Comparison of silages from Napier Pakchong1 and Sweet grass fermented with agricultural waste from banana cultivars

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Abstract

Silage is fermented forage that can be preserved for a long time, this can alleviate the problem of insufficient roughage in the dry season for ruminant farms. Bananas contain post-harvest agricultural waste that can be used in animal feed. The objective of this experiment was to compare the physical characteristics, chemical composition and ensiling products from Napier Pakchong1 (NP1) and Sweet grass (SG) fermented with banana cultivars. The experiment was conducted as a 2 x 5 factorial in a completely randomized design consisting of 10 treatments of 4 replicated treatments each, with two species of grass NP1, and SG, and five banana cultivars (control (without banana), Tani banana (TB), Hin banana (HB), Nam Thai banana (NTB), and Thep Parod banana (TPB)). The grass and bananas were fermented at a ratio of 80:20 for 21 days. The results showed that the overall silage quality of NP1 fermented with TB, HB or NTB, and SG fermented with TB or HB achieved a high score. Thus, the combined use of bananas ensiled with grasses can reduce the loss of dry matter better than NP1 grass silage alone. All the silage groups contained 11.12-12.06% crude protein (CP). The use of bananas fermented with grass had a lower effect on the production of lactic acid bacteria (LAB) than NP1 grass fermented alone. However, there was a statistically significant interaction between the two species of grass fermented with banana cultivars in reducing sugar (RS), total sugar (TS), lactic acid (LA), acetic acid (C_2) concentrations, neutral detergent fiber (NDF), gross energy, and ammonia nitrogen (NH_3-N), especially with the treatment that used fermented SG with HB which resulted in an increased RS, TS, C_2, NDF. Furthermore, a decrease in pH and NH_3-N was found to have a beneficial effect on the efficiency of fiber digestion. The treatment that used fermented NP1 grass with HB and NP1 grass fermented with NTB resulted in an increased RS, TS and a decrease in NH_3-N. Leaves with petioles of bananas can be used as an alternative material to ferment together with grass to feed ruminants and reduce the amount of agricultural waste.

Keywords: Grass silage, Local agricultural waste, Fermentation, Ensiling products, Silage quality

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Introduction

Currently, the utilization of agricultural waste (AW) as an alternative material in animal feed has become popular and is accepted by farmers due to the reductions in feed cost and the environmental impact. In the past, there have been efforts to identify AW benefits in various fields. AW can also be used to feed animals. However, there may be restrictions on quality and shelf life due to perishability. Some guidelines have been established for utilizing AW through a fermentation process with other forage crops (grass, corn or legumes) in ranaerobic conditions to form 70-75% moisture, with pH of about 4.2. The preservation conditions required for this silage fermentation (SF) process allow the fermented forage to retain its good condition and properties that can be preserved for a long time. In addition, this process can alleviate the problem of roughage in the dry season for ruminant farms. Moreover, it helps to reduce environmental pollution by disposing of AW. AW acquired from bananas after harvesting usually consists of the petiole, leaf blade, and pseudo stem, which are abundant since they grow well in all regions of Thailand in both household and economic cultivation, resulting in enough production for it to be used as animal feed. Jaitrong and Srisaikham (2018) reported that TPB leaves had 11.25%CP, 4786.19 calories/g, and high crude fiber (CF) (27.26%) which is more than 18%CF of dry weight, and it therefore has properties that can be used as roughage for ruminants. Ruminants can eat all banana parts. Astuti (2015) reports that smallholder farmers use banana peel widely as a supplement for tropical ruminants, which is rich in vitamins, pectin, sugar, and lignin (Mohaputra et al., 2010). However, the moisture content of pseudo-stems and leaves of bananas was greater than 90 and 70% respectively, according to Jaitrong and Srisaikham (2018) even when the proportion of leaves is less than grass, the fermentation of banana alone affects silage quality and shelf life. The properties of grass are often useful for silage production. Napier Pakchong1 (*Pennisetum purpureum* cv. Pakchong1; NP1) is a tropical grass of good quality and it is the primary source of roughage for ruminants, since it is fast growing and has a high yield (Boonkoed et al., 2018). At a cutting age of 60 days it yields a dry weight of 6420 kg/hectare per time and an average of dry matter (DM), CP, NDF and acid detergent fiber (ADF) with 15.5, 13.4, 66.2 and 41.4%, respectively (Boonruangkao and Waipanya, 2017). SG (*Pennisetum purpureum* cv. Mahasarakham) is now popular with farmers because it grows well in Thailand, and has yields similar to NP grass. Mapato and Wanapat (2016) reported that SG contains 15.2%CP, 12.1% non-fiber carbohydrate (NFC) and ADF (34.9%). At 42 days of harvest, it was possible to improve rumen fermentation, reduce methane production and enrichment levels for ruminants in tropical countries (Mapato and Wanapat, 2018).

Consequently, silage from these two species of grass which can be grown with a high yield per area and provides protein suitable for feeding ruminants together with banana leaves and petiole should increase the efficiency benefits of AW utilization from bananas and encourage its use as an alternative material for silage to feed ruminants during the dry season.

Therefore, this research aimed to compare the effects of NP1 and SG species fermented with banana cultivars on the physical characteristics, chemical composition, and ensiling products of AW.

Research Methodology

NP1 (*Pennisetum purpureum* cv. Pakchong1) grass is a cross between NP grass (*Pennisetum purpureum*) and Pearl millet (*Pennisetum americanum*) and Sweet grass (*Pennisetum purpureum* cv. Mahasarakham; SG) grass grown on farms of the Faculty of Agricultural Technology, on the Sa Kaeo Campus at Burapha University (BUU) in Thailand. The soil is classified in the Sakaeo series as Ska, which is sandy loam, planting distance 1*1 meter. 0.5 liters of manure per plant were added after planting and applied once a year. A chemical fertilizer formula of 15-15-15 was applied at the rate of 312.5 kg per hectare after planting and urea fertilizer (46-0-0) at the rate of 62.5 kg per hectare after every cut, and watered every 7 days during the dry season. The entire field was mowed at a height of 5 cm from the soil surface which was counted as day 0, and two species of grass were harvested after 45 days. Cultivars of banana trees (TB, HB, NTB and TPB) were collected at Sa Kaeo Campus on the BUU farm. The banana leaves with petioles of four banana cultivars and two species of grass (NP1 and SG) were chopped to 2-3 cm. After chopping, they were fermented together in ratio according to the experimental plan. A 2x5 factorial in a completely randomized design (CRD) experiment was assigned, consisting of 10 treatments, 4 replications each, 2 levels of two species grass and 5 levels of banana cultivars (control, TB, HB, NTB and TPB) (Table 1).

Two species of grass and four banana cultivars in each experimental group were mixed in proportion to each other and then packed at 15 kg weight into silage bags of 2 layers. The outer layer was a synthetic fiber bag and the inner layer was a 24" x 44" gray extra thick plastic silage bag. A pump was used to suck out as much air as possible and string was tied tightly around the inner and outer bags in the absence of oxygen (O_2) in anaerobic conditions and then ensiled for 21 days at room temperature. A 1000 g of samples were taken from all the bags at 4 specific points: top, middle, side, and bottom and then the samples were pooled and mixed together into the same bag in order to evaluate the physical characteristics, chemical composition and ensiling products of each experimental silage treatment.

Table 1 Proportion of species grass and banana cultivars used in the experiment (%)

Treatment (T)	Proportion of grass species fermented with banana cultivars for 21 days (%)
T1	100% NP1 silage
T2	80% NP1 fermented with 20% TB
T3	80% NP1 fermented with 20% HB
T4	80% NP1 fermented with 20% NTB
T5	80% NP1 fermented with 20% TPB
T6	100% SG silage
T7	80% SG fermented with 20% TB
T8	80% SG fermented with 20% HB
T9	80% SG fermented with 20% NTB
T10	80% SG fermented with 20% TPB

Note: NP1 = Napier Pakchong1; SG = Sweet grass, TB = Tani banana, HB = Hin banana, NTB = Nam Thai banana, TPB = Thep Parod banana

Assessment of physical characteristics

Smell, texture, color, and pH were scored according to a physical fermentation quality assessment form of the Animal Feed Division, Department of Livestock Development (Department of Livestock Development, 2004). A sample of silage (100 g of fresh matter (Figure 2)) was mixed with 100 ml of distilled water and blended in a blender jar for 1 min. The liquid was immediately measured with a pH meter (PH-009(I) (Pen type pH Meter).

Color measurement

The silage samples were heated at 60°C for 48 h, and then ground through a 1-mm screen for color measurement. Briefly, the color values of the dried samples were measured by a spectrophotometer (Hunter Lab- UltraScan VIS (USVIS2329, USA)). The International Commission on Illumination (CIE) system was evaluated by L* or brightness (0 = black, 100 = white), a* (+ a = red, -a = green) and b* (+ b = yellow, -b= blue). The color terms for the hues (h*) were applied: H^0 approaches 0° (degree) = the object was assigned to the red group, H^0 (hue angle) approaches 90° = the yellow group, H^0 approaches 180° = the green group, H^0 approaches 270° = the blue group, and chroma of the hue (C*) (the apparent color intensity).

Chemical analysis

Fresh and silage samples of two species of grass (NP1 and SG) and four banana cultivars (TB, HB, NTB and TPB (leaves and petiole ratio 1:1) were dried at 60°C for 48 h, then ground through a 1-mm screen and subjected to proximate analysis. Dry matter (DM) was analyzed in a hot air oven at 100±5°C for 4 h. Ash was determined by Furnace (CARBOLITE CWF 11/23, England) according to the method of the Association of official Analytical Chemists (AOAC, 1990). Crude protein (CP) was determined by the Kjedahl method (AOAC, 1990) by using a Kjeltec auto analyzer. Ether extract (EE) and crude fiber (CF) were determined by the Weende method (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using a detergent analysis method (Van Soest et al., 1991). Gross energy (GE) was determined using an automatic adiabatic bomb calorimeter (AC 500, Leco, St. Joseph, Michigan, USA).

The concentration of volatile fatty acids (acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), valeric acid (C_5), and lactic acid (LA) were measured using a Gas chromatograph (GC) (Nexis

GC-2030: SHIMADZU, Shimadzu Corp., Kyoto, Japan) equipped with a capillary column (molecular sieve 13X, 30/60 mesh, Alltech Associates Inc., Deerfield, IL, USA), at a column temperature of 190°C; at a flow rate of 1.80 ml/min. An analysis for NH_3-N was conducted according to the method of Chen et al*. (*1994*).*

Dried and ground samples (DGS) were added to 30 ml of water and incubated in a water bath at 90°C for 30 min, then the volume was adjusted to 50 ml and it was centrifuged at 10000 rpm for 10 min, then filtered with Whatman No.1 filter paper. The reducing sugar (RS) was determined by the Somogyi-Nelson method (Somogyi, 1952) and total sugar (TS) was determined by the Phenol-sulfuric acid method (AOAC, 1990) and calculated using a standard glucose curve.

Scanning electron microscope

The shape, appearance and external surface of DGS were observed using a 3D scanning electron microscope (3D-SEM) (FESEM: Carl Zeiss model Auriga, German).

Lactic acid bacteria (LAB)

The LAB was analyzed according to Thai Industrial Standards (THAI INDUSTRIAL STANDARD (2005); TIS 2239-2548; ISO 15214: 1998; microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria- Colony count technique at 30°C).

Statistical analysis

The data were analyzed using the Analysis of variance (ANOVA). Duncan's new multiple-range test was used for a comparison of means by R statistics program (R Core Team, 2019). A 95% confidence interval was considered statistically significant.

Results

At a cutting interval of 45 days, NP1 prior to ensiling contained higher OM and CP and less ash content than those obtained from SG (Table 2). The pH was determined at 4.5 and the color parameters of the two species of grass revealed a hue angle (H^0) which approached 180 degrees (shade of green). With regard to the other color parameters, a* was negative (green) and b* was positive (yellow), and C* showed a clear yellow-green direction. It was found that of the four banana cultivars HB had the highest moisture content, followed by TPB, NTB and TB. The OM of TB and TPB were similar and lower than that of HB and NTB. The CP, EE and CF were in the range of 9.04-9.80%, 3.08-5.51% and 21.59-23.65%, respectively (Table 2). The pH and color values were similar. All the banana cultivars had yellowish-green tones, especially TB and HB, while NTB and TPB had H^0 approaching 90 degrees (yellow) which was consistent with the results observed with the naked eye. TB had the lowest a* values and the highest b* and C* values, indicating that the leaves with petiole of TB had a tendency towards a greenish-yellow color which was consistent with the appearance observed with the naked eye when compared to other banana cultivars.

Physical characteristics, chemical composition and ensiling products at day-21 of fermentation

Table 3 shows that the smell, color, and quality parameters of the experimental silage were related to the effective interaction of species grass and banana cultivars. T2, T3, T4, T7 and T8 had a fruity alcoholic or vinegary scent. It was found that the TPB fermented with the two species of grass was very pungent with an unpleasantly acidic smell (slightly smelly) which caused the score to fall statistically, while the grass silage alone (T1 and T6) was found to have a slightly pungent smell that was not fragrant. None of the experimental silages texture was observed to have any interaction with the banana cultivars. The score for the experimental silage was about 4 (firm texture, leaves and stems and free of impurities), with no adverse characteristics such as rotting leaves or stems of fermented plants with slime or any impurities. Of the ten experimental silages, T1, T5, T6, T8 and T10 were found to have a pH (Table 3 and Figure 1) which conformed to the chemistry standards of silage (Department of Livestock Development, 2004). However, when assessed on the overall quality based on the scores for smell, texture, color and pH, it was found that they were in the range of good (T1, T5, T6 and T9) to very good (T2, T3, T4, T7 and T8), whereas T10 had the lowest level.

The experimental silage had a moisture content of more than 70% in all treatments as it derived directly from freshly cut plants at the harvest, and was then chopped and ensiled immediately. DM of T5 was the highest. T3, T4, T6, T7, T8, T9 and T10 had more DM than T2 and T1. T1 had the least DM. The average CP of the experimental silages was between 11.12-12.06%. TPB fermented with the two species of grass had a higher fat content than those that were used with the other banana cultivars. The CF of T1 was the highest, followed by T5 and T10 which had the lowest CF. On average, the experimental treatments which used bananas with the two species of grass had the lowest CF, and those without bananas were fermented with the two species of grass which resulted in the highest average CF. The NDF of T1 was significantly higher than that of T6. The treatment that used HB and NTB fermented with the two species of grass (62.06 and 61.95% respectively) showed that NDF was statistically significantly higher than the treatments without bananas (61.23%) or TB (59.71%) and TPB (61.38%) (Data not shown in Table). T8 had the highest NDF, which was consistent with the mean NDF of the treatment that used HB fermented with the two species of grass mentioned above. ADF and cellulose were abundant in T6. The highest ADL was in T3 and T8. When considering the ADL of HB fermented with both grasses, the results were in the same direction as NDF, and it was found that of the interactions between the two types of grass and the bananas on the NFC, T8 had the highest hemicellulose value. Grass silage, the two species of grass and/or fermented with TB, were found to have the lowest hemicellulose. T6 had the lowest gross energy (GE) and T3 had the highest. The TS was highest in T8, while T3 and T4 had the highest RS and lowest in T1. The NH_3-N concentration was abundant in T1 and T2. The T1, T2, and T8 had greater C_2 than the other treatments (Table 3 and Figure 1). C_3 and C_5 were not detected in T10, and T7, T8 and T10 respectively. The highest C_4 for T6 was 21.74%,

followed by T10 and T9, with 20.19% and 18.59%, respectively.

The results of shape, appearance, and external surface of the experimental silage through 3D-SEM are shown in Figure 3 and the sample structure in T1 and T6 had rod, hexagonal shapes with a few brittle cracks and pores scattered on the external surface. Bar and square shapes were found in T2, T3, T4 and T7. However, there were brittle marks and cracks throughout the structure with more porosity and disintegration spread along the external surface, especially in T3, T4 and T7, and the fiber delamination on the external surface was high. In contrast to T8, the structure was brittle and ruptured and had less fiber delamination at the external surface than T3. Although the external surface of T9 and T10 was porous, the structure was brittle and cracked, while the delamination of the fibers was not as widely distributed as in T6 and T8.

Discussions

After fermentation, five of the ten experimental treatments with pH, namely, T1, T5, T6, T8 and T10, conformed to the silage plant chemical standard of between 3.5-4.2 (Department of Livestock Development, 2004). In accordance with McDonald et al. (1991), the pH of good quality silage should range from 3.8-4.2. The pH conditions of the silage varied, perhaps due to the diversity and number of initial epiphytic bacteria or aerobic fungi attached to the plants, types of forage crops or other ingredients which they were fermented with which could have affected the growth of microorganisms in each. Acid-producing bacteria thrive in acidic (low pH) conditions (Kongbangkerd, 2003). Good fermentation requires anaerobic conditions so that fermented forage crops can be kept in a good condition with their normal characteristics so that they can be stored for a long time. Immediately the fermentation begins, LAB attach themselves to the outer surface of fresh plants in large quantities, then they divide rapidly and convert water soluble carbohydrate (WSC) into organic acids (OA), mainly LA. This accumulation of LA results in lower pH, which is an important factor affecting the activity of microorganisms such as yeast and mold which are the cause of silage spoilage (Woolford, 1990). The optimal conditions for anaerobic activity of the bacteria (*Lactobacilli* and *Sterptococci*) in the fermentation depend on the quantities of initial sugar, and pH which should be approximately 4.2 or less. All the experimental treatments had pH below 4.2, between 2.98-3.95.

Overall, all experimental silage treatments after fermentation had a yellow tone (H^0 which approached 90 degrees (Table 3), which was consistent with the color observed with the naked eye which was olive yellow. This indicated that fermentation played a role in the color change. Plant cell respiration continues to occur early in fermentation, using O_2 and carbohydrates, and produced carbon dioxide, water, and generating heat. Adesogan and Newman (2014) indicated that temperature rises during the first phase of SF shows that the heat may increase the temperature in a sealed silo by 8.3-11.1°C. There were reports in Thailand that the temperature inside a fermentation tank can

be as high as 45°C or more during the early fermentation stage (Bureenok et al. 2021), depending on the amount of air available. This may be the result of a chemical reaction that causes the silage to change color, which is consistent with Charoenphun and Pakeechai (2019), who reported that a chemical browning reaction can occur during heating, for example, baked food or food products undergoing the maillard reaction will turn brown. In the first case, a non-enzymatic browning reaction occured between reducing sugars and proteins in the plants, amino acids (AA) or other nitrogenous compounds. Heat is catalyzed in high temperature conditions, and it produces a variety of compounds that cause a brown color and a different flavor which is both desirable and undesirable (Rattanapanone, 2006). All experimental treatments were measured for color by heating to 60°C for 48 h until dry for color measurement. In the second case, the pigmentation or color intensity that occurs in the silage may be due to a browning reaction which is related to the enzymatic browning reaction. This is an oxidation reaction that occurs when living cells are exposed to O_2 in the air, such as the peel or skin of fruit or plants containing phenolic compounds, such as phenolase enzymes and O_2 (Rattanapanone, 2002) as precursors. Amounts of phenolic compounds, condensed tannins and hydrolysed tannins were found in the TPB leaves followed by inflorescence and pseudo-stems, with phenolic compounds between 13.01-45.40 mg gallic acid/ml (Jaitrong and Srisaikham, 2018). Therefore, part of the browning process is associated with the enzymes on the surface of the leaves, grass and leaves with petioles of bananas when exposed to O_2 in the air. Silage processing requires grasses and bananas to be chopped into pieces of 2-3 cm in length to enable the plants to be tightly compacted. This allows the air to be purged more easily and the sugars are released more quickly, which in turn allows the enzymes and reactants to react quickly and O_2 is able to come into contact with these in an oxidation reaction. Monophenol is oxidized to diphenol (both monophenol and diphenol were colorless), and it was then further oxidized to form o-quinone which became brown after o-quinone interacted with AA or proteins and eventually turned brown in color (Rattanapanone, 2014).

Some of the data from the present study suggest that SF process was able to change the external surface of all the objects of experimental silages. According to a report by Manwicha et al. (2014), it was found that internal the structure of longan peel, leaves and stems of rice straw treated with urea by SEM were loosened. Since alkalinity by urea is degraded by microbial urease to ammonia and to ammonium hydroxide when dissolved in water (Wanapat and Cherdtong, 2009), cellulose was better utilized by microorganisms (Wanapat et al., 2009) cited by Manwicha et al. (2014).

DM of all experimental silages was less than NP1 silage at 60 days after cutting and fermentation for 21 days (22.14%DM) (Boonkoed et al., 2018) and slightly higher than NP1 grass after 45 days and fermented for 30 days (13.27%DM) (Noosen and Lounglawan, 2018). In

this study, the treatment that used HB, NTB and TPB, which were fermented with two types of grass without being inoculated with LAB had significantly more DM than other treatments (16.04-16.72%DM).

Table 2 Chemical composition, pH and color measurement of the two species of grass and banana cultivars prior to ensiling.

Item	Napier Pakchong1	Sweet grass	Tani Banana	Hin Banana	Nam Thai Banana	Thep Parod Banana
Moisture[1/]	87.11±1.93	86.65±1.30	76.90±0.84	83.47±1.64	78.01±0.67	79.42±1.96
DM 1[2/]	12.89±1.93	13.35±1.30	23.10±0.84	16.53±1.64	21.99±0.67	20.58±1.96
DM 2[3/]	99.97±1.35	99.51±0.69	98.05±0.57	98.57±0.24	96.62±2.01	98.10±0.39
OM[4/]	83.88±0.02	80.22±0.47	92.25±0.14	93.41±0.03	93.60±0.74	92.33±0.01
Ash (%)	16.12±0.02	19.78±0.47	7.75±0.14	6.59±0.03	6.40±0.74	7.67±0.01
CP[5/] (%)	14.10±0.28	10.36±0.07	9.04±0.08	9.69±0.08	9.80±0.13	9.42±0.13
Ether extract (%)	2.52±0.03	2.69±0.06	4.23±0.13	3.08±0.09	4.13±0.13	5.51±0.05
CF (%)	22.59±0.10	20.64±0.18	22.42±0.05	23.65±0.06	21.59±0.07	22.24±0.03
NFC[6/] (%)	7.14±1.31	8.01±2.19	16.52±1.53	19.56±0.82	17.05±1.19	16.43±0.42
NDF[7/] (%)	60.13±1.03	59.16±1.60	62.46±1.34	61.09±1.03	62.63±0.18	60.98±0.35
ADF[8/] (%)	25.66±0.14	25.95±0.49	31.85±0.84	32.95±0.73	38.32±0.68	35.27±0.35
ADL[9/] (%)	2.52±0.00	2.79±0.03	9.76±0.28	11.32±0.47	11.80±0.16	9.65±0.14
Hemicellulose (%)[10/]	34.47±1.17	33.22±1.11	30.62±0.50	28.14±0.30	24.31±0.49	25.71±0.71
Cellulose (%)[11/]	23.14±0.14	23.17±0.46	22.09±0.57	21.63±0.26	26.52±0.52	25.62±0.21
pH (Fresh)	4.50±0.07	4.50±0.07	5.80±0.07	5.70±0.07	5.70±0.07	5.80±0.07
L*	50.56±0.25	51.34±0.28	48.22±0.62	48.88±0.27	50.91±0.24	46.06±0.65
a*	-2.14±0.01	-2.28±0.05	-2.20±0.10	-0.15±0.07	0.12±0.06	1.82±0.02
b*	26.21±0.55	26.74±0.31	30.01±0.16	23.76±0.22	28.49±0.11	28.39±0.23
C*	26.29±0.54	26.84±0.31	30.09±0.15	23.76±0.22	28.49±0.11	28.45±0.23

H°	94.67±0.08	94.88±0.16	94.19±0.21	90.37±0.19	89.77±0.11	86.32±0.03

Note: [1]/Moisture content (%) = 100-%DM; [2]/Dry matter 1 (% of fresh); [3]/Dry matter 2 (%); [4]/Organic matter (%) = 100 - %ash; [5]/Crude protein (%); [6]/Crude fiber (%); [6]/Non fiber carbohydrate = 100 - (%NDF + %crude protein + %fat + %ash); [7]/Neutral detergent fiber; [8]/Acid detergent fiber; [9]/Acid detergent lignin; [10/]Calculated as %NDF - %ADF; [11/]Calculated as %ADF - %ADL

Table 3 The interaction of each of the species of grass and banana cultivars with regard to physical characteristics, chemical composition and ensiling products after an ensiling period of 21 days.

Characteristics	NP1 grass					SG grass					SEM[11/]	Grass	Banana	GxB[12/]
	NP1[1/]	TB[2/]	HB[3/]	NTB[4/]	TPB[5/]	SG[6/]	TB[7/]	HB[8/]	NTB[9/]	TPB[10/]				
Smell[13/]	8^{b}	12^{a}	10^{ab}	12^{a}	8^{b}	8^{b}	12^{a}	12^{a}	8^{b}	4^{c}	0.42	***	***	***
Texture[14/]	4	4	4	4	4	4	4	4	4	4	0.00	ns	ns	ns
Color[15/]	3^{a}	3^{a}	3^{a}	2.50^{ab}	3^{a}	3^{a}	2^{b}	2^{b}	2^{b}	3^{a}	0.08	***	***	***
pH[16/]	3.88^{ab}	3.10^{d}	3.15^{cd}	2.98^{d}	3.95^{a}	3.70^{abc}	3.23^{cd}	3.50^{abcd}	3.33^{bcd}	3.55^{abcd}	0.06	ns	***	***
Quality[17/]	18.88^{cd}	22.10^{a}	20.15^{abc}	21.48^{ab}	18.95^{bcd}	18.70^{cd}	21.23^{abc}	21.50^{ab}	17.32^{d}	14.55^{e}	0.37	***	***	***
L*	49.90^{ab}	48.59^{abc}	48.47^{abc}	48.66^{abc}	45.75^{d}	48.57^{abc}	47.83^{bcd}	50.28^{ab}	50.62^{a}	47.14^{cd}	0.28	*	***	***
a*	2.38^{f}	3.76^{c}	4.67^{a}	4.51^{b}	4.77^{a}	3.11^{e}	3.59^{d}	3.50^{d}	3.26^{e}	4.40^{b}	0.14	***	***	***
b*	25.88^{de}	26.83^{cd}	29.35^{a}	26.24^{d}	27.67^{bc}	24.99^{e}	26.71^{cd}	27.85^{b}	27.47^{bc}	27.36^{bc}	0.22	**	***	***
C*	25.99^{ef}	27.09^{bcd}	29.72^{a}	26.62^{de}	28.08^{b}	25.17^{f}	26.95^{cde}	28.07^{b}	27.66^{bc}	27.71^{bc}	0.22	***	***	***
H°	84.73^{a}	82.01^{d}	80.96^{e}	80.26^{f}	80.22^{f}	82.92^{bc}	82.35^{d}	82.85^{c}	83.24^{b}	80.86^{e}	0.26	***	***	***
Moisture[18/]	85.58^{a}	85.17^{ab}	84.00^{abc}	83.74^{abc}	82.58^{c}	84.47^{abc}	84.56^{abc}	83.92^{abc}	83.31^{bc}	83.98^{abc}	0.20	ns	***	**
DM 1[19/]	14.42^{c}	14.83^{bc}	16.00^{abc}	16.26^{abc}	17.42^{a}	15.53^{abc}	15.44^{abc}	16.08^{abc}	16.69^{ab}	16.02^{abc}	0.20	ns	***	**
DM 2[20/]	99.16^{abc}	98.52^{abc}	99.53^{a}	98.61^{abc}	99.05^{abc}	99.71^{a}	98.31^{bc}	99.12^{abc}	99.48^{ab}	98.03^{c}	0.14	ns	*	*
OM[21/] (%)	84.64^{d}	85.62^{cd}	89.12^{a}	87.32^{b}	86.09^{c}	80.58^{f}	82.89^{e}	85.68^{c}	83.47^{e}	83.05^{e}	0.54	***	***	**
Ash (%)	15.36^{c}	14.38^{cd}	10.88^{f}	12.68^{e}	13.91^{d}	19.42^{a}	17.11^{b}	14.32^{d}	16.53^{b}	16.95^{b}	0.54	***	***	**
CP[22/] (%)	11.68^{ab}	11.50^{ab}	12.01^{ab}	12.06^{a}	11.35^{ab}	11.45^{ab}	11.23^{ab}	11.70^{ab}	11.12^{b}	11.58^{ab}	0.07	***	**	**
EE[23/] (%)	2.48^{d}	2.93^{cd}	2.90^{cd}	1.85^{e}	4.87^{a}	3.24^{c}	3.39^{bc}	3.83^{b}	3.33^{bc}	3.87^{b}	0.18	***	***	***

CF[24/] (%)	26.85[a]	23.81[cd]	24.01[c]	22.69[e]	24.52[b]	23.79[cd]	23.40[d]	22.72[e]	22.30[e]	19.71[f]	0.40	***	***	***
NFC[25/] (%)	7.55[c]	10.12[b]	13.26[a]	11.05[b]	6.95[c]	6.39[c]	9.95[b]	7.00[c]	7.51[c]	7.79[c]	0.49	***	***	***
NDF[26/] (%)	62.95[ab]	61.08[c]	60.95[c]	62.38[b]	62.93[ab]	59.51[d]	58.33[e]	63.16[a]	61.52[c]	59.82[d]	0.36	***	***	***
ADF[27/] (%)	34.33[cd]	34.49[c]	32.82[e]	34.61[c]	35.38[b]	36.49[a]	33.93[d]	34.09[cd]	35.47[b]	33.85[d]	0.22	***	***	***
ADL[28/] (%)	2.33[g]	3.24[de]	4.57[a]	3.79[c]	4.24[b]	2.77[f]	3.48[d]	4.87[a]	3.86[c]	2.95[ef]	0.18	*	***	***
Hemicellulose	28.62[ab]	26.59[d]	28.13[bc]	27.77[c]	27.55[c]	23.02[f]	24.41[e]	29.07[a]	26.05[d]	25.98[b]	0.42	***	***	***
Cellulose	32.00[b]	31.25[cd]	28.25[g]	30.82[de]	31.14[cd]	33.72[a]	30.45[e]	29.23[f]	31.61[bc]	30.90[de]	0.32	***	***	***
GE[29/]	4339.75[b]	4352.89[b]	4749.89[a]	3904.41[cd]	4027.57[c]	3503.08[f]	3670.00[ef]	3922.20[c]	3677.76[ef]	3722.40[de]	84.56	***	***	***
LA[30/]	8.37[f]	13.23[de]	15.20[bc]	14.33[cd]	12.88[e]	15.54[bc]	16.03[b]	7.54[f]	7.17[f]	17.69[a]	0.67	ns	***	***
C_2[31/]	87.51[a]	81.88[ab]	73.06[c]	73.35[c]	78.41[bc]	61.95[d]	74.64[bc]	87.28[a]	75.22[bc]	79.81[abc]	1.66	***	***	***
C_3[32/]	3.58[ab]	4.81[ab]	6.84[a]	3.70[ab]	5.56[ab]	9.43[a]	7.63[a]	3.90[ab]	5.87[a]	0.00[b]	0.63	ns	ns	**
C_4[33/]	6.68[g]	10.13[f]	15.33[d]	17.50[c]	12.21[e]	21.74[a]	17.74[c]	8.80[f]	18.59[bc]	20.19[ab]	1.12	***	***	***
VA[34/]	2.24[f]	3.19[e]	4.78[c]	5.46[b]	3.80[d]	6.81[a]	0.00[g]	0.00[g]	0.33[g]	0.00[g]	0.55	***	***	***
NH_3-N[35/]	1.80[ab]	1.97[a]	1.36[abc]	0.77[c]	1.18[bc]	1.02[c]	0.95[c]	1.02[c]	1.21[bc]	0.94[c]	0.09	***	***	***
LAB[36/]	$2.0x10^{8a}$	$5.9x10^{3b}$	$5.7x10^{3b}$	$8.8x10^{4b}$	$2.3x10^{6b}$	$1.3x10^{7b}$	$2.4x10^{3b}$	$1.4x10^{6b}$	$2.5x10^{b}$	$1.3x10^{5b}$	$1.1x10^{7}$	***	***	***
TS[37/]	1329.64[g]	2339.78[b]	2227.20[b]	2027.61[c]	1585.07[f]	1909.51[de]	2066.46[c]	2635.80[a]	1856.43[e]	2017.22[cd]	65.21	***	***	***
RS[38/]	295.40[e]	447.77[b]	605.33[a]	622.32[a]	289.75[e]	343.43[d]	314.19[de]	392.86[c]	309.15[de]	404.82[c]	21.69	***	***	***

Note: Value in each row marked by the same letter were not significantly different at P<0.05

[1]/100% Napier Pakchong1 grass silage; [2]/80% Napier Pakchong1 grass fermented with 20% Tani banana; [3]/80% Napier Pakchong1 grass fermented with 20% Hin banana; [4]/80% Napier Pakchong1 grass fermented with 20% Nam Thai banana; [5]/80% Napier Pakchong1 grass fermented with 20% Thep Parod banana; [6]/100% Sweet grass silage; [7]/80% Sweet grass fermented with 20% Tani banana; [8]/80% Sweet grass fermented with 20% Hin banana; [9]/80% Sweet grass fermented with 20% Nam Thai banana; [10]/80% Sweet grass fermented with 20% Thep Parod banana; [11]/Standard error of mean; [12]/Interaction of species of grass and bananas cultivars; [13]/Smell (vinegar = 12, slightly pungent = 8, slightly stinky = 4, rancid smell =0); [14]/Texture (The leaves and stems remain the same = 4, the leaves and stems are little maceration = 2, the leaves and stems are very maceration = 1, the leaves and stems are mucilage and soft= 0); [15]/Color (yellow = 3, green = 2, golden brown = 1,

brown-black = 0); [16]/pH (3.5-4.2 = 6, 4.4-4.7 = 4, 4.7-5.1 = 2, >5.1 = 0); [17]/Quality level (very good = 20-25, good = 15-19, medium = 6-14, low = 0-5); [18]/Dry matter 1 (% of fresh silage); [19]/ Moisture content; [20]/Dry matter 2 (%); [21]/Organic matter; [22]/Crude protein; [23]/Ether extract; [24]/Crude fiber; [25]/Non-fiber carbohydrates; [26]/Neutral detergent fiber; [27]/Acid detergent fiber; [28]/Acid detergent lignin; [29]/Gross Energy; [30]/Lactic acid (%); [31]/Acetic acid (%); [32]/Propionic acid (%); [33]/Butyric acid (%); [34]/Valeric acid (%); [35]/Ammonia nitrogen (g/kgDM); [36]/Lactic acid bacteria (CFU/g); [37]/Total sugar (mg/100g); [38]/Reducing sugar (mg/100g)

Figure 1 The pH and organic acid content (lactic acid, acetic acid and butyric acid) of the experimental silages.

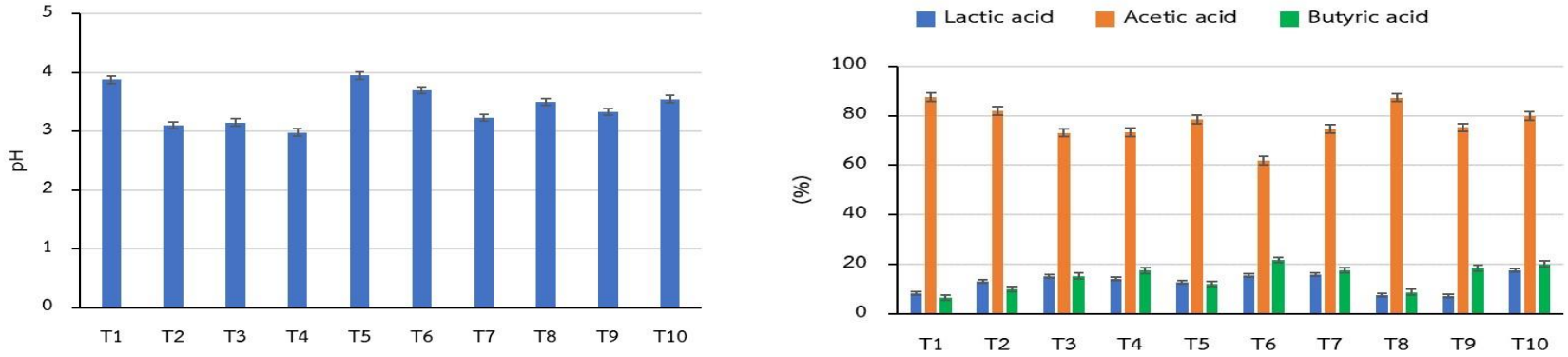
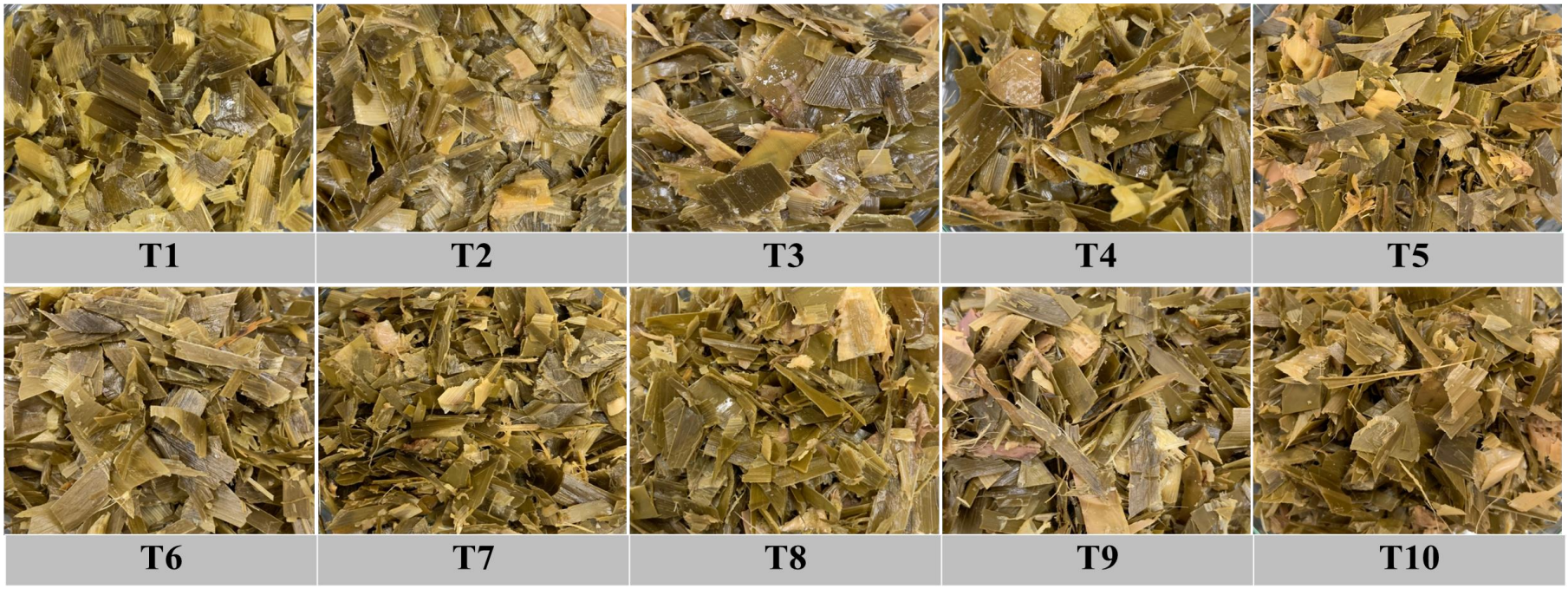
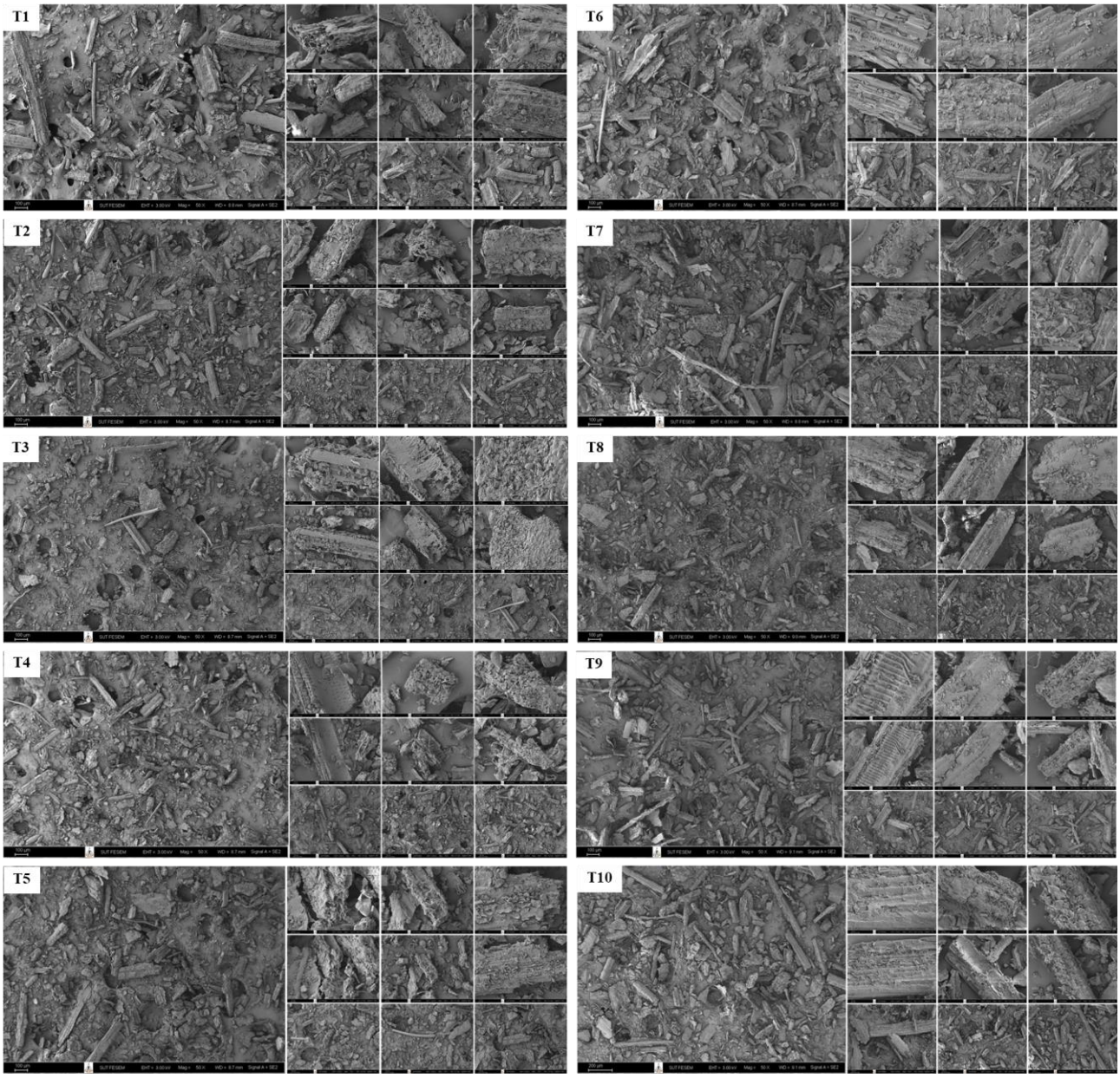


Figure 2 The color of the experimental silages.



Note: T1 = 100% Napier Pakchong1 grass silage; T2 = 80% Napier Pakchong1 grass fermented with 20% Tani banana; T3 = 80% Napier Pakchong1 grass fermented with 20% Hin banana; T4 = 80% Napier Pakchong1 grass fermented with 20% Nam Thai banana; T5 = 80% Napier Pakchong1 grass fermented with 20% Thep Parod banana; T6 = 100% Sweet grass silage; T7 = 80% Sweet grass fermented with 20% Tani banana; T8 = 80% Sweet grass fermented with 20% Hin banana; T9 = 80% Sweet grass fermented with 20% Nam Thai banana; T10 = 80% Sweet grass fermented with 20% Thep Parod banana

Figure 3 The shape and surface characteristics of the experimental silage treatments as revealed by a scanning electron microscope (SEM)



Note: T1 = 100% Napier Pakchong1 grass silage; T2 = 80% Napier Pakchong1 grass fermented with 20% Tani banana; T3 = 80% Napier Pakchong1 grass fermented with 20% Hin banana; T4 = 80% Napier Pakchong1 grass fermented with 20% Nam Thai banana; T5 = 80% Napier Pakchong1 grass fermented with 20% Thep Parod banana; T6 = 100% Sweet grass silage; T7 = 80% Sweet grass fermented with 20% Tani banana; T8 = 80% Sweet grass fermented with 20% Hin banana; T9 = 80% Sweet grass fermented with 20% Nam Thai banana; T10 = 80% Sweet grass fermented with 20% Thep Parod banana

They were also higher DM than NP grass silage inoculated with *Lactobacillus buchneri* (LB), *Lactobacillus plantarum* (LP), or combined with LB and LP (Noosen and Lounglawan, 2018) and Yokota et al. (1992) who reported that NP grass (*Pennisetum purpureum* Schum.) silage ensiled with 4% of

molasses had 14.75%DM, but that it was similar to NP grass silage alone (Kaewpila et al., 2020). This may due to the banana cultivars having more DM than the grasses (Table 2), so when they were fermented with the two types of grasses it resulted in a higher level of DM. Thus, these results show that using these banana cultivars can reduce the DM loss of silage when compared the non-use treatments. T3 had the highest CP and OM and the high OM in HB resulted in increased OM of the two species of grass fermented with HB which were significantly greater than other treatments. This was consistent with the results for using ash with fermented grass with HB, when the content of ash was low, the amount of OM that animals can use increased. CP of T1 decreased compared to the fresh NP1 including T2 to T5, whereas SG (T6) slightly increased compared to fresh SW, which was consistent with Kaewpila et al. (2020). The SF with high protein legumes can increase CP in silage as reported by Bureenok et al. (2011) and Muhammad et al. (2008), thus the protein of 4 fresh banana cultivars ranged from 9.04 to 9.80% CP which is associated with the decrease of CP in the silage. NDF and ADF of these experimental silages were slightly variegated and the direction indicated that they varied according to NDF and ADF in the banana leaves with petioles.

T4 and T8 had increased NDF, while T2, T3, T4 and T8 had increased and RS and decreased pH to below 4.0. Noosen and Lounglawan (2018) found that although the concentration of *L. buchneri* had no influence on the changes in CF, NDF, ADF and ADL, the pH was lower than 4.2 in NP silage and RS and LAB had increased with the concentration of *L. buchneri*, resulting in the production of LA, which has a rather strong acidic property. Therefore, the quality of silage can be preserved, because it inhibits the growth of other microorganisms (McDonald et al., 1991; Weinberg et al., 2003) which has a beneficial effect on the digestion process in the rumen, increasing the efficiency of fiber digestion (Weinberg et al., 2007). These treatments (T2, T3, T4 and T8) fermented without *L. buchneri* had LAB between $5.7x10^3$-$1.4x10^6$ CFU/g, which was higher than those of Noosen and Lounglawan (2018). Nsereko et al. (2008) reported that LAB affects the activity of the ferulic esterase enzyme, resulting in hemicelluloses. These are components of cell walls, which are water insoluble carbohydrates and separate from the lignin structure, which is the part that strengthens the plant cell wall and increases with the age of plants and the animals were not able to utilize them. Likewise, the increase in NDF is due to the activity of ferulic acid esterase from the use of *L. buchneri* (Kang et al., 2009). According to Nsereko et al. (2008) and Kang et al. (2009) *L. buchneri* in silage has a beneficial effect on the efficiency of fiber digestion, especially NDF. DM intake, digestibility and rumen capacity in ruminants were negatively correlated with NDF and ADF, but positively correlated with chewing time (NRC, 1988), as cited by Thongnum et al. (2021). Therefore, in a diet with high NDF content, there is less feed intake. On the other hand, if the amount of NDF in the diet is low, the animals are less ruminant than on a high NDF diet, which affects the end-products in fermentation, such as a decrease in C_2 and an increase in C_3 (Beauchemin et al., 1994).

The anaerobic fermentation process relies on LAB to convert WSC to LA, however, the ensiling products from our experimental silages were variable and directionally unstable. T1 had significantly higher LAB than all the other treatments. LA was highest in

T10, followed by T7, T3 and T6, however, T1, T8 and T9 were the lowest. The LA of T1, T2, T5, T8 and T9 were in the range of 3-13%, which can be classified as good quality silage (Tudsri, 2005). The data showed that C_2 was higher than LA in all the experimental silage treatments. Lukkananukool et al. (2018) and Boonkoed et al. (2018) reported that C_2 was the main OA occurring in silage made from tropical forage crops.

The results show that the overall quality of T2, T3, T4, T7 and T8 was very good These five experimental silages showed an increase in RS, TS, LA and C_2 (between 73.06-87.28% of the total OA), and a reduction in NH_3-N, although the LAB was lower than T1. In general, the fermentation of silage caused an accumulation of LA through LAB (epiphytic LA bacteria), which are the bacteria that produced LA under anaerobic fermentation conditions and converted WSC into LA. The ensiling process progresses over a period of time, so that the accumulation of LA increased, while the silage pH is lower. Spoilage such as *Clostridium spp*., yeast and fungi are inhibited (Wang et al., 2014 cited by Jaipolsaen et al., 2021). These properties lead to silage that can be stored for a long time and maintain a good quality (olive-yellow color) with an aroma similar to fruity alcoholicity or vinegar, firm flesh with leaves and stems that remain intact according to the standards of good quality silage (Department of Livestock Development, 2004). The spoilage microorganisms of fermented feed can grow if the fermentative processing of the silage pH condition is lower, it degrades sugars and converts LA to C_4, with the loss of protein in the form of ammonia (McDonald et al., 1991). Good silage should not contain or minimize C_4 (less than 0.1%) (Department of Livestock Development, 2004). Lower pH of silage also affects the reduction of NH_3-N (g/kg DM) (Filya, 2003). All the experimental silages in this study showed that pH was lower than 4.2, indicating that starch and sugar which are the primary carbohydrates in the plants were sufficient to enable LAB, or LA-producing bacteria to be completely utilized in the fermentation process without utilizing protein as an energy source. This resulted in higher NH_3-N consistent with lower protein and slightly higher NH_3-N concentration in fermented kale inoculated with *L. buchneri* at 10^6 CFU/g. This may be due to the low content of starch and sugar which was found. The protein is therefore used by these over-supplied microorganisms as a source of energy (Fraser et al., 2001). Most plants store energy in the form of glucose and store glucose in starch form, which is a polysaccharide. Jaipolsaen et al (2021) reported that the NP grass ensiling process required inoculation of a starter culture such as *L. plantarum* J39 at 10^7 CFU/g fresh grass, and *L. plantarum* J39: *L. brevis* BCC42336: *P. pentosaceus* TBRC7603 (2:1:1) at 10^7 CFU/g fresh grass). This agrees with Pitiwittayakul et al. (2021) who found that the inoculation of single or combined cultures, such as *L. plantarum* G4, *L. fermentum* N4 and *P. pentosaceus* R5, improved the quality of NP grass silage by inhibiting Clostridial bacteria growth, as NP grass has a low predominance of bacteria both in diversity and quantity which is rare in tropical forage crops (Cai et al., 1998) and is mainly a heterofermentation process (Cai et al., 1999). Examples of tropical forage crops include Brachiaria, Panicum, Paspalum, Pennisetum and Sorghum. Oliveira et al. (2017) reported that silage quality was consistent if microbial inoculants were used in production. Therefore, the inoculation of LAB in

silage production may have led to a reduction in the variability of ensiling products from the fermentation process in this study.

CONCLUSION

NP1 grass fermented with TB, HB or NTB, and SG fermented with TB or HB in 80:20 ratios after 21 days resulted overall in very good silage quality that can be used as an alternative silage for ruminants, and the interactions between grass fermented with banana were statistically significant in SG fermented with HB.

Suggestion

The combined use of fermented banana with NP1 grass silage can reduce the loss of DM better than the use of NP1 grass silage alone. However, guidelines for the use of other types of local AW with high WSC, or supplementation with molasses or ground grain (corn), or the addition of the inoculation of LAB of both types and levels appropriate to quality improvement should also be considered. These different types of treatment should be compared in terms of their cost effectiveness.

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