DMP was used as an additive. Perhaps, the high WSC content of DMP stimulated the production of LA, thereby, counteracting the depressant effect of total phenolics on LAB counts. On the other hand, it must be taken into account that the use of DMP not only provided WCS but also increased the DM content of the silage. It is well known that higher DM contents of silage improve the process of ensiling (McDonald *et al.,* 1991). Clearly, the current data do not causally explain the higher LA contents of the silages treated with DMP.

Across time, both FJLB and LPP were found to be equally effective in raising the mean LA content of silage, irrespective the use of DMP. This result appears to be in contrast with the observation that the fraction of LA of total fermentation acids (TFa), after 30 d of ensiling, was influenced by DMP × LAB. This apparent discrepancy can probably be explained by the different time patterns of the LA content during the 30 d ensiling period (Fig. 1, panel C). The influence of the last two LA values on the mean LA content of silage treated with DMP, was probably too small to significantly influence the interaction between DMP and LAB on the mean LA content. Dried mao pomace *versus* FJLB and LPP, was found to be a more effective additive to produce sufficient LA to reduce silage pH. This result is in line with the current observation that TFa after 30 days of ensiling was higher when DMP was applied. These results support the idea that the WSC content of Stylo limits the process of LA formation. Furthermore, the silages treated with DMP had low NH_3-N contents (i.e. < 43 g/kg total N). Perhaps, current pH values have inhibited the growth of proteolytic Clostridia, thereby, preventing the formation of ammonia (Umana *et al.,* 1991).

Umana *et al.* (1991) suggested that well preserved silages should contain pH values < 4.5 and NH_3-N < 100 g/kg total N, and the ratio of LA/TFa, HAc/TFa and HBut/TFa should be > 0.70, < 0.20, and < 0.025, respectively (Ojeda *et al.,* 1991). The current results show that a good silage quality was already achieved when DMP instead of FJLB or LPP was used as silage additive. In case DMP was added, the values with respect to pH, NH_3-N and butyric acid (Umana *et al.,* 1991; Ojeda *et al.,* 1991) were already met. However, in the silages treated with DMP, LA/TFa was found to be on average 0.69 and this was associated with an average HAc of total TFa fraction of 0.30. Even though the aerobic stability of the silages is not specifically evaluated in the current study, the relative high HAc fractions may indicate that the aerobic stability of the silage (Weinberg *et al.,* 1993; Danner *et al.,* 2003) was somewhat compromised. On the other hand, the pH and DM contents of the DMP silages were on average 3.7 and 336 g kg^{-1}, respectively and this combination of silage pH and DM content generally ensures a sufficient stability of the silage (McDonald *et al.,* 1991). The current data cannot explain the relatively high fraction of HAc of total TFa. However, it is likely not related to the DMP as the fractions of HAc were high in all silages. Stylo contains approximately 200 g hemicellulose/kg DM and it can be speculated that the HAc originated from hemicellulose acetyl groups (Bergen *et al.,* 1991). Further research, however, is warranted to substantiate this suggestion. The addition of DMP was associated with virtually zero HBut contents which can be explained by the low pH values of the silages in question. The DMP silages had pH values < 4.0 and such low pH values are known to inhibit the

growth of clostridia species and thereby the production of HBut (Lindgren *et al.,* 1985).

Next to DMP, the addition of either FJLB or LPP also increased the TFa content of the silages. Although caution is warranted to generalize this result, it can be speculated that the number of epiphytic LAB in Stylo, next to its low WSC content, also limit the process of fermentation during ensiling. This is in an agreement with results of Bureenok *et al.* (2005, 2006) who suggested that the application of FJLB to tropical forages such as Napier grass and Guinea grass, improved the quality of the respective silages. In legumes, low levels of butyrate are commonly observed (Ward, 2008) but in the current study, the fraction of HBut of TFa was much too high (Ojeda *et al.,* 1991) when Stylo was ensiled without LAB and DMP. The epiphytic LAB in Stylo may be heterolactic bacteria and produce also a weak acid such as acetic acid. The production of HAc prevents a rapid decline of the pH during ensiling (Liu *et al.,* 2012) thereby hindering the growth of Clostridia species (Kaiser and Weiss, 1997) so as to keep HBut/total fermentation acids < 0.025 (Ojeda *et al.,* 1991).

Conclusions: Ensiling of Stylo without DMP or LAB leads to inferior silage quality. The use of DMP as a silage additive to Stylo results in a good quality silage, irrespective the use of LAB in the form of FJLB or LPP.

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REFERENCES

AOAC (1995). Official Methods of Analysis, Inc. 12th Ed., Arlington, VA, USA.

Bergen, W.G., T.M. Byrem, and A.L. Grant (1991). Ensiling characteristics of whole-crop small grains harvested at milk and dough stages. J. Anim. Sci. 69: 1766-1774.

Bureenok, S., T. Namihira, Y. Kawamoto, and T. Nakada (2005). Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guinea grass (*Panicum maximum* Jacq.) silage. Grassl. Sci. 51: 243-248.

Bureenok, S., T. Namihira, S. Mizumachi, Y. Kawamoto, and T. Nakada (2006). The effect of epiphytic lactic acid bacteria with or without different byproduct from defatted rice bran and green tea waste on napiergrass (*Pennisetum purpureum* Schumach) silage. J. Sci. Food Agric. 86: 1073-1077.

Bureenok, S., W. Suksombat, and Y. Kawamoto (2011). Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on digestibility and rumen fermentation characteristics of ruzigrass (*Brachiaria ruziziensis*) silages. Livest. Sci. 138: 266-271.

Bureenok, S., K. Sisaath, C. Yuangklang, K. Vasupen, and J.T. Schonewille (2016). Ensiling characteristics of silages of Stylo legume (*Stylosanthes guianensis*), Guinea grass (*Panicum maximum*) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages. Small Rumin. Res. 134: 84-89.

Butkhup, L., and S. Samappito (2011). Phenolic constitutes of extract from Mao Luang seeds and skin-pulp residue and its antiradical and antimicrobial capacities. J. Food Biochem. 35: 1671–1679.

Cai, Y. (2004). Analysis method for silage. In: Japanese Society of Grassland Science (ed), Field and Laboratory Methods for Grassland Science. Thosho Printing, Tokyo, Japan, 279-282. (In Japanese.)

Danner, H., M. Holzer, E. Mayrhuber, and R. Braun (2003). Acetic acid increases stability of silage under aerobic conditions. Appl. Envir. Microbiol. 69: 562-567.

Dubois, M., K.A. Giles, J.K. Hamilton, P.A. Rebbers, and F. Smith (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Kaiser, E., and K. Weiss (1997). Fermentation process during the ensiling of green forage low in nitrate. 2. Fermentation process after supplementation of nitrate, nitrite, lactic-acid bacteria and formic acid. Arch. Anim. Nutr. 50: 187-200.

Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H Yi, S. Won, and J. Chun (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int. J. Syst. Evol. Microbiol. 62: 716-721.

Kozaki, M., T. Uchimura, and S. Okada (1992). Experimental Manual of Lactic Acid Bacteria. Asakurasyoten, Tokyo, Japan. (In Japanese.)

Lindgren, S., K. Pettersson, A. Johnsson, P. Lingvall, and A. Kaspersson (1985). Selected strains, temperatures, wilting and practical application. Swed. J. Agr. Res. 15: 9-18.

Liu, Q., M. Chen, J. Zhang, S. Shi, and Y. Cai (2012). Characteristics of isolated lactic acid bacteria and their effectiveness to improve Stylo (*Stylosanthes guianensis* Sw.) silage quality at various temperatures. Anim. Sci. J. 83: 128-135.

Liu, Q., J. Zhang, S. Shi, and Q. Sun (2011). The effects of wilting and storage temperature quality and aerobic stability of Stylo silage. Anim. Sci. J. 82: 549-553.

McDonald, P., A.R. Henderson, and S.J.E. Heron (1991). The Biochemistry of Silage, second ed. Chalombe Publications, Marlow, England.

Ojeda, F., O. Cáceres, and M. Esperance (1991). Sistema de evaluación para ensilajestropicales, Conservación de Forrajes, Pueblo y Educación, Ciudad de La Habana, Cuba.

Pang, H., G. Qin, Z. Tan, Z. Li, Y. Wang, and Y. Cai (2011). Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and *recA* gene analysis. Syst. Appl. Microbiol. 34: 235-241.

Pang, H., Z. Tan, G. Qin, Y. Wang, Z. Li, Q. Jin, and Y. Cai (2012). Phenotypic and phylogenetic analysis of lactic acid bacteria isolated from forage crops and grasses in the Tibetan Plateau. J. Microbiol. 50: 63-71.

Phaikaew, C., and M.D. Hare (2005). Stylo adoption in Thailand: three decades of progress. Trop. Grassl. 39: 216.

SAS (1994). Users Guide. Fourth edition. SAS Institute Inc., Carry, NC, USA, Version 6.

Samappito, S. and Butkhup, L. 2008. An analysis on organic acids contents in ripe fruits of fifteen Mao Luang (*Antidesma bunius*) cultivars, harvested from dipterocarp forest of Phupan valley in Northeast Thailand. Pakistan J. Biol. Sci. 11: 974-981.

Tamada, J., H. Yokota, M. Ohshima, and M. Tamaki (1999). Effect of additives, storage temperature and regional difference of ensiling on the fermentation quality of Napier grass (*Penisetum purpureum* Schum.) silage. Asian-Austr. J Anim. Sci. 12: 28-35.

Umana, R., C.R. Staples, D.B. Bates, C.J. Wilcox, and W.C. Mahanna (1991). Effects of a microbial inoculant and (or) sugarcane molasses on the fermentation, aerobic stability, and digestibility of bermudagrass ensiled at two moisture contents. J. Anim. Sci. 69: 4588-4601.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis (1991). Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Wang, S., X. Yuan, Z. Dong, J. Li, G. Guo, Y. Bai, J. Zhang, and T. Shao (2017). Characteristics of isolated lactic acid bacteria and their effects on the silage quality. Asian-Austr. J. Anim. Sci. 30: 819-827.

Ward, R.T. (2008). Fermentation analysis of silage: use and interpretation. [accessed February 16, 2018]: [18 p.]. http://www.foragelab.com/media/fermentation-silage-nfmp-oct-2008.pdf.

Waterhouse, A.L. (2005). Polyphenolics. In: R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz, C.F. Shoemaker, D. Smith and P. Sporns (eds.) Handbook of Food Analytical Chemistry, London, UK, John Wiley and Sons.

Zhang, J.G., S. Kumai, and R. Fukumi (1997). Effects of lactic acid bacteria and cellulase additives at different temperatures on the fermentation quality of corn (*Zea mays* L.) and Guineagrass (*Panicum maximum* Jacq.) silages. Acta Prataculturae. Sinica.6: 66–75.

Zhang, M., Y. Wang, Z. Tan, Z. Li, Y. Li, H. Lv, B. Zhang, and Q. Jin (2017). Microorganism profile, fermentation quality and rumen digestibility in vitro of maize-stalk silages produced at different maturity stages. Crop Pasture Sci. 68: 225-233.

Weinberg, Z., G. Ashbell, Y. Hen, and A. Azrieli (1993). The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Microbiol. 75: 512-518.