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Research Article

Effects of fermented juice of epiphytic lactic acid bacteria from dragon fruit (*Hylocereus undatus*) peel addition on the characteristics and *in vitro* digestibility of sugar palm peel silage

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Abstract - Sugar palm peel silage is utilized as a ruminant feed in Thailand. However, quality improvement of the silage is demanded for development of ruminant production. Fermented juice of epiphytic lactic acid bacteria (FJLB) prepared from natural resources is known as a useful inoculant for successful silage production. Although dragon fruit is widely grown in the country, its peel (DFP) is not utilized meaningfully and becomes a waste. In addition, the characteristics of FJLB from DFP (FJLB-DFP) as an inoculum for ensiling are unknown. Thus, this study had objectives to investigate the characteristics of FJLB-DFP as a silage additive and to clarify the effects of FJLB-DFP addition on the chemical composition and digestibility of sugar palm peel silage. The FJLB-DFP was prepared by a 96-hour anaerobic incubation of DFP (T1) or DFP mixed with additional 5% molasses (T2). Although the counts of epiphytic lactic acid bacteria (LAB) had no significant difference between the treatments, the pH value was lower in T2 than in T1. The addition of 5% molasses to DFP was evaluated to make a better inoculant. The silage was prepared without addition (S1), with 1% FJLB-DFP (S2) and with 1% molasses (S3). The silage with inoculants decreased pH value and increased crude protein, total volatile basic nitrogen and non-fibrous carbohydrate concentrations. The lowest cellulose content among the treatments was obtained in S2. No adverse effects were found in digestibility and

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gas production of the silage with FJLB-DFP. Thus, the addition of FJLB-DFP possibly induces the better nutritional quality of sugar palm peel silage.

Keywords: Dragon fruit peel, epiphytic lactic acid bacteria, fermented juice, silage, sugar palm peel

1. Introduction

Feed resources are important materials for livestock production and easily affect animal performances. However, feeding and nutrition have been reported to be the major constraints to livestock production in Southeast Asia (Devendra & Leng, 2011). Thus, much attention to feed resources is necessary for the development of livestock production in the area. Two critical periods of feed shortage for ruminants in tropical areas were reported (Chantalakhana & Skunmun, 2002). One of them is the period during the dry and hot season when most ruminants should move a long distance for grazing. The other period is cropping season when most farm areas are cultivated and leave no area for grazing. Therefore, the efficient utilization of locally available feed resources becomes one of the countermeasures for preventing from the feed shortage.

Preservation of locally available feed resources during the rainy season is a substantial activity for maintaining feed supply throughout the year. Feed materials can be conserved through the fermentation process for ensilage. Epiphytic lactic acid bacteria (LAB) and water-soluble carbohydrates (WSC) are useful resources for successful ensiling with producing enough lactic acid for pH reduction (Rooke, 1990). Although the numbers of LAB in tropical feed resources may be low (Ohmomo et al., 2002), the addition of fermented juice of LAB (FJLB) to tropical diets improved the quality of their silage (Bureenok et al., 2005; 2006). The total mixed ration (TMR) silage prepared from agricultural and industrial by-products also improved the fermentation quality by FJLB from Italian ryegrass (Yanti & Yayota, 2019). The FJLB was produced by fermentation of macerated natural resources such as grasses or peels in sterilized distilled water and is known as a novel additive for ensiling. In addition, molasses is a common resource in tropical areas for providing WSC easily (Yokota et al., 1991).

Sugar palms and dragon fruits are abundantly produced in Thailand. Palmyra palm (*Borassus flabellifer*, Linn) is widely grown for sugar production in southern Thailand, especially in Phetchaburi and Songkhla provinces. The main product of the palm is the palmyra sap which includes sucrose (Naknean et al., 2010), and the processing of sap yields fibrous sugar palm peel as one of by-products (Rungrodnimitchai, 2011). This by-product is treated as agricultural waste and their accumulation causes detrimental effects on the environment (Saenphoom et al., 2016). Therefore, the sugar palm peel has been traditionally utilized as a ruminant feed in southern Thailand. On the other hand, dragon fruit (*Hylocereus undatus*) is a major fruit grown in Thailand. In general, dragon fruit peel (DFP) becomes a waste from the fruit industries and is used as fertilizer. However, DFP has the potential to improve the quality of locally available feed resources. In fact, DFP was reported to have high amounts of phytonutrients such as betacyanins, flavonoids, and phenolic acids (Tenore et al., 2012). Thus, this fruit peel may have bioactive compounds for fermentation. However, the characteristics of FJLB from DFP (FJLB-DFP) as an inoculant for ensiling are still unknown. Although molasses is known as a major resource to supply energy for the growth of LAB (Keady, 1996), the effects of molasses addition to

FJLB-DFP on its characteristics are not yet clear. In addition, the effects of FJLB-DFP addition on the chemical composition and digestibility of sugar palm peel silage are still ambiguous. If FJLB-DFP possesses the significant characteristics for making high quality silage of locally available feed resources, it can contribute to efficient and effective developments of livestock production in the tropical area.

Therefore, this study had an objective to investigate the characteristics of FJLB-DFP with or without molasses for identifying a better silage additive. In addition, another objective is to clarify the effects of FJLB-DFP addition on the chemical composition and digestibility of sugar palm peel silage to improve its quality. The FJLB-DFP may contribute to reducing waste and induce an effective utilization of DFP. Furthermore, the improvement of nutritional quality of sugar palm peel silage with FJLB-DFP for supplying better feed resources to livestock farmers is anticipated.

2. Materials and methods

2.1 FJLB preparation

The experiment for identifying the characteristics of FJLB-DFP consisted of 2 treatments with 4 replicates. FJLB was prepared from DFP itself (T1) and DFP mixed with additional 5% molasses on a fresh matter basis (T2). Bureenok et al. (2005) reported that the FJLB prepared from guineagrass (P*anicum maximum* Jacq cv. Gatton) with 5% molasses showed pH 4.12, 1.85×10^9 LAB and 15.42 mg/mL lactic acid. This FJLB with 5% molasses was identified as a suitable inoculant for guineagrass silage compared to the other additions such as 1% glucose or 1% sucrose in terms of pH and lactic acid concentration. Thus, the addition of 5% molasses with FJLB was settled in this study. The molasses was sterilized before usage. Fresh dragon fruits were obtained from Phetchaburi province in Thailand. The fruits were washed with water and peeled by hand. The DFP was cut approximately 2 to 3 cm in length ground by an electric mixer and filtered through sterilized double cheesecloth just before the usage. The DFP (1,000 g) was mixed with distilled water (2,000 g) in each treatment. In T2, the molasses (150 g) was added to the mixture of DFP and water. The mixture was filtered through a sterilized double cheesecloth and transferred into glass bottle. The extract (40 g) was collected in a sealable capped glass bottle and incubated at 30°C for 0, 12, 18, 24, 30, 36, 48, 72 and 96 hours in an anaerobic condition.

2.2 Measurement of pH and counts of lactic acid bacteria in FJLB-DFP

The pH of FJLB in T1 and T2 was measured using a pH meter (Cyberscan, Eutech instrument, Singapore) at 0, 12, 18, 24, 30, 36, 48, 72, and 96 hours after the beginning of incubation. The LAB is grown in Lactobacillus MRS agar using pour plate technique. The FJLB-DFP samples (100 μL) at 0, 12, 18, 24, 30, 36, 48, 72 and 96 hours during the incubation was serially diluted with 0.9 mL of 0.85% NaCl (10 times, 10^2 times, and 10^3 times). The sample and culture medium were poured into a petri dish and incubated at 30°C for 18-24 hours in an anaerobic condition. The colonies of the culture medium were counted and calculated as colony forming unit (cfu) / mL according to the formula of Kozaki et al. (1992).

2.3 Processing of sugar palm peel silage

Sugar palm peel (24 kg) was collected in Phetchaburi province in Thailand. The peel was cut approximately 2 to 3 cm length. The experiment for identifying the characteristics of silage consisted of 3 treatments with 4 replicates. The silage was prepared without addition of any inoculant as control (S1), with 1% of FJLB-DFP (S2) and with 1% molasses (S3). The FJLB-DFP used in S2 was mixed with 5% molasses on a fresh matter basis and contained 1.5

× 10^6 cfu/mL of total LAB. The FJLB-DFP was prepared by the 24-hour incubation using the mixture of 400 g of DFP, 800 g of distilled water, and 60 g of molasses in a sealable capped glass bottle. The mixture in a two-layer plastic bag was put into a 3,000 mL plastic container and incubated at 30°C for 30 days in an anaerobic condition using airtight plastic pouches.

2.4 Chemical composition of sugar palm peel silage

The silage samples were oven dried at 60ºC for 48 hours for measuring dry matter (DM) content. The dried samples were milled to pass a 1 mm screen for the proximate analyses. Chemical composition of the samples was measured according to the standard methods of the Association of Official Analytical Chemist (AOAC, 2016), including DM, crude protein (CP), ether extract (EE), crude fiber (CF), and crude ash (CA). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined based on a previously described method (Van Soest et al., 1991). Organic matter (OM) and non-fibrous carbohydrate (NFC) were obtained using the following formulae: OM (%) = 100 - CA (%), NFC (%) = 100 - NDF (%) - CP (%) - EE (%) - CA (%).

2.5 Measurement of pH and volatile basic nitrogen content in sugar palm peel silage

The sample of silage (15 g) on DM basis was mixed with 140 mL distilled water in an Erlenmeyer flask and cap the flask with wrapping film. The mixture was shaken for 24 hours and the extract liquid was filtered by filter papers. The pH of the extract liquid was measured using pH meter (Cyberscan, Eutech instrument, Singapore). Total volatile basic nitrogen (TVBN) was determined by steam distillation (Cai, 2004).

2.6 *In vitro* digestibility of sugar palm peel silage and gas production during the digestion

In vitro digestibility of DM (IVDMD), OM (IVOMD), and NDF (IVNDFD) of the sugar palm peel silage was determined by the methods of Tilley and Terry (1963). The rumen fluid was collected using an oral stomach tube from healthy mature 6 goats before the feeding on the collection day. The procedures for rumen fluid collection were approved by the Institutional Animal Care and Use Committee of Silpakorn University (Approval No. 06/2565), and were performed according to the guidelines for the care and use of animals at Silpakorn University. The goats consumed rice straw and Leucaena leaves *ad libitum* every day. The fluid was strained through a sterilized four-layer cheesecloth. In addition, the artificial saliva was prepared as described by McDougall (1948). The strained fluid (1,000 mL) and artificial saliva (4,000 mL) were mixed. Approximately 0.5 g of dried sample was added into 40 mL of the mixture of rumen fluid and artificial saliva in a 50 mL serum bottle. The bottles were capped with rubber stoppers, crimp sealed, and incubated in a water bath at 39°C in an anaerobic condition. The bottles were gently shaken for 30 minutes at the beginning of incubation and at the time of three-hour intervals for 12 hours. The incubation was conducted for 72 hours. Gas production was measured by recording the amount of gas volume after incubation using 100 mL glass syringe connected to the incubation bottle. Reading of gas production was recorded at 6, 12, 24, 48, and 72 hours after the beginning of incubation. The incubated samples were separated from the mixture solution and determine IVDMD. The OM and NDF contents of incubated samples were analyzed for showing IVOMD and IVNDFD, respectively.

2.7 Statistical analysis

The mean comparison between treatments was compared using the probability of differences. Results of the characteristics of FJLB-DFP were analyzed by Student's t-test to show the effect of molasses's addition to FJLB-DFP. Analysis of variance (ANOVA) and Tukey's multiple comparison test for showing the effect of inoculants on the chemical composition and *in vitro* digestibility of the silage. All calculations were made using a commercially available computer program (Excel Statistics; SSRI Co., Ltd., Tokyo, Japan).

3. Results

3.1 pH value and counts of lactic acid bacteria in FJLB-DFP

The pH of FJLB-DFP is shown in Table 1. The pH values of FJLB-DFP in both treatments decreased with the lapse of the fermentation period. Although the pH value in T2 was lower than in T1 at the beginning of measurement ($P < 0.05$), no significant differences were identified between the treatments at 12, 18 and 24 hours after the commencement of incubation. The variation of pH value during the first 12 hours was larger in T1 than in T2 (0.6 vs. 0.4 on average). The pH value was lower again in T2 than in T1 after 30 hours from the start of incubation ($P < 0.05$). The pH value reached the minimum at 72 hours later from the start of incubation and became stable until 96 hours later.

Table 1. pH of FJLB-DFP.

Hour	T1	T2	SEM	P value
0	5.1[a]	4.9[b]	0.01	<0.01
12	4.5	4.5	0.01	0.34
18	4.4	4.4	0.03	0.89
24	4.4	4.3	0.02	0.06
30	4.3[a]	4.2[b]	0.02	0.03
36	4.2[a]	4.0[b]	0.02	<0.01
48	4.1[a]	3.8[b]	0.03	<0.01
72	3.9[a]	3.7[b]	0.02	<0.01
96	3.9[a]	3.7[b]	0.02	<0.01

Note: [ab]Different superscripts in the same row show significant difference ($P < 0.05$). T1: FJLB-DFP itself, T2: FJLB-DFP mixed with 5% molasses.

The LAB count in FJLB-DFP is shown in Table 2. The number of LAB was increased with the lapse of the fermentation period. The increase in average number was 2.2×10^5 in T1 and 7.5×10^4 in T2, respectively, during 96 hours of incubation. However, no significant differences were identified during almost all the measurement time except at 30 hours after the commencement of the incubation ($P > 0.05$).

Table 2. Lactic acid bacteria count in FJLB-DFP (cfu/mL).

Hour	T1	T2	SEM	P value
0	3.3×10^{2}	1.1×10^{3}	4.4×10^{2}	0.12
12	8.9×10^{4}	1.1×10^{5}	1.1×10^{5}	0.86
18	2.8×10^{5}	2.4×10^{5}	1.7×10^{6}	0.16
24	3.3×10^{5}	1.5×10^{6}	1.4×10^{6}	0.40
30	$8.4\times10^{5\,b}$	$1.7\times10^{6\,a}$	3.0×10^{5}	0.02
36	3.7×10^{6}	3.4×10^{6}	1.3×10^{6}	0.87
48	1.0×10^{7}	1.3×10^{7}	1.2×10^{7}	0.83
72	1.2×10^{7}	2.2×10^{7}	4.8×10^{6}	0.09
96	7.2×10^{7}	8.2×10^{7}	4.2×10^{7}	0.83

Note: [ab]Different superscripts in the same row show significant difference ($P < 0.05$).
T1: FJLB-DFP itself, T2: FJLB-DFP mixed with 5% molasses.

3.2 Characteristics and chemical composition of sugar palm peel silage

The characteristics and chemical composition of sugar palm peel silage are shown in Table 3. The pH was lower in S2 and S3 than in S1 ($P < 0.05$). On the other hand, the concentration of TVBN in total nitrogen (TN) was higher in S2 and S3 than in S1 ($P < 0.05$). No significant differences among the treatments were identified in DM and CA ($P > 0.05$). Although S3 showed the highest concentration of CP and EE among the groups, the concentration of ADF, NDF, and hemicellulose in S3 was lower than in S1 and S2 ($P < 0.05$). The rate of NFC was lower in S1 than in S2 and S3 ($P < 0.05$). In addition, S2 showed the lowest cellulose content and the highest ADL content among the treatments ($P < 0.05$).

Table 3. Characteristics and chemical composition of sugar palm peel silage.

	S1	S2	S3	SEM	P value
pH	3.60[a]	3.40[b]	3.40[b]	0.04	<0.01
TVBN/TN(g/kg)	203.3[b]	298.20[a]	282.20[a]	26.00	<0.01
DM (%)	15.40	15.00	15.50	0.36	0.16
CP (%)	3.83[c]	4.39[b]	4.75[a]	0.03	<0.01
EE (%)	1.15[b]	1.18[b]	5.95[a]	0.21	<0.01
CF (%)	35.96	36.81	33.87	2.00	0.10
ADF (%)	49.34[a]	48.03[b]	45.81[c]	0.77	<0.01
NDF (%)	73.84[a]	71.05[b]	65.31[c]	0.56	<0.01
ADL (%)	18.86[b]	24.43[a]	17.76[b]	1.65	<0.01
Cellulose (%)	30.48[a]	23.64[b]	28.36[a]	1.84	<0.01
Hemicellulose (%)	24.50[a]	23.02[a]	19.50[b]	0.91	<0.01

Table 3. Characteristics and chemical composition of sugar palm peel silage. (cont.)

	S1	S2	S3	SEM	P value
NFC (%)	17.18[b]	19.46[a]	19.60[a]	0.74	<0.01
CA (%)	4.00	3.92	4.39	0.44	0.23

Note: [abc]Different superscripts in the same row show significant difference (P < 0.05). TVBN/TN: total volatile basic nitrogen in total nitrogen, DM: dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, ADF: acid detergent fiber, NDF: neutral detergent fiber, ADL: acid detergent lignin, NFC: non-fibrous carbohydrate, CA: Crude ash. S1: no addition, S2: with 1% FJLB-DFP, S3: with 1% molasses.

3.3 *In vitro* digestibility of sugar palm peel silage and gas production during the digestion

The *in vitro* digestibility of sugar palm peel silage is shown in Table 4. No significant difference was obtained in IVDMD among the treatments (P > 0.05). Although the IVOMD was higher in S3 than in S1, the IVNDFD digestibility was lower in S3 than in S1 (P < 0.05). The gas production during *in vitro* digestion of sugar palm peel silage is shown in Table 5. The gas was yielded continuously over time. However, no significant difference was identified among the treatments (P > 0.05).

Table 4. *In vitro* digestibility of sugar palm peel silage (%).

Hour	S1	S2	S3	SEM	P value
DM	48.95	50.30	51.44	3.32	0.20
OM	50.98[b]	52.28[ab]	53.44[a]	2.18	0.03
NDF	39.76[a]	39.15[ab]	37.57[b]	2.12	0.04

Note: [ab]Different superscripts in the same row show significant difference (P < 0.05). S1: no addition, S2: with 1% FJLB-DFP, S3: with 1% molasses, DM: dry matter, OM: organic matter, NDF: neutral detergent fiber.

Table 5. Gas production during *in vitro* digestion of sugar palm peel silage (mL/g substrate).

Hour	S1	S2	S3	SEM	P value
6	8.30	7.70	9.10	6.50	0.76
12	21.90	19.00	21.90	15.20	0.75
24	35.40	31.60	33.60	24.00	0.86
48	47.90	45.60	46.80	30.40	0.97
72	57.60	54.40	55.90	33.50	0.95

Note: S1: no addition, S2: with 1% FJLB-DFP, S3: with 1% molasses.
No significant difference was identified among the treatments (P > 0.05).

4. Discussion

In general, dragon fruit itself has acidity with a pH value between 4 to 5 (Islam et al., 2012; Tan et al., 2023). On the other hand, molasses, a by-product of sugar cane, is also characterized by an acidic pH. The pH value of molasses in the present study might have been lower than that of DFP and induced lower pH in FJLB-DFP with 5% molasses at the beginning of fermentation. Molasses has sucrose as a main sugar content (Palmonari et al., 2020) and is known as a suitable resource for producing lactic acid (Dumbrepatil et al., 2008; Vidra et al., 2017). Usually, Lactobacillus species utilize glucose and fructose which are hydrolyzed from sucrose for converting to lactic acid. Thus, lactic acid might have already been produced in the molasses in this study because the molasses had possibility to contain some strains of LAB in advance. In fact, the LAB count in FJLB-DFP with 5% molasses tended to be higher than that in FJLB-DFP itself at the beginning of fermentation (1.1×10^3 vs. 3.3×10^2 on average, $P = 0.12$). However, the increase of CFU during the first 12 hours' incubation was larger in FJLB-DFP itself than in FJLB-DFP with 5% molasses (2.7×10^2 vs. 1.0×10^2 on average). This difference probably induced the faster reduction of pH value during the first 12 hours of incubation in FJLB-DFP itself than in FJLB-DFP with 5% molasses (0.6 vs 0.4 on an average). The CFU continued to rise with the lapse of the incubation period. However, the rate of CFU increase stagnated between 24 hours and 30 hours of incubation time compared to the previous 6 hours (6.25 times vs. 1.13 times in T2). Although the reasons for this variation were not clear, the concentration of glucose and/or fructose utilized for LAB was possibly reduced after the 24 hours' incubation. Thus, the FJLB-DFP with 24 hours incubation was used for the ensiling in this experiment. The pH value was lower in FJLB-DFP with 5% molasse than in FJLB-DFP itself after 30 hours from the start of incubation. Bureenok et al. (2005) reported that the pH value and lactic acid concentration of FJLB prepared from guineagrass (P*anicum maximum* Jacq cv. Gatton) were 5.83 and 0 mg/mL after the incubation at 30ºC for 3 days, respectively. However, the FJLB with 5% molasses had pH 4.12 and 15.2 mg/mL lactic acid after the same condition of incubation. The pH value of FJLB-DFP with 5% molasses after 30 hours of incubation was lower than that of FJLB-DFP itself. On the other hand, no significant difference was shown in LAB count between the groups. LAB can be classified as either homolactic or heterolactic based on the fermentation type. Although the homofermentative LAB develops lactic acid and rapidly reduces pH, the heterofermentative LAB produces lactic acid, ethanol or acetic acid, and carbon dioxide. The different populations of each type of LAB possibly affected the pH between the groups. The FJLB-DFP with 5% molasses might have induced a higher population of homolactic fermentation during the incubation in this study. Thus, the present study also clearly showed that the addition of molasses induced lower pH values. On the other hand, the LAB in both treatments increased over time and reached more than 7.0×10^7 cfu/mL on average in both treatments after the 96-hour incubation. In the case of FJLB prepared from guineagrass, the number of LAB was more than 1.96×10^8 cfu/mL after 3-day incubation (Bureenok et al., 2005). The lower LAB in the present study might be due to the different resource, DFP, for processing FJLB.

Quality silage made from tropical herbage species has a pH value 4.2 or below (Catchpoole & Henzell, 1971; McDonald et al., 1991). According to this standard, all the silage in the present experiment can be evaluated to have good pH values as silage. However, the content of TVBN/TN was more than 200 g/kg of TN in all the silage. Umana et al. (1991) reported ammonium nitrogen (NH_3-N) concentration of well-preserved silage should be no more than 11% of TN. In general, most contents

of TVBN in silage can be considered as NH_3-N. Thus, all the silages in the present study are supposed to have relatively higher NH_3-N than the recommended value. This indicates degradation of the protein fraction in all the silage. The undesirable fermentation quality of silage regarding TVBN is probably due to the high moisture content of the sugar palm peel.

No significant differences among the treatments were identified in DM concentration of the silage. The moisture content of silage in the present study was around 85%, though the appropriate moisture for making silage is from 60% to 70% in general. Saenphoom et al. (2016; 2017) reported that the DM of sugar palm peel contained 82% moisture, which nearly coincide with the DM of silage in the present study. This indicates the silage has higher moisture than the standard and easily induces the degradation of protein fraction in all the silage. The best fermentation process was indicated to comprise a substrate with high water-soluble carbohydrate content (at least 2%) and low moisture content (Bureenok et al., 2011). However, the sugar palm peel silage is common among ruminant farmers located in southern areas of Thailand as a locally available feed resource. The publicity of manageable methods for reducing moisture contents of sugar palm peel before silage processing should be considered. For example, the reduction of moisture of the peel can be conducted by dehydration with sunshine before ensiling.

The silage with FJLB-DFP or molasses showed a higher concentration of CP and NFC than the silage itself. The inoculant as FJLB-DFP or molasses probably added WSC and induced better fermentation compared with the silage without inoculant. However, the protein in the silage might have been degraded during the fermentation due to the high TVBN production in the present study. The concentration of ADF and NDF in the silage with inoculant was lower than the control silage. The activity of bacteria for fermentation was probably promoted with the addition of FJLB-DFP or molasses. The active fermentation possibly induced the degradation of fiber content such as hemicellulose or cellulose. Saenphoom et al. (2016) indicated the acidic condition of the sugar palm peel silage induced partial acid hydrolysis of hemicelluloses, contributing to the lower fiber content of the silage. Yahaya et al. (2004a) also reported that the silage with FJLB from tropical legumes showed low ADF content due to the decrease in pH value caused by the fermentation of silage. The reduction of NDF content was found with the addition of molasses in silage (Huisden et al., 2009), which may be due to the enzymatic or acid hydrolysis of the cell wall fraction. Interestingly, the silage with FJLB-DFP showed the lowest cellulose content among the treatments. The FJLB-DFP might have possessed more bacterial strains that digest cellulose. The lower cellulose concentration was attributed to the activated cellulolytic bacteria with FJLB-DFP. Bureenok et al. (2011) found that the cows fed ruzigrass silage treated with FJLB had more counts of viable cellulolytic bacteria in their rumen than the cows fed untreated silage. On the other hand, cellulolytic bacteria are sensitive to low pH and are unable to grow at pH 6 (Weimer, 1996). Thus, FJLB-DFP might have contained more cellulolytic bacteria or the silage treated with FJLB-DFP has slowly reduced pH value during the fermentation compared with the other treatments, inducing the lower cellulose concentration in the silage.

The addition of FJLB-DFP or molasses did not change IVDMD of the silage. Bureenok et al. (2011) reported that apparent DM digestibility of ruzigrass silage in cows was not increased by the addition of FJLB from ruzigrass or molasses, agreeing with the present results. The addition of FJLB-DFP did not show a significant difference in IVOMD and IVNDFD of the silage compared with the silage without inoculant. The addition of FJLB was reported to increase the digestibility of

NDF in tropical elephant grass compared with the control (Yahaya et al., 2004b). In addition, Takahashi et al. (2005) found that FJLB treatment and crushing improved the digestibility of fibrous contents in whole-crop rice straw compared to the control. An increased rate of NDF degradation in the rumen was reported by Salawu et al. (2001) by inoculating pea–wheat bi-crop silages with *Lactobacillus Plantarum*. On the other hand, Yanti et al. (2019) reported that the apparent digestibility of DM, OM, and NDF of TMR silage produced by rice straw, corn stover silage, brewer grain, tofu waste, steam-flaked corn and a mineral-vitamin mix was not affected by FJLB from Italian ryegrass. These disagreements were likely due to the different resources for FJLB among the studies. Additionally, the application doses of FJLB in this present study have possibility to be insufficient for improving the digestibility. On the other hand, the IVOMD of the silage with molasses was higher than that of the control silage. The silage with molasses had the highest CP and EE among the treatments. The OM such as protein and fat was probably digested well in the silage with molasses in this study. In fact, the increase in IVOMD of Napier grass silage with 5% molasses application was reported by Bureenok et al. (2012). However, the silage with molasses showed lower IVNDFD than the silage without inoculant in the present study. The silage with molasses had the lowest NDF, ADF, and hemicellulose among the silage in this experiment. Fiber fractions in the silage with molasses have already been digested during the silage fermentation. The content of digestible fiber for rumen bacteria might have been limited in the silage with molasses and induced the lower IVNDFD. Abubakr et al. (2021) found that *Brachiaria obtusiflora* grass silage treated by the mixture of 5% molasses and 1% FJLB prepared from *Brachiaria obtusiflora* induced higher CP digestibility, though the digestibility of DM and OM was not improved. In addition, the addition of FJLB prepared from wild reed plant with molasses improved the fermentation quality of wheat straw silages, aerobic stability, and DM losses (Hussian & Saeed, 2023). Further study using the mixture of molasses and FJLB as an inoculant is needed for the improvement of digestion.

The volume of gas production was not changed by the different treatments in this study. Saenphoom et al. (2016) reported the gas production from the fermented sugar palm peel with pineapple peel after 21-day fermentation was around 110 mL/0.5 g substrate. The production in the present study was extremely lower than that. The lower gas production in the present study may be due to the different resources of rumen fluid and mixing materials.

5. Conclusion

The addition of 5% molasses in FJLB-DFP induced desirable characteristics as a silage inoculant. Then, the mixture of FJLB-DFP with 5% molasses promoted better acidic condition, increased NFC and decreased fiber contents, especially cellulose, in sugar palm peel silage. The better acidic condition was probably induced by 5% molasses addition. Although the increase of CP concentration was also found with the FJLB-DFP with 5% molasses, the protein was degraded during the silage fermentation. The moisture control of raw sugar palm peel for silage processing is required for better silage quality. No adverse effects were found in digestibility and gas production with 1% of FJLB-DFP which was mixed in advance with 5% molasses on a fresh matter basis and contained 1.5×10^6 cfu/ml of total LAB. The addition of FJLB-DFP to sugar palm peel silage was not so effective on the quality compared with the addition of molasses. However, further study is necessary for the effects of bioactive compounds such as phytonutrients with the FJLB-DFP addition to the silage.

Animal ethics declaration

The procedures for rumen fluid collection were approved by the Institutional Animal Care and Use Committee of Silpakorn University (Approval No. 06/2565), and were performed according to the guidelines for the care and use of animals at Silpakorn University.

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