EFFECTS OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS BUCHNERI* ON NAPIER GRASS SILAGE QUALITIES AND AEROBIC STABILITY

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ผลของ *Lactobacillus plantarum* และ *Lactobacillus buchneri* ต่อคุณภาพของหญ้าเนเปียร์หมัก และความคงสภาพในสภาวะที่สัมผัสกับอากาศ

นายภัทรพงศ์ ใจบุญลือ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in fulfillment of the requirements for a Master's Degree.

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ภัทรพงศ์ ใจบุญลือ : ผลของ *Lactobacillus plantarum* และ *Lactobacillus buchneri* ต่อคุณภาพของหญ้าเนเปียร์หมัก และความคงสภาพในสภาวะที่สัมผัสกับอากาศ (EFFECTS OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS BUCHNERI* ON NAPIER GRASS SILAGE QUALITIES AND AEROBIC STABILITY)
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วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อศึกษาผลของการใช้ *Lactobacillus plantarum* และ *L. buchneri* ในหญ้าเนเปียร์ที่ทำการใส่กากน้ำตาล ต่อคุณภาพการหมัก ปริมาณการสูญเสียของวัตถุแห้ง และความคงทนของพืชหมักเมื่อสัมผัสอากาศ การทดลองนี้ได้จัดการทดลองแบบแฟกทอเรียล และใช้แผนการทดลองแบบสุ่มสมบูรณ์ โดยมี 2 ปัจจัย (*L. buchneri* และ *L. plantarum*) และมีปัจจัยละ 3 ระดับ (0, 1×10^5 และ 1×10^6 cfu/g นน. สดหญ้าสด) และได้ทำการหมักที่ 24 วัน ซึ่งปริมาณการสูญเสียวัตถุแห้ง คุณภาพการหมักของพืชหมัก (pH ปริมาณกรดแลคติก ปริมาณกรดไขมันระเหยง่าย แอมโมเนียไนโตรเจน ปริมาณน้ำตาลคงเหลือจากการหมัก) และจุลินทรีย์ในกลุ่มที่ไม่ใช้อากาศ (Lactic acid bacteria, Enterobacteria และ *Clostridium* spp.) ได้ทำการวิเคราะห์ในวันที่ 24 ของการหมัก หลังจากนั้นได้ทำการวิเคราะห์ความคงทนของหญ้าเนเปียร์หมักเมื่อสัมผัสอากาศที่ 2 4 และ 6 วัน โดยใช้ค่า pH ปริมาณ WSC คงเหลือ และจุลินทรีย์ในกลุ่มที่ใช้อากาศ (ยีสต์ lactate-assimilating yeast และรา) ซึ่งจากผลของการศึกษาครั้งนี้ได้บ่งบอกถึงการใช้ *L. buchneri* ในระดับที่สูงที่สุด พบว่าสามารถเพิ่มความคงทนของหญ้าหมักเมื่อสัมผัสอากาศได้ตลอด 6 วัน แต่อย่างไรก็ตามการใช้ *L. buchneri* ในระดับที่สูงที่สุดทำให้ส่งผลเสียต่อคุณภาพการหมัก คุณค่าทางโภชนะของหญ้าหมัก และส่งผลทำให้มีการสูญเสียของวัตถุแห้งเพิ่มขึ้น แต่การใช้จุลินทรีย์ในกลุ่มแลคติกทั้ง 2 ชนิดในระดับสูงที่สุด สามารถแก้ไขปัญหาดังกล่าวข้างต้นได้ นอกจากนี้ยังสามารถเพิ่มความคงทนของหญ้าหมักเมื่อสัมผัสอากาศได้ตลอด 6 วัน ได้อีกด้วย ดังนั้นการใช้ 1×10^6 cfu *L. buchneri*/g นน. สดหญ้าสด ร่วมกับ 1×10^6 cfu *L. plantarum*/g นน. สดหญ้าสด สามารถปรับปรุงคุณภาพการหมัก คุณค่าทางโภชนะของพืชหมัก และยังสามารถเพิ่มความคงทนของพืชหมัก เมื่อเทียบกับการใช้ *L. buchneri* ที่ระดับสูงสุดเพียงอย่างเดียว

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____
ปีการศึกษา 2562 ลายมือชื่ออาจารย์ที่ปรึกษา_____

PATTARAPONG JAIBOONLUE : EFFECTS OF *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS BUCHNERI* ON NAPIER GRASS SILAGE QUALITIES AND AEROBIC STABILITY. THESIS ADVISOR : ASST. PROF. PIPAT LOUNGLAWAN, Ph. D., 66 PP.

FERMENTATION QUALITY/DRY MATTER LOSS/AEROBIC STABILITY

The aim of this study was to study the inoculation effects of both lactic acid bacteria into Napier grass added molasses on silage fermentation, dry matter loss, and aerobic stability. The experiment was designed 3 × 3 factorials experiment in CRD as 3 levels (0, $1 \times 10^5$and 1×10^6 cfu/g fresh forage weight) of each factor (*Lactobacillus buchneri* and *L. plantarum*) was ensiled to 24 days. The silage dry matter loss, fermentation characteristics (pH, lactic acid, VFAs, NH_3-N, residual WSC), nutritive values (DM, CP, EE, Ash), insoluble fiber fractions (NDF, ADF, ADL) and anaerobic microbial profiles (Lactic acid bacteria, Enterobacteria and *Clostridium* spp.) were determined at 24 days of ensiling. Then, silage was exposed to air through 2, 4 and 6 days to determine the aerobic stability characteristics (pH, residual WSC) and aerobic microbial profiles (yeast, lactate-assimilating yeast and mold). The results showed the inoculation at the highest levels of *L. buchneri* can improve the silage aerobic stability characteristics. However, there was increased dry matter loss and the fermentation quality was affected. Nevertheless, the combination at the highest level of both lactic acid bacterial species had been able to solve the problem of dry matter loss and the overall fermentation qualities. Also, the combination at the highest levels of both lactic acid bacterial species had improved the silage aerobic stability characteristic compared with the sole inoculation of *L. buchneri.* Thus, the combinations at the

highest level of both lactic acid bacterial species had improved the dry matter loss, fermentation qualities and silage aerobic stability of Napier grass silage added molasses.

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Pattarapong Jaiboonlue

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CHAPTER I

INTRODUCTION

The appropriate ensiling process requires the suitable of forage conditions such a forage moisture content, a water-soluble carbohydrate (WSC) and a buffering capacity to enhance the suitable of ensiling process (Wilkinson, 2005). Napier grass is recognized as its high nutritive value and production yield per area especially under the theoretical manipulation. Therefore, Napier grass is became to primary roughage for feed to ruminant animals in Tropical area (Wilkinson and Hanna, 1995). However, the forage conditions of Napier grass have not suited for ensiling process since its high moisture content. Consequently, the ensiling process of a higher moisture content in Napier grass needs a lower silage pH value since its need to inhibit the *Clostridium* spp. and Enterobacteria fermentation than the suitable level of forage moisture content. Thus, Napier grass ensiling process needs more WSC concentration as serve for lactic acid bacteria to produce more a concentration of lactic acid, which purposes to reach a lower pH level. The addition of molasses at 5% of fresh forage weigh had accepted as increases the level of WSC resulted to enhance the lactic acid production (Yokota et al., 1991; Arbabi and Ghoorchi, 2008). On the other hand, the residue silage WSC as a resulted from molasses addition is caused to silage spoilage in aerobic phase by aerobic bacteria such yeast, lactate-assimilating yeast, and mold proliferations (Moon, 1983; Guan et al., 2002). Therefore, the silage has contaminated the aflatoxin caused from mold proliferation after silage was exposed to air (Ferrero et al., 2019). The inoculation of *Lactobacillus buchneri* can produce a lot of acetic

acid concentration in silage as its belonged to the Heterofermentative lactic acid bacteria group. Acetic acid is recognized that had been able to inhibit the aerobic bacteria proliferations (Giannattasio et al., 2013). Nevertheless, the using of *L. buchneri* only had led to silage dry matter lose in CO_2 form (Holzer et al., 2003), contributed to economic loss (Goeser et al., 2015). However, the prior studies have shown the inoculation of *L. buchneri* combination with *Lactobacillus plantarum* has contributed to solve the corn silage dry matter loss since *L. buchneri* has ability to convert lactic acid to acetic acid in silage acidic condition instead of the acetic acid production via pentose fermentation pathway, which its caused to silage dry matter lose (Oude Elferink et al., 2001). Although the prior research studies have been demonstrating the combination of *L. buchneri* with *L. plantarum* have the potential to decrease the silage dry matter loss. However, it is unclear which has not confirmed the application of *L. buchneri* combined with *L. plantarum*, and which the concentration (cfu/ g fresh weight) of both species are appropriated for inoculating into the Napier grass added molasse to solve the problems were descripted above. Because of the different in forage characteristics of another forage crop and Napier grass resulted to different in the silage fermentation pattern of each. Consequently, the silage qualities have different according to forage crop. Thus, this study needs to evaluate the inoculation of *L. buchneri* combine with *L. plantarum* in Napier grass added molasses on silage dry matter loss, silage fermentation characteristics, and silage aerobic stability.

1.1 Research questions

1.1.1 Will the combinations of both lactic acid bacterial species at different levels of both do affect to the qualities of Napier grass added molasses were ensiled at 24 days? And, how do these combinations affect to the silage quality?

1.1.2 Will the combinations of both lactic acid bacterial species have been able to decrease the silage dry matter loss when compared with *L. buchneri* solely inoculated in Napier grass added molasses were ensiled at 24 day? And, how do these combinations affect to silage dry matter loss?

1.1.3 Will the combinations of both lactic acid bacterial species at different levels of both do improve the silage aerobic stability when compare with only inoculation of *L. buchneri* of Napier grass silage added molasses were ensiled at days 24 and were exposed to air through 6 days? And, how do these combinations can improve to the silage aerobic stability?

1.1.4 What the level of combinations are appropriate for inoculating in Napier grass added molasses while mainly regards with the silage dry matter lose and silage qualities at day 24 of ensiled, and aerobic stability at 2, 4, and 6 days after exposed to air?

1.2 Research aim

The aim of this study is to assess the combination effect of both lactic acid bacterial species at different level inoculated in Napier grass added molasses on silage dry matter loss, silage qualities at day 24 of ensiled, and silage aerobic stability at days 2, 4, 6 after exposed to air.

1.3 Research objectives

1.3.1 To evaluate the silage dry matter loss, silage fermentation characteristics such silage pH value, lactic acid, acetic acid, propionic acid, butyric acid, NH_3-N and microbial profiles such lactic acid bacteria, Enterobacteria,

Clostridium spp. of each combination of both lactic acid bacterial species inoculated in Napier grass silage added molasses at days 24 of ensiling.

1.3.2 To evaluate the silage aerobic stability characteristics of each combination of both lactic acid bacterial species inoculated in Napier grass added molasses ensiled at day 24 are measured from pH values, dry matter loss, aerobic microbial profiles such yeast, lactate-assimilating yeast, and mold at 2, 4, 6 day after exposed to air.

1.3.3 To evaluate the silage nutritive values and fiber fractions at day 24 of ensiled.

1.4 Research hypothesis

All inoculations of *L. buchneri* are combined with *L. plantarum* in Napier grass added molasses at 5% of fresh forage weight were hypothesized that;

1.4.1 Silage fermentation characteristics of Napier grass silage has inoculated a higher levels of *L. buchneri* combines with a lower levels of *L. plantarum* combine have expected may have been able to affect to the silage qualities, because of may have been a higher in silage pH value, propionic acid, butyric acid, NH_3-N, and may have a lower lactic acid concentration than the inoculations at a higher levels of *L. plantarum* combine with several levels of *L. buchneri*.

14.2 The inoculation at a higher levels of *L. plantarum* combine with the several levels of *L. buchneri* in Napier grass added molasses may have been able to decrease the silage dry matter loss than Napier grass silage inoculated the combination of both species at a lower level of *L. plantarum* combine with the several levels of *L. buchneri*.

14.3 The inoculation at a higher levels *L. buchneri* combine with the several levels of *L. plantarum* into the Napier grass added molasses may have been able to improve the silage aerobic stability after the silage exposes to air than Napier grass has inoculated with the lower levels *L. buchneri* combine with the several levels of *L. plantarum.*

14.4 The inoculation at the highest level of *L. plantarum* combines with the highest level of *L. buchneri* might has been able to improve silage qualities and silage aerobic stability than the inoculation at a lower levels of *L. plantarum* combine with the highest level of *L. buchneri*.

1.5 Scope and limitation of the study

This study was conducted to study the effect of treatment combination of *L. buchneri* and *L. plantarum* inoculated in Napier grass added molasses at 5 % of fresh forage weight. Both of lactic acid bacterial species were used for this study are *L. buchneri* TISTR753 and *L. plantarum* TISTR1284 in which were brought from the Thailand Institute of Scientific and Technological Research (TISTR). Napier grass was cut at Suranaree University of technology's farm. This study has used Napier grass was harvested at around 45 days of regrowth, and was planted, fertilized, and irrigated under the theoretical field manipulation. The among of 500 g of 1 to 2-centimeter length of Napier grass were used for each replication. This study was made the anaerobic condition by vacuum sealing machine to close the plastic bag. And this study had used the Napier grass chemical composition indicates in table 3.1 shows in chapter 3.

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CHAPTER II

LITERATURE REVIEW

2.1 Napier grass

Napier grass (*Pennisetum purpureum*) is favorably planted for Ruminant farming in Tropical area, as its high production per area, high responds to fertilizer and irrigation, and high pest resistant ability (Farrell et al., 2002). Previous studies had observed the maturity stages for cutting affected to Napier grass while regarded to nutritional values, digestibility and also forage yield (Table 2.1). The effect of forage maturity stages for cutting the Napier grass. Consequence to forage dry matter content, water soluble carbohydrate, NDF, ADF had trended to increase as resulted from stages of maturity for cut increased. Conversely, forage crude protein content, forage dry matter digestibility had trended to reduce since the maturity stage for cutting increased. Moreover, the decreasing of maturity in Napier grass for cutting resulted to promote to have a higher production yield per years (especially for intensive irrigation area). And, Napier grass was cut at the earlier maturity stage had resulted to the higher nutritional values than cutting at maturity of Napier grass.

Table 2.1 The results of several maturity stage at cutting of Napier grass on nutritional values, dry matter digestibility.

Crop	days[1]	DM	CP	WSC	NDF	ADF	DMD[3]	Ref.
		%						
Napier grass	60	16.40^{c}	12.10^{a}	-	54.60^{a}	36.00^{b}	63.90^{a}	Tessema et al. (2010)
	90	25.80^{b}	10.80^{b}	-	54.70^{a}	39.80^{a}	62.90^{a}	
	120	31.70^{a}	8.00^{c}	-	54.80^{a}	41.00^{b}	56.90^{b}	
Napier grass	30	13.37^{c}	12.62^{a}	-	66.18^{a}	39.25^{a}	-	Lounglawan et al. (2014)
	45	17.16^{b}	10.13^{b}	-	70.13^{a}	46.99^{b}	-	
	60	18.39^{a}	8.64^{c}	-	76.49^{b}	41.03^{a}	-	
Napier grass	14	14.30	20.40^{a}	8.60^{b}	70.40^{a}	36.00	72.80^{c}	Manyawu et al. (2003)
	28	16.60	14.30^{b}	11.50^{a}	73.50^{b}	36.50	70.50^{b}	
	42	14.90	12.60^{c}	13.60^{a}	75.90^{b}	37.50	69.40^{b}	
	56	18.50	9.20^{d}	12.80^{a}	78.50^{c}	39.80	63.60^{a}	

$^{a, b, c, d}$ Means in the same row with different superscript differed (P<0.05).

[1] Day = Regrowth interval, [2]WSC = water-soluble carbohydrate.

[3]DMD = forage dry matter digestibility.

2.2 The factors influence to silage fermentation quality

As descripted in previous section, the earlier maturity stages have higher nutritional values, digestibility, and higher production per years than the maturity stage of Napier grass, thus the cutting at the earlier maturity stage had been more the suitable and higher valuable outcome than maturity stage to feed to Ruminant animal (Lee et al., 1991). However, Napier grass was cut at the earlier maturity stage resulted to high moisture content. Consequence, unsuitable for ensiling process. The previous

study showed in figure 2.1 demonstrated that the forage has higher moisture content is needed more lower silage pH value to inhibiting the fermentation of *Clostridium* spp. and Enterobacterium than the forage has lower moisture content (Muck, 2010). Which, these microorganisms have affected to silage quality since have ability to producing butyric acid, forage protein deterioration and compete to using the substrate such WSC instead fermented by lactic acid bacteria. Consequently, the forage has higher moisture content resulted to higher butyric acid, ammonia nitrogen, pH value, and lower in silage lactic acid concentration. In which confirmed from the study of Santos et al. (2011) was studied the different in maturity of signalgrass *Brachiaria decumbens* (cv. Basiliski) was cut at different regrowth interval (30, 40, 50, 60, 70 day) on silage fermentation characteristics were showed that the earlier cut silage has higher pH value than late cut silage (figure 2.1). Hence, the earlier cut silage has higher the concentration of acetic acid, butyric acid than late cut silage, as resulted from lower in concentration of lactic acid.

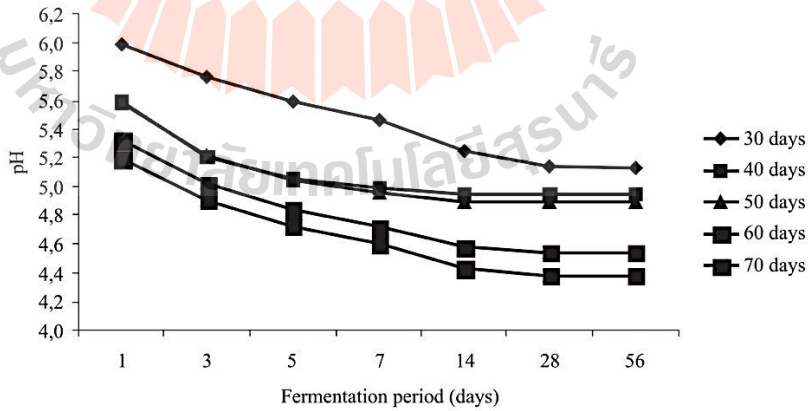

Figure 2.1 The silage pH value in each different of regrowth cutting interval of signalgrass *Brachiaria decumbens* (cv. Basiliski) adapted from Santos et al. (2011).

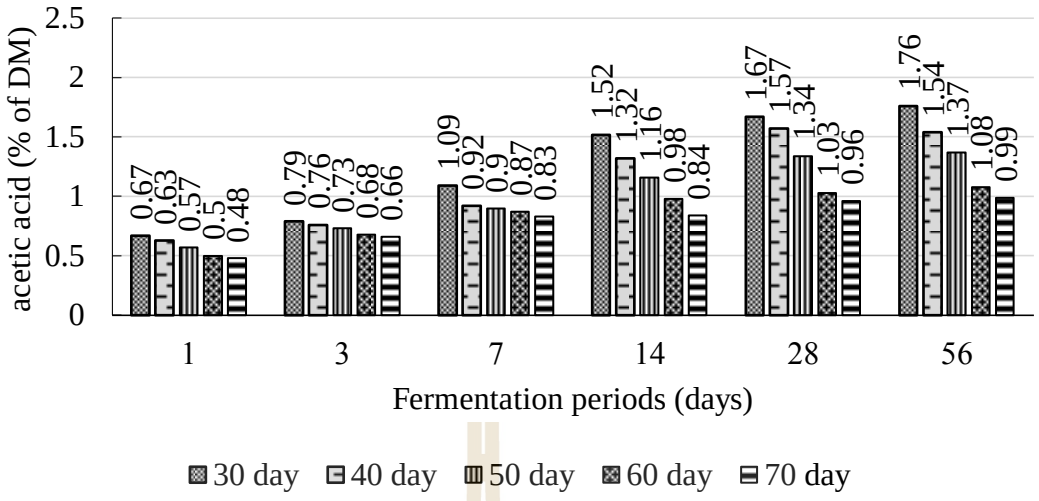

Figure 2.2 The concentration of acetic acid in each different of regrowth cutting interval of signalgrass *Brachiaria decumbens* (cv. Basiliski) adapted from Santos et al. (2011).

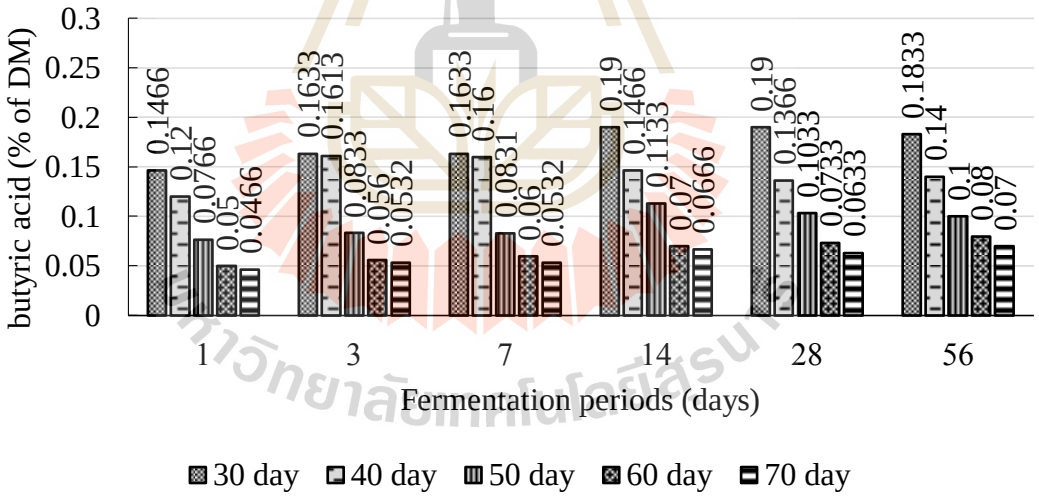

Figure 2.3 The concentration of butyric acid in each different of regrowth cutting interval of signalgrass *Brachiaria decumbens* (cv. Basiliski) adapted from Santos et al. (2011).

2.3 Silage additive

Normally, the ensiling process doesn't need the silage additive to improve or to solve the fermentation qualities since the appropriate of the natural fermentation as results from the suitable of forage conditions before ensiling (described above). Thus, the unsuitable of forage conditions before ensiling is need the silage additive to solve the silage quality. As described above, the well ensiling needs a lower pH to inhibit an undesirable of anaerobic microorganism such a *Clostridium*, *Enterobacteria*. The silage that have a lower pH caused from the high concentration of lactic acid. Lactic acid almost is produced by lactic acid bacteria. Lactic acid bacteria have been able to convert only simple sugar (water-soluble carbohydrate, WSC) as for their energy, then has released a lactic acid as their byproduct. Thus, the abundant of forage WSC might has been able to get the high quality as reasons that it enough of WSC to serve to the fermentation process of lactic acid bacteria then release a lot of lactic acid, finally the silage has a low pH value resulted to inhibit the unsuitable of anaerobic microorganism. In previous research had shown the use of lactic acid bacteria as inoculants, formic acid addition had been able to solve the silage quality as to promote the silage acidic condition of the forage had the unsuitable condition before ensiling. The studies of Santos et al. (2014) and Fukagawa et al. (2016) had shown the use of silage additive such an lactic acid bacteria had been able to solve the silage quality. The table 2.2 shows the pH values of each had indicated that the treatment used of silage additive had lower pH than non-used of silage additive as resulted from the higher concentration of lactic acid. Thus, the use of lactic acid bacteria as inoculates as silage additive has a higher concentration of lactic acid (table 2.2). However, the results had shown the use of lactic acid bacteria as inoculants had not been able to

inhibit the fermentation of undesirable anaerobic microorganism. Table 2.2 shows the concentration of butyric acid concentration of each treatment indicated that the use of lactic acid bacteria had non-statistically significant difference in butyric acid concentration between the treatment had used and non-used of the lactic acid bacteria, although the use of lactic acid bacteria as inoculants had solved the silage acidic conditions than without lactic acid bacteria inoculation but it has not enough for inhibiting the fermentation of *Clostridium*, and *Enterobacteria*. The study of Mayne (1990) had studied about the usage of varies silage additive such as formic acid, lactic acid bacteria, and absorbent polymer into forage before ensiling had shown the use of formic acid had the lowest concentration of butyric acid and NH_3-N than the other treatment as indicated the reasons that, the use of formic acid had a rapid production of lactic acid, and a quicker decline in pH while observed with control and inoculants treated silage (Mayne, 1990). For this reason, the silage that had a quicker acidic condition results to has been able to inhibit the *Clostridium*, and *Enterobacteria* fermentations. Thus, the silage additive is used to promote a quicker of silage acidic condition. The inoculants treated silage had recognized that have ability to produce a rapid of lactic acid and quicker acidic condition if enough of WSC, as serve to lactic acid bacteria has inoculated into the forage before ensiling.

Table 2.2 The influence of lactic acid bacteria inoculation and forage dry matter content on the silage fermentation quality.

Crops	%DM, Treatments[1]	pH	NH3-N (% of Total N)	References
Guinea grass	19.75, WT	5.15[a]	9.59[a]	(Santos et al., 2014)
	19.75, W	5.04[b]	8.78[b]	
	19.91, WT	5.09[a]	8.91[a]	
	19.91, W	4.96[b]	8.38[b]	
	20.50, WT	4.84[a]	8.22[a]	
	20.50, W	4.83[b]	7.83[b]	
	25.10, WT	4.63[a]	6.71[a]	
	25.10, W	4.44[b]	6.09[a]	
Perennial grass	15.40, Control	4.14[ab]	10.20[b]	(Mayne, 1990)
	15.90, FA	3.94[b]	6.60[d]	
	15.70, In	4.12[ab]	8.20[c]	
	16.00, In+Abs	4.21[a]	11.10[a]	
Dwarf Napier grass	13.5%, LAB+AC	3.59[c]	7.19[b]	(Fukagawa et al., 2016)
	13.5%, FJLB	3.73[b]	16.90[a]	
	13.5%, Control	4.46[a]	15.40[ab]	

[1]WT = without inoculant; W, In = with inoculant; FA = Formic acid; In+Abs = inoculant + absorbent polymer (ammonium polyacrylamide); LAB+AC = lactic acid bacteria + *Acremonium* cellulase; FJLB = fermented juice lactic acid bacteria.

Table 2.2 (Continue).

Crops	%DM, Treatments[1]	Lactic acid	Acetic acid	Butyric acid	References
		(% of DM)			
Guinea grass	19.75, WT	2.54[b]	1.34[a]	0.070[a]	Santo et al., (2014)
	19.75, W	3.04[a]	1.15[b]	0.056[b]	
	19.91, WT	3.15[b]	1.16[a]	0.050[a]	
	19.91, W	3.61[a]	0.77[b]	0.043[b]	
	20.50, WT	3.49[b]	0.92[a]	0.040[a]	
	20.50, W	4.45[a]	0.68[b]	0.040[a]	
	25.10, WT	4.27[a]	0.89[a]	0.043[a]	
	25.10, W	4.56[a]	0.68[b]	0.040[a]	
Perennial grass	15.40, Control	17.60[a]	3.75[a]	0.22[c]	Mayne (1990)
	15.90, FA	12.60[c]	2.58[d]	0.06[d]	
	15.70, In	15.80[bc]	2.85[c]	0.40[b]	
	16.00, In+Abs	15.30[b]	3.08[b]	0.44[a]	
Dwarf Napier grass	13.5%, LAB+AC	2.40[a]	0.245[b]	-	Fukagawa et al., (2016)
	13.5%, FJLB	2.16[a]	0.323[b]	-	
	13.5%, Control	0.69[b]	0.638[a]	-	

[1]WT = without inoculant; W, In = with inoculant; FA = Formic acid; In+Abs = inoculant + absorbent polymer (ammonium polyacrylamide); LAB+AC = lactic acid bacteria + *Acremonium* cellulase; FJLB = fermented juice lactic acid bacteria.

Table 2.3 Effects of molasses addition and with or without inoculation of lactic acid bacteria on pH values and NH_3-N of Napier grass silage, adapted from Yunus et al. (2000)

Crop	**Treatments**[1]	**pH**	**NH_3-N**[2]
Napier grass	0% molasses - LAB	5.44	19.04
	0 % molasses + LAB	4.82	17.49
	2 % molasses - LAB	4.18	4.26
	2 % molasses + LAB	4.21	4.52
	5 % molasses - LAB	3.59	1.98
	5 % molasses + LAB	3.62	4.57
Statistical significance		---------- *P* – values ----------	
Molasses level		< 0.01	< 0.05
LAB addition		NS	< 0.05
Molasses × LAB addition		NS	< 0.01

[1] % molasses on fresh weight with/ without LAB inoculation (2×10^4 cfu of *Lactobacillus plantarum* on g fresh weight); [2] (% of Total N in %DM)

Table 2.4 Effects of molasses addition and with/ without inoculation of lactic acid bacteria on %CP and lactic acid of Napier grass silage, adapted from Yunus et al. (2000).

Crop	**Treatments**[1]	**% Crude Protein**	**Lactic acid**[2]
Napier grass	0% molasses - LAB	13.56	18.1
	0% molasses + LAB	16.56	39.4
	2% molasses - LAB	16.81	127.8
	2% molasses + LAB	13.68	141.3
	5% molasses - LAB	12.25	138.2
	5% molasses + LAB	13.00	160.4
Statistical significance		---------- *P* – values ----------	
Molasses level		< 0.01	< 0.01
LAB addition		NS	< 0.01
Molasses × LAB addition		NS	NS

[1] % molasses on fresh weight with/ without LAB inoculation (2×10^4 cfu of *Lactobacillus plantarum* on g fresh weight); [2] g/kg DM

Table 2.5 Effects of molasses addition and with or without inoculation of lactic acid bacteria on acetic acid and butyric acid of Napier grass silage, adapted from Yunus et al. (2000).

Crop	Treatments[1]	Acetic acid[2]	Butyric acid[2]
Napier grass	0% molasses - LAB	4.50	0.00
	0% molasses + LAB	3.60	0.00
	2% molasses - LAB	2.30	0.20
	2% molasses + LAB	1.80	0.30
	5% molasses - LAB	1.30	0.10
	5% molasses + LAB	1.20	0.20
Statistical significance		---------- *P* – values ----------	
Molasses level		< 0.01	NS
LAB addition		NS	NS
Molasses × LAB addition		NS	NS

[1] % molasses on fresh weight with/ without LAB inoculation (2×10^4 cfu of *Lactobacillus plantarum* on g fresh weight); [2] g/kg DM

2.4 Aerobic stability

As recall from previous described above, the inoculation of lactic acid bacteria had been able to improve the silage fermentation quality even the forage WSC had enough for lactic acid bacterial fermentation. The addition of molasses had promoted the rapid of lactic acid production and decline pH quicker since molasses is able to increase the simple sugar to serve for lactic acid bacterial fermentation. However, there had reported about after open the silo or silage bunker had resulted to silage

spoilage. Because of the secondary fermentation by yeast and mold. Also, the high quality of silage that had a lower VFAs and had a higher of lactic acid as resulted easily to spoilage from yeast and mold. Yeast and Mold had been able to ferment the residual molasses from ensilage. Also, lactate assimilating yeast had been able to ferment the lactic acid to use as their energy caused to increase the pH after silage exposed to air. The increasing of pH then had followed by yeast and mold proliferation (Wilkinson and Davies, 2013). As well known that mold can produce the aflatoxin. So, the silage was contaminated with aflatoxin from mold. Moreover, the silage had contaminated the aflatoxin was fed to the animals (Scudamore and Livesey, 1998). It had been able to directly negative effect to animal health and animal production. And there have indirect negative effect to animal products also, because the product had contaminated with aflatoxin. So, finally can be strongly effects to the human food security.

2.5 The inoculation of Heterofermentative lactic acid bacteria combines with Homofermentative lactic acid bacteria on silage fermentation quality, dry matter loss and aerobic stability

As previous described above, the addition of molasses had been able to improve the silage fermentation quality. On the other hand, the residual molasses from ensiling caused to silage spoilage was occurred by yeast and mold proliferations. Acetic acid has recognized as have ability to against the aerobic microorganism (Moon, 1983). However, the good quality of silage is need to lower in VFAs and higher in lactic acid. Thus, the good quality of silage has a lower aerobic stability than the poor quality of silage (Weinberg et al., 1993). Table 2.8 shown the inoculation of Homofermentative lactic acid bacteria group such a *Lactobacillus plantarum*,

Pediococcus pentosaceus resulted to spoil from yeast, and mold after silage exposed to air than the control groups. The inoculation of Homofermentative lactic acid bacteria had recognized that have ability to the rapid lactic acid production then resulted to had a lower the pH values than untreated group (Oliveira et al., 2017). Consequently, the inoculation of Homofermentative lactic acid bacteria had a higher the silage fermentation quality than untreated group since it has a lower of VFAs thus it easy to spoil from yeast and mold. In previous studies had inoculated the *Lactobacillus buchneri*, the results shown its ability had been able to solve the silage was spoiled from aerobic microorganism after exposed to air. *L. buchneri* had been able to convert the 1 mol. of lactic acid to 0.5 mol. of acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). Table 2.7 shows the concentration of acetic acid of each study, the results of the treatment was treated with *L. buchneri* had the highest of acetic acid concentration, also had the lowest of yeast and mold than the control groups and the treatment was treated with Homofermentative lactic acid bacteria. However, from previous described about the ability of *L. buchneri* that had been able to convert lactic acid to other was confirmed in the table 2.7, the treatments treated with *L. buchneri* had a lower of lactic acid than other treatment resulted to had a higher of pH values. Finally, the inoculation of *L. buchneri* had not been able to inhibit the proteolytic microorganism since a higher in pH value.

Table 2.6 Inoculation effects of Homo- combined with/ without Heterolactic acid bacteria in several forage crops on silage pH, and residuals WSC.

Crops	Treatments	pH values	retained WSC (g/kg DM)	References
Maize (1.5 L) (90 day)	Control	3.72[b]	31.50[a]	Filya (2003a)
	LB[1]	4.13[a]	6.40[b]	
	LP[2]	3.64[b]	25.40[a]	
	LB+LP[3]	3.80[b]	10.30[b]	
Maize (1.5 L) (60 day)	Control	3.60 ± 0[b]	29.00 ± 2.0[a]	Filya (2003b)[16]
	LB[4]	3.90 ± 0[a]	8.00 ± 1.0[b]	
	LP[5]	3.60 ± 0[b]	26.00 ± 2.0[a]	
	LB+LP[6]	3.70 ± 0[b]	11.00 ± 1.0[b]	
Perennial ryegrass (1 L) (90 day)	Control	4.19[c]	42.00[b, c]	Driehuis et al. (2001)
	LB[7]	4.40[a]	15.00[d]	
	LB[8]	4.31[b]	25.00[c, d]	
	PL[9]	4.06[d]	124.00[a]	
	LB+PL[10]	3.95[f]	59.00[b]	
	LB+PL[11]	4.02[e]	59.00[b]	
Maize (1.5 L) (90 day)	Control	3.60 ± 0.0[b]	18.00 ± 5.0	Weinberg et al. (2002)[16]
	PL[12]	3.60 ± 0.0[c]	14.00 ± 2.0	
	LB[13]	3.90 ± 0.1[a]	11.00 ± 1.0	
	LP+LB[14]	3.90 ± 0.1[b]	13.00 ± 3.0	
Maize (20 L) (120 day)	Untreated	3.61[b]	10.90[a]	Schmidt and Kung (2010)
	LB[15]	3.69[a]	7.90[b]	
	LBPP[16]	3.68[a]	7.60[b]	

[1,4]LB = *L. buchneri* (1×10^6 cfu/g of fresh forage); [2,5]LP = *L. plantarum* (1×10^6 cfu/g); [3,6]LB+LP = LB (1×10^6 cfu/g) + LP (1×10^6 cfu/g); [7]LB = *L. buchneri* (1×10^5 cfu/g); [8]LB = *L. buchneri* (3×10^5 cfu/g); [9]PL = mixture of (1×10^5 cfu/g) *P. pentosaceus* and *L. Plantarum*; [10]LB+PL = LB (*L. buchneri* 1×10^5 cfu/g)+[9]PL; [11]LB+PL = LB (*L. buchneri* 3×10^5 cfu/g)+[9]PL; [12]LP = *L. plantarum* (0.5×10^6 cfu/g of forage); [13]LB = (0.5×10^6 cfu/g of forage); [14]LP+LB = LP (0.5×10^6 cfu/g of forage)+LB (0.5×10^6 cfu/g of forage); [15]LB = *L. buchneri* 40788 (4×10^5 cfu/g of forage); [16]LBPP = [15]LB+ *Pediococcus pentosaceus* (1×10^5 cfu/g of forage),[16]mean ± SE

Table 2.7 Inoculation effects of Homo- combined with/ without Heterolactic acid bacteria in several forage crops on silage lactic acid, acetic acid, NH_3-N.

Crops	Treatments	Lactic acid (g/kg DM)	Acetic acid (g/kg DM)	NH_3-N (% of total N)	References
Maize (1.5 L) (90 day)	Control	40.40^{c}	12.70^{b}	2.62^{b}	Filya (2003a)
	LB[1]	27.60^{d}	38.90^{a}	2.85^{a}	
	LP[2]	79.40^{a}	3.30^{c}	2.11^{c}	
	LB+LP[3]	55.50^{b}	31.70^{a}	2.20^{c}	
Maize (1.5 L) (60 day)	Control	37.00±3.0^{b}	11.00±1.0^{b}	0.11±0.007^{a}	Filya (2003b)[16]
	LB[4]	24.00±1.0^{c}	21.00±1.0^{a}	0.110±0.006^{a}	
	LP[5]	51.00±4.0^{a}	3.00±1.0^{c}	0.081±0.003^{b}	
	LB+LP[6]	32.00±2.0^{bc}	20.00±2.0^{a}	0.087±0.003^{b}	
Perennial ryegrass (1 L) (90 day)	Control	74.50^{d}	31.70^{c}	0.10^{a}	Driehuis et al. (2001)
	LB[7]	41.40^{f}	51.00^{a}	0.097^{a}	
	LB[8]	48.40^{e}	38.40^{b}	0.086^{b}	
	PL[9]	97.50^{b}	9.30^{e}	0.085^{b}	
	LB+PL[10]	100.00^{a}	22.30^{d}	0.079^{c}	
	LB+PL[11]	89.00^{c}	21.90^{d}	0.083^{b}	
Maize (1.5 L) (90 day)	Control	43.00±3.0^{a}	10.00±1.0^{bc}	-	Weinberg et al. (2002)[16]
	PL[12]	33.00±1.0^{ab}	9.00±1.0^{c}	-	
	LB[13]	21.00±6.0^{bc}	17.00±1.0^{ab}	-	
	LP+LB[14]	19.00±5.0^{c}	22.00±4.0^{a}	-	
Maize (20L silo) (120 day)	Untreated	57.30^{a}	12.30^{b}	0.094	Schmidt and Kung (2010)
	LB[15]	53.20^{b}	19.50^{a}	0.096	
	LBPP[16]	54.40^{ab}	18.00^{a}	0.098	

[1,4]LB = *L. buchneri* (1×10^6 cfu/g of fresh forage); [2,5]LP = *L. plantarum* (1×10^6 cfu/g); [3,6]LB+LP = LB (1×10^6 cfu/g) + LP (1×10^6 cfu/g); [7]LB = *L. buchneri* (1×10^5 cfu/g); [8]LB = *L. buchneri* (3×10^5 cfu/g); [9]PL = mixture of (1×10^5 cfu/g) *P. pentosaceus* and *L. Plantarum*; [10]LB+PL = LB (*L. buchneri* 1×10^5 cfu/g)+[9]PL; [11]LB+PL = LB (*L. buchneri* 3×10^5 cfu/g)+[9]PL; [12]LP = *L. plantarum* (0.5×10^6 cfu/g of forage); [13]LB = (0.5×10^6 cfu/g of forage); [14]LP+LB = LP (0.5×10^6 cfu/g of forage)+LB (0.5×10^6 cfu/g of forage); [15]LB = *L. buchneri* 40788 (4×10^5 cfu/g of forage); [16]LBPP = [15]LB+ *Pediococcus pentosaceus* (1×10^5 cfu/g of forage), [16]mean ± SE

Moreover, the use of *L. buchneri* had a rapid of silage dry matter loss. The sugar metabolism of *L. buchneri* have not been able to ferment the simple sugar via glycolytic pathway as since *L. buchneri* have not aldolase enzyme. Thus, the simple sugar fermentation of *L. buchneri* had used the phosphogluconate/phosphoketolase pathway instead of glycolytic pathway. The end product of phosphogluconate/phosphoketolase pathway from the simple sugar fermentation are CO_2, lactic acid, acetic acid, or ethanol. For this reason, *L. buchneri* had grouped into Obligate heterofermentative lactic acid bacteria (Salvetti et al., 2012). The inoculation of *L. buchneri* caused to the silage dry matter loss in form of CO_2 than other treatments are shown in table 2.8. However, the use of *L. buchneri* combines with *Lactobacillus plantarum* had been able to decrease the silage dry matter loss. Since, the inoculation of *L. plantarum* had recognized that it is be able to produce a rapid of lactic acid has accorded is described by. Consequently, a silage had a quicker reducing in pH values. Therefore, the use of *L. plantarum* had ability to quick inhibit the other anaerobic microorganism such another *lactobacillus* spp., proteolytic microorganism such a *Clostridium*, other pathogen such an Enterobacteria. Thus, the inoculation of *L. buchneri* combine with *L. plantarum* had been able to solve the silage dry matter loss problem since the rapid lactic acid production ability of *L. plantarum* has affected to inhibit the sugar metabolism of *L. buchneri* is described by Driehuis et al. (2001). However, as described above, the ability of *L. buchneri* had been able to covert 2 mol. of lactic acid in to a 1 mol. of acetic acid, and 1 mol. of 1,2 propanediol. Thus, the combination of both had reduced the yeast, mold and had improved the silage aerobic stability as consistence with the only inoculation of *L. buchneri* (Table 2.8).

Table 2.8 Inoculation effects of Homo- combined with/ without Heterolactic acid bacteria in several forage crops on silage dry matter loss, Yeast, Mold.

Crops	Treatments	DM loss (%)[20]	Yeasts[17] (log cfu /g DM)	Mold[18] (log cfu /g DM)	References
Maize (1.5 L jar) (90 day)	Control	1.65[b]	3.86	3.26	Filya (2003a)
	LB[1]	3.26[a]	< 2.00	< 2.00	
	LP[2]	0.75[c]	4.45	3.08	
	LB+LP[3]	1.14[b, c]	< 2.00	< 2.00	
Maize (1.5 L jar) (60 day)	Control	0.80 ± 0.1[c]	6.5	3.3	Filya (2003b)[19]
	LB[4]	2.50 ± 0.1[a]	< 2.00	< 2.00	
	LP[5]	0.80 ± 0.0[c]	7.7	3.8	
	LB+LP[6]	1.60 ± 0.2[b]	2.00	< 2.00	
Perennial ryegrass (1 L jar) (90 day)	Control	3.20[c]	4.50	3.80	Driehuis et al. (2001)
	LB[7]	4.78[a]	< 2.00	2.20	
	LB[8]	3.91[b]	< 2.00	< 2.00	
	PL[9]	1.51[e]	5.60	2.90	
	LB+PL[10]	2.25[d]	2.40	3.40	
	LB+PL[11]	2.27[d]	< 2.00	< 2.00	
Maize (1.5 L jar) (90 day)	Control	0.40 ± 0.0[b]	3.10	3.00	Weinberg et al. (2002)[19]
	PL[12]	0.30 ± 0.0[b]	3.30	< 2.00	
	LB[13]	0.30 ± 0.1[a]	< 2.00	2.20	
	LP+LB[14]	0.30 ± 0.2[a]	< 2.00	3.20	
Maize (20 L silo) (120 day)	Untreated	3.15	-	-	Schmidt and Kung (2010)
	LB[15]	3.26	-	-	
	LBPP[16]	2.83	-	-	

[1,4]LB = *L. buchneri* (1×10^6 cfu/g of fresh forage); [2,5]LP = *L. plantarum* (1×10^6 cfu/g); [3,6]LB+LP = LB (1×10^6 cfu/g) + LP (1×10^6 cfu/g); [7]LB = *L. buchneri* (1×10^5 cfu/g); [8]LB = *L. buchneri* (3×10^5 cfu/g); [9]PL = mixture of (1×10^5 cfu/g) *P. pentosaceus* and *L. Plantarum*; [10]LB+PL = LB (*L. buchneri* 1×10^5 cfu/g)+[9]PL; [11]LB+PL = LB (*L. buchneri* 3×10^5 cfu/g)+[9]PL; [12]LP = *L. plantarum* (0.5×10^6 cfu/g of forage); [13]LB = (0.5×10^6 cfu/g of forage); [14]LP+LB = LP (0.5×10^6 cfu/g of forage)+LB (0.5×10^6 cfu/g of forage); [15]LB = *L.*

buchneri 40788 (4×10^5 cfu/g of forage); [16]LBPP = [15]LB+ *Pediococcus pentosaceus* (1×10^5 cfu/g of forage). [17,18]Microbiological analysis was performed on single sample. Therefore, no statistical analyses are available, [19]mean ± SE, [20]DM loss was analyzed at open the silo

2.6 References

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CHAPTER III

MATERIALS AND METHODS

3.1 Experimental design

The study was designed to the 3 × 3 factorials experiment in completely randomize design (CRD), with 2 factors (*Lactobacillus plantarum* (LP), and *Lactobacillus buchneri* (LB), and each factor had 3 levels of colonies forming unit (cfu)/ g of fresh forage weight (g FW) (0, 1×10^5, 1×10^6 cfu/g FW). The treatment combinations were concluded in Table 3.1

Table 3.1 Experimental treatment combinations of both lactic acid bacterial species.

Lactobacillus buchneri	***Lactobacillus plantarum* (cfu/g fresh forage weight)**		
(cfu/g fresh forage weight)	**0 (LP0)**	**1×10^5 (LP5)**	**1×10^6 (LP6)**
0 (LB0)	LP0LB0	LP5LB0	LP6LB0
1×10^5 (LB5)	LP0LB5	LP5LB5	LP6LB5
1×10^6 (LB6)	LP0LB6	LP5LB6	LP6LB6

3.2 Forage and ensiling process

Napier grass was harvested in December, 2018 at approximately 25% of Dry matter content, at Farm of Suranaree University of Technology. Napier grass was chopped to a theoretical cut length of 1.5 cm, then was added molasses at 5% of fresh forage weight. Each of 500 g of fresh forage weight added molasses was treated the inoculant according to

the 3 × 3 factorials experimental design was described above. The inoculants of each treatment were prepared by dissolving in deionized water, and were mixed uniformly accorded to the treatment combination into the chopped forage. All treatment combinations had made to 4 replications, and each of replication was weighted to 500 g of fresh forage weight, and was sealed into the polyethylene bag, and stored at 25°C. The silage was opened at day 24 of ensiled for analyzed the silage fermentation qualities, dry matter loss, aerobic stability characteristics, and microbial profiles had further descripted below.

Table 3.2 The nutrient composition and microbial profiles of Napier grass added/ non-added molasses at 5% of fresh forage weight.

Constituent	**Napier grass added molasses**[1]	**Napier grass**
DM (% fresh weight)	22.17	26.24
CP (% DM)	8.26	8.44
EE (% DM)	3.53	2.75
Ash (% DM)	10.79	9.47
NDF (% DM)	55.24	70.01
ADF (% DM)	30.14	39.31
ADL (% DM)	8.53	10.34
WSC (g WSC/ kg DM)	164.7	62.92
Buffering capacity (meq NaOH/ kg DM)	86.50	90.00
Lactic acid bacteria (log10 cfu/ kg fresh weight)	9.36	9.88
Enterobacteria (log10 cfu/ kg fresh weight)	9.40	9.79
Clostridium spp. (log10 cfu/ kg fresh weight)	7.52	8.02

[1]Napier grass was added molasses at 5% of forage weight.

3.3 Chemical analysis

The dry matter content of each sample had used the freeze dry method. Then, the percentage of dry matter loss (%DM) was calculated by subtracting of the difference in % DM content before and after ensiling. Then, dried samples were grounded by laboratory blender into 1 mm. particle length.

Water soluble carbohydrate determination had performed the sugar extraction before accorded to method of Chow and Simon (2004) then sugar content in sugar extracted was determined by the procedure of Dubosis et al. (1956).

The nutrient compositions had analyzed crude protein, ether extract, and ash by used the standard procedure accorded to AOAC (1995), The determinations of fiber fractions had used the detergent fiber analysis for analyzed NDF, ADF, and ADL accorded to Van Soest et al. (1991).

The 10x dilutions of each sample had extracted to determine forage buffering capacity accorded to Playne and McDonald (1966), NH_3-N, pH values, lactic acid, VFAs. Thoroughly mixed of sample, 50 g. had sampled then was made to the 10x dilution by addition of 450 ml. of deionized water into 50 g. of fresh sample, then mixed thoroughly by laboratory blender.

The concentration of lactic acid and VFAs in silage samples were determined by used GC (Agilent 7890B GC) and had used the same GC column (Agilent CP-Sil 5 CB, 0.32 mm x 25 m fused silica) for both analyses, but had differenced in GC condition, were implied in Agilent application note for lactic acid, and VFAs C_2-C_7 analysis. NH_3-N was determined by adapted from Weatherburn (1967).

3.4 Aerobic stability characteristic determinations

The silage sample was sampled from days 24 of ensiled for silage aerobic stability characteristics determination, adapted from (Ashbell et al., 1987; Ashbell et al., 1991). The silage was exposed to air through 2, 4, 6 days. Then, the silage exposed to air was samples to made 10x dilution (described above) to determine the pH values, aerobic microbial profiles such yeast, lactate assimilating yeast, and mold. The residual WSC, as use to WSC loss determination, by subtracting the WSC at day 24 of ensiled with silage WSC at 2, 4, 6 days exposed to air.

3.5 Microbial enumerations

Each of 10x dilution of each sample was made to serial dilution technique by 10^{-1} to 10^{-10} dilutions. Then, 0.1 ml. of each serial dilution was pipetted to each of culture media (described below), then had used pour plate technique to mix thoroughly.

The culture media for *Lactobacillus* spp. enumerations. This study had used Lactobacillus MRS agar. MRS Agar preparation had suspended 67.17 g. in 1000 ml. distilled water. Then, it was boiled to dissolving the medium to complete. Sterilized by autoclaving at 121°C. Then, waiting for agar to cool at 60°C, add cycloheximide 0.4 g/1000 ml. agar. Mix thoroughly.

Reinforced Clostridium Medium Base (RCM) was used to *Clostridium* spp. enumeration. Suspended 30.50 g. of RCM in 1000 ml. of distilled water. Heat to boiling to dissolve the medium completely then sterilized by autoclaving at 121°C. Then, waiting for agar cool at 60°C, added 200 mg. D-Cycloserine in 20 ml.

phosphate buffer/1000 ml. agar, then added methyl red 50 mg/1000 ml. agar. Mix thoroughly.

Violet Red Bile Glucose Agar was used for Enterobacterium species enumeration. Suspend 38.53 g. in 1000 ml. distilled water. Heat to boiling to dissolve the medium completely but do not autoclave.

Yeast, and Mold determination in silage exposed to air was used Malt extract agar. The pH agar was adjusted to 3.5 by the addition of 50 ml/l of 10% lactic acid. And, 30 mg/l of penicillin G, 30 mg/l of streptomycin sulfate was added into Malt extract agar as use for antibiotic. And, the enumeration was observed the different in colonies characteristic of Yeast, and Mold.

Lactate-assimilating yeast was determined by Yeast Nitrogen base agar, the agar contained 2% agar, and added 5% of lactic acid as purpose to the sole source of Carbon for Lactate-assimilating yeast. And, antibiotic was used as same as malt extract agar.

The colonies plate count had enumerated only the plate had shown the colonies more than 30 colonies and less than 300 colonies. The results were expressed to $\log_{10}$ cfu/g fresh weight.

3.6 Statistical Analysis

The raw data had adjusted to ANOVA assumptions by used SPSS versions 23 (IBM Corp, 2016). Standardized residual determination was used for Normality testing by Shapiro-Wilk (P>0.05), and Kurtosis, Skewness statistic of standardized residual were ranged in -0.5 to 0.5. Then, if the standardized residual data had failed to ANOVA assumptions, then the raw data needed to perform data transformation.

All variables adjusted data (excepted the microbial profiles) had analyzed the statistically significant difference to indicate, did the results have affected from the LP, or LB main effect or have affected from the LP × LB interaction (P<0.05), by used the analysis of variance (ANOVA) in SPSS versions 23 (IBM Corp, 2016). Turkey's HSD had used to analyze the treatment mean analysis for the main effect of both, and the interaction effect (P<0.05). However, microbial profiles had not determined the statistically significant difference because of their had 1 replicated of each treatment.

3.8 References

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CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Napier grass silage fermentation qualities at 24 days of ensiling

In this study investigated whether inoculation of both lactic acid bacterial species at different levels will do have affected to fermentation quality. The results shown that the combinations of both lactic acid bacterial species, especially the inoculation at the highest level of *L. buchneri* had affected to the silage fermentation quality.

The substantial of silage acetic acid content at day 24 of ensiling was found in the inoculation of LB6LP0, LB6LP5, and LB6LP6 as the result arose from the main effect of *L. buchneri* (shows in table 4.1). Thus, the silage acetic acid concentration at day 24 of ensiling was not depended on the inoculation of *L. plantarum* since *L. buchneri* has been able to anaerobically degrade lactic acid to acetic acid (Oude Elferink et al., 2001). Thus, the inoculation of *L. buchneri* at the highest level resulted to degrade more lactic acid to acetic acid under an anaerobic condition than a lower inoculation of *L. buchneri*. As for this reason, the highest acetic acid content found in LB6LP5 resulted to there was the lowest of lactic acid lactic acid content. The result was consistence with the silage review of Kung et al. (2018) had indicated the inoculation of *L. buchneri* resulted to there was a higher acetic acid content and a lower lactic acid content.

Table 4.1 The results of treatment combinations on acetic acid, propionic acid, and butyric acid (mean ± SE) in Napier grass silage at 24 days of ensiling.

Treatment[1]		Acetic acid	Propionic acid	Butyric acid
		(g /kg DM)		
LB0	LP0	21.06 ± 0.27	5.57 ± 1.27	10.36 ± 0.27
	LP5	24.66 ± 3.30	6.82 ± 0.13	10.36 ± 3.30
	LP6	22.58 ± 0.04	8.49 ± 1.54	10.65 ± 0.04
LB5	LP0	24.29 ± 1.79	4.95 ± 0.16	10.13 ± 1.79
	LP5	21.40 ± 2.17	6.99 ± 0.21	11.08 ± 2.17
	LP6	21.51 ± 1.40	12.33 ± 4.02	10.60 ± 1.40
LB6	LP0	32.94 ± 0.28	5.86 ± 0.16	10.11 ± 0.28
	LP5	46.43 ± 7.96	9.91 ± 1.70	13.39 ± 7.80
	LP6	41.05 ± 1.28	13.85 ± 0.72	13.23 ± 1.28
LB main effect				
LB0		22.77 ± 1.08^{q}	6.96 ± 0.74	10.45 ± 0.24^{q}
LB5		22.47 ± 1.08^{q}	7.88 ± 1.63	10.61 ± 0.20^{q}
LB6		40.14 ± 3.24^{p}	9.87 ± 1.54	12.24 ± 0.73^{p}
LP main effect				
LP0		26.10 ± 2.29	5.46 ± 0.37^{q}	10.20 ± 0.29^{q}
LP5		30.83 ± 5.47	7.91 ± 0.77^{pq}	11.61 ± 0.60^{p}
LP6		28.45 ± 4.04	11.35 ± 1.49^{p}	11.50 ± 0.57^{p}
***P*- values**				
LB		< 0.01	NS	< 0.01
LP		NS	< 0.01	< 0.01
LP × LB		NS	NS	NS

$^{a, b, c, d; p, q, r}$ statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 are the concentration of *L. plantarum* at 0, 1 × 10^{5}, and 1 × 10^{6} cfu/ g fresh weight, respectively. LB0, LB5, and LB6 are the concentration of *L. buchneri* at 0, 1 × 10^{5}, and 1 × 10^{6} cfu/ g fresh weight, respectively.

The reason for the inoculation of *L. plantarum* as it was grouped into the Homofermentative lactic acid bacteria group (Salvetti et al., 2012). The inoculation of Homofermentative lactic acid bacteria group has been accepted to rapid and tremendous lactic acid production (Muck and Kung, 1997; Kung, 1998; Oliveira et al., 2017). So, the higher inoculation of *L. plantarum* had been able to quicker decline silage pH resulted to the metabolism of epiphytic lactic acid bacteria including with *L. plantarum*, and the other anaerobic microorganism were inhibited by the silage acidic condition faster than non-inoculated *L. plantarum*. Thus, the inoculation of *L. plantarum* was expected to solve the problem of a lower lactic acid content caused from an anaerobically lactate degradation to acetic acid by *L. buchneri.*

However, the results of this study found the inoculation of *L. plantarum* had not been able to solve this problem. As was supported by the results, as the increasing of the level of *L. plantarum* had not increased lactic acid content. Moreover, the higher inoculation of *L. plantarum* combined with LB5 was found a lower lactic acid content than non-inoculated *L. plantarum* (LB5LP0). Nevertheless, the inoculation at the highest level of *L. buchneri* combined with the several levels of *L. plantarum* had not statistically decreased the lactic acid concentration with increasing the levels of *L. plantarum*. Consequently, the higher pH was affected by the inoculation at the highest level of *L. buchneri* (LB6). As resulted from there was a highest acetic acid content, and a low of lactic acid content (shows in table 4.3). Thus, the secondary anaerobic fermentation was activated by the inoculation at the highest level of *L. buchneri*, as the higher pH has effected on activate the fermentations of *L. buchneri* and the epiphytical lactic acid bacteria, also have activated the metabolism of undesirable anaerobic microorganism (Wilkinson, 2005; Oliveira et al., 2017).

Table 4.2 Residual WSC, lactic acid, acetic acid, propionic acid, and butyric acid (mean ± SE) in Napier grass silage at 24 days of ensiling.

Treatment[1]		residual WSC	Lactic acid
		(g /kg DM)	
LB0	LP0	23.74 ± 0.84	70.87 ± 1.14[b]
	LP5	25.78 ± 0.07	62.78 ± 0.21[b]
	LP6	28.23 ± 0.38	55.94 ± 0.38[b]
LB5	LP0	24.58 ± 2.67	73.74 ± 0.39[a]
	LP5	24.83 ± 1.66	60.17 ± 0.98[b]
	LP6	29.49 ± 0.62	56.36 ± 0.03[b]
LB6	LP0	21.91 ± 0.57	67.07 ± 4.78[b]
	LP5	19.81 ± 0.02	53.40 ± 0.86[b]
	LP6	21.63 ± 0.06	63.83 ±5.33[b]
LB main effect			
LB0		25.92 ± 1.87[p]	61.43 ± 2.75
LB5		26.30 ± 1.31[p]	63.19 ± 3.35
LB6		21.12 ± 0.44[q]	63.42 ± 3.21
LP main effect			
LP0		23.41 ± 0.89[q]	70.55 ± 1.76[p]
LP5		23.48 ± 1.26[q]	58.78 ± 1.80[q]
LP6		26.45 ± 1.56[p]	58.71 ± 2.13[q]
***P*- values**			
LB		< 0.01	NS
LP		< 0.01	< 0.01
LB × LP		NS	< 0.05

[a, b, c, d; p, q, r] statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 are the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. LB0, LB5, and LB6 are the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively.

The results of residual WSC of this study had been consistent with the study of Huisden et al. (2009) had studied the use of the inoculant included with *L. buchneri* for corn crop, the inoculant included with *L. buchneri*-treated forage had a lower of residual WSC than the control group as resulted from the forage WSC in the inoculant-treated forage was used more exhaustively for the lactic acid fermentation by lactic acid bacteria. The residual WSC results of this study was significantly affected by the main effect of LB and LP (shows in table 4.2). The lowest of the residual WSC was found in the inoculation at the highest level of *L. buchneri* as WSC is used exhaustively for the fermentation to lactic acid production. As the secondary anaerobic fermentation caused by the inoculation at the highest level of *L. buchneri*, WSC was used by anaerobic microorganism.

However, the results of NH_3-N of this study had resulted from the main effect of *L. buchneri* and *L. plantarum* (shows in table 4.3), the decreasing of NH_3-N was affected by increasing the levels of both lactic acid bacterial species. As resulted from the abundant of WSC caused from molasses addition, the inoculation at a higher levels of both had been able to ferment WSC more than a lower levels of both lactic acid bacterial species, consequently to rapid lactic acid production then the silage pH quickly lower, resulted to inhibit plant proteolytic enzymes at initial of ensiling, and proteolytic bacteria at initial of ensiling and secondary anaerobic fermentation (Rooke and Hatfield., 2003; Oliveira et al., 2017). Thus, the reduction of silage NH_3-N have been decreased with increasing the levels of *L. plantarum* as silage pH quickly lower than the inoculation at lower levels of both lactic acid bacterial species.

Table 4.3 The results of treatment combinations on silage dry matter lose, pH value, NH_3-N (mean ± SE) in Napier grass silage at 24 days of ensiling.

Treatment[1]		**DM losses (%)**	**pH**	**NH_3-N**[3]
	LP0	10.77 ± 0.20^{ab}	3.80 ± 0.20	13.03 ± 0.99
LB0	LP5	17.66 ± 0.13^{a}	3.78 ± 0.35	9.62 ± 0.67
	LP6	13.08 ± 0.40^{a}	3.78 ± 0.40	8.12 ± 1.34
	LP0	8.81 ± 0.35^{b}	3.83 ± 0.35	12.08 ± 0.21
LB5	LP5	13.21 ± 0.15^{a}	3.83 ± 0.15	8.39 ± 0.14
	LP6	12.48 ± 0.24^{a}	3.80 ± 0.24	8.88 ± 0.50
	LP0	15.18 ± 0.31^{a}	3.88 ± 0.31	9.01 ± 0.81
LB6	LP5	10.72 ± 0.47^{ab}	3.96 ± 0.47	6.60 ± 0.23
	LP6	10.81 ± 0.14^{ab}	3.87 ± 0.14	6.55 ± 0.42
LB main effect				
LB0		13.83 ± 1.30^{p}	3.78 ± 0.01^{r}	10.26 ± 1.03^{p}
LB5		11.50 ± 0.93^{q}	3.81 ± 0.01^{q}	9.02 ± 1.03^{pq}
LB6		12.24 ± 1.00^{pq}	3.90 ± 0.02^{p}	8.15 ± 0.57^{q}
LP main effect				
LP0		11.58 ± 1.26^{q}	3.83 ± 0.01	11.38 ± 0.84^{p}
LP5		13.86 ± 1.31^{p}	3.85 ± 0.03	8.96 ± 0.31^{q}
LP6		12.12 ± 0.50^{pq}	3.82 ± 0.02	7.09 ± 0.49^{r}
***P*- values**				
LB		< 0.05	< 0.01	< 0.05
LP		< 0.05	NS	< 0.01
LB × LP		< 0.01	NS	NS

[a, b, c, d; p, q, r] statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 are the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. LB0, LB5, and LB6 are the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. [3]NH_3-N = ammonia nitrogen (% of total N).

As recall in previously described above, the highest silage pH had found in the inoculation at the highest level of *L. buchneri* as resulted from the considerable of acetic acid content was consistent with previous meta-analysis of Kleinschmit and Kung (2006). Acetic acid was produced from the fermentation of *L. buchneri* has less stronger acid than lactic acid that was mainly produced from the fermentation of *L. plantarum* (Kleinschmit and Kung, 2006; Muck et al., 2018b). For this reason, the inoculation at the highest level of *L. buchneri* had not inhibited the secondary anaerobic fermentation of undesirable anaerobic microorganism such as *Clostridium* spp. and Enterobacteria when compared with untreated both of lactic acid bacterial species (LP0LB0). There was confirmed by the results of butyric acid content.

The substantial of butyric acid content at day 24 of ensiling was found in the inoculation at highest level of both lactic acid bacterial species (shows in table 4.1). As resulted from *L. plantarum* has been slowly growth rate at pH > 5, at the initial stage of ensiling (Kung, 2011). Normally, saccharolytic clostridia, and proteolytic clostridia are two major groups have been found in silage. Silage clostridia grow best at pH 7.0-7.4, and wet forage before ensiling are required for clostridia growth (McDonald et al., 2010). Thus, the initial of ensiling, and wet forage before ensiling are preferred to encourage for Clostridia growth.

Thus, the increasing of silage pH caused to secondary anaerobic fermentation resulted to activate the undesirable of anaerobe microorganism. Saccharolytic clostridia has been able to break lactic acid down to acetic acid and butyric acid (McDonald et al., 2010). From theory, the inoculation at the highest levels of *L. plantarum* have been able to produce lactic acid than the inoculation at a lower levels

of *L. plantarum*. Consequently, saccharolytic clostridia might had been able to broken lactic acid to butyric acid than the inoculation at a lower levels of *L. plantarum*.

The microbial enumerations in Napier grass silage added molasses at day 24 of ensiled (non-statistical significance test since has not replicated for each treatment combinations, shows in appendix table 1) inoculated according to the treatment combinations of both lactic acid bacterial species at different levels had been used for supplement the results of silage fermentation at 24 days of ensiling. As previous described, *L. plantarum* was slow growing at the higher pH, thus Clostridia had not been inhibited suddenly at the initial ensiling by acidic condition, also including with the inoculation at highest levels of *L. buchneri* affected to the silage pH has a higher than the other treatment combinations. Also, the highest level of *L. plantarum* has produced more lactic acid as serve for saccharolytic clostridia. Thus, the results have shown there was a higher *Clostridium* spp. in the inoculation of *L. plantarum* combined with the highest levels of *L. buchneri*.

Although, the silage pH values of both treatment combination have not been able to inhibit the undesirable of anaerobic bacterial fermentation when compared with the untreated or other treatment combination of both lactic acid bacterial species. Whereas, the range of silage butyric acid content at day 24 of ensiling of both treatment combinations were ranged in the standard had been recommended by Agriculture and Food Development Authority (Teagasc) and Kung et al. (2018) (shows in appendix tables 5, 6). The standard recommendation for the optimum concentration of silage butyric acid should not be more over than the range of 10-40 g of butyric acid/ kg DM silage. As a consequently of the silage pH of both treatment combinations (LP5LB6, LP6LB6) were ranged in the standard has recommended that

the range of silage pH values should not be over than 4.5, and silage pH should be lower than 3.7, that could be have ability to inhibit the fermentation of the undesirable anaerobic microorganism.

Thus, the treatment combinations of both lactic acid bacterial species had not affected to the overall silage fermentation qualities. As the reason that, all of the silage analytical parameters were ranged in the standard had recommended. However, the treatment combinations at the highest level of *L. buchneri* had affected to the silage fermentation qualities when compared with the untreated of both of lactic acid bacterial species.

4.2 Napier grass silage dry matter loss at 24 days of ensiling

The result of treatment combinations shows that there has a LP × LB interaction effect on the reduction of silage dry matter loss in Napier grass added molasses at 24 days of ensiling. The result showed the inoculation at the higher levels of *L. plantarum* combined with the highest level of *L. buchneri* (LB6LP5, and LB6LP6) had been able to reduce the silage dry matter loss when compared with other treatment combination, but non-statistically different from LB6LP0, excepted with treatment combination at LB5LP0 had the lowest silage dry matter loss. On the other hand, the inoculation at the lower levels of *L. buchneri* combined with a higher levels of *L. plantarum* (LB0LP5, LB0LP6, LB5LP5, and LB5LP6) had a higher silage dry matter loss than the non-inoculation of *L. plantarum* (LB0LP0, LB5LP0) (shows in table 4.3).

For the reason that, the sole inoculation at the highest level of *L. buchneri* (LB6LP0) had the highest of silage dry matter loss at day 24 of ensiled. As since, *L.*

buchneri is grouped an Obligate heterolactic acid bacterium (Het-LAB) (Salvetti et al., 2012). The Het-LAB group had been able to ferment the forage simple sugar into lactic acid and carbon dioxide (Oude Elferink et al., 2001; Pahlow et al., 2003; Borreani et al., 2017).

Thus, the results this study have indicated that, the use of a higher levels of *L. plantarum* inoculated with the highest level of *L. buchneri* (LB65LP5, LB6LB6) had been able to reduce the silage dry matter loss when compared with solely inoculation of *L. buchneri* at the highest level (LB6LP0) and the results had been consistence with Driehuis et al. (2001); Weinberg et al. (2002); Filya (2003b, 2003a); Schmidt and Kung (2010). Also, the results was accorded to the concept had been interpreted by Driehuis et al. (2001).

4.3 Napier grass silage nutritional values at 24 days of ensiling

The silage nutritive values were affected by the inoculation of both lactic acid bacterial species as influenced from silage dry matter loss. The silage dry matter loss resulted to there was a silage nutritive lose.

As previous described, the decreasing of the silage dry matter loss at the highest level of *L. buchneri* was solved by the combination with *L. plantarum* (LB6LP5, and LB6LP6) as resulted from the silage acidic condition that caused by the inoculation of *L. plantarum* has inhibited to the *L. buchneri* metabolism. Thus, the combination at the highest level of *L. buchneri* combined with the several levels of *L. plantarum* resulted to there have been a higher silage dry matter content at 24 days of ensiled than the non-inoculated *L. plantarum* (shows in table 4.4).

The inoculation of both lactic acid bacterial species into Napier grass added molasses were ensiled to 24 days found there was not the effect of treatment combinations on the silage fiber fractions of NDF, ADF. But, had affected to increase ADL content. As since, lactic acid bacteria has not been fermentable the forage fibers as there were not the fibrolytic enzyme (Dewar et al., 1963; Pahlow et al., 2003). Thus, the inoculation of lactic acid bacteria of this study had not affected to decrease the silage NDF, ADF. Moreover, the inoculation of both lactic acid bacterial species had affected to silage dry matter loss, the losing of silage dry matter as the substrate was able to ferment by silage anaerobic microorganism. Thus, insoluble fiber fractions (NDF, ADF) had trended to increase but non-statistical differenced, but ADL had statistical differenced as since the decreasing of fermentable fractions.

Also, the silage crude protein content was affected by the inoculation of both lactic acid bacterial species. Silage crude protein content was found as resulted from the effect of LB × LP interaction effect. As since, the decreasing of silage crude protein content might have resulted from the increasing the levels of *L. plantarum* when combined with non-inoculated *L. buchneri* as resulted from the initial fermentation the silage pH has slower declined than other group (the reason has described above), thus the proteolytic clostridia might have been fermentable the forage crude protein in to other product at the initial fermentation (Oliveira et al., 2017). And the silage crude protein content had decreased with the increasing of silage dry matter loss. As the results was confirmed by the silage dry matter loss, the highest silage dry matter loss was found in the sole inoculation of *L. plantarum* resulted to there were a lower silage crude protein content. Moreover, the silage crude protein content was found in the inoculation at the highest level of *L. buchneri* had increased according to increase the levels of *L. plantarum* as consistent with the increasing of silage dry matter recovery (shows in table 4.4).

Table 4.4 The results of treatment combination dry matter content, crude protein, and ether extract (mean ± SE) in Napier grass silage at 24 day of ensiling.

Treatment[1]		Dry matter	Crude protein	Ether extract
		(% of fresh weight)	(% of DM)	
	LP0	23.42 ± 0.20^{ab}	9.01 ± 0.09^{ab}	3.49 ± 0.43
LB0	LP5	21.61 ± 0.13^{b}	8.54 ± 0.00^{cd}	2.11 ± 0.01
	LP6	22.40 ± 0.40^{ab}	8.20 ± 0.17^{d}	2.02 ± 0.32
	LP0	24.07 ± 0.35^{a}	9.05 ± 0.10^{ab}	3.46 ± 0.88
LB5	LP5	22.78 ± 0.15^{ab}	8.66 ± 0.07^{de}	3.91 ± 0.41
	LP6	22.97 ± 0.24^{ab}	9.13 ± 0.03^{a}	1.58 ± 1.17
	LP0	22.26 ± 0.31^{b}	8.72 ± 0.01^{abc}	3.51 ± 0.94
LB6	LP5	23.71 ± 0.47^{ab}	9.14 ± 0.01^{bc}	2.56 ± 0.42
	LP6	23.41 ± 0.14^{ab}	9.12 ± 0.01^{a}	1.50 ± 0.17
LB main effect				
LB0		22.47 ± 0.35	8.58 ± 0.16^{q}	2.55 ± 0.46
LB5		23.27 ± 0.28	8.93 ± 0.09^{p}	2.98 ± 0.48
LB6		23.12 ± 0.32	8.99 ± 0.09^{p}	2.53 ± 0.48
LP main effect				
LP0		23.25 ± 0.36	8.93 ± 0.07	3.49 ± 0.14
LP5		22.70 ± 0.41	8.78 ± 0.12	2.86 ± 0.52
LP6		22.92 ± 0.22	8.80 ± 0.20	1.71 ± 0.29
***P*- values**				
LB		NS	< 0.01	NS
LP		NS	NS	NS
LP × LB		< 0.01	< 0.01	NS

$^{a, b, c, d; p, q, r}$ statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively.

Table 4.5 The results of treatment combination on ash, NDF, ADF, ADL (mean ± SE) in Napier grass silage at 24 days of ensiling.

Treatment[1]		Ash	NDF	ADF	ADL
		(% of DM)			
LB0	LP0	12.24 ± 0.03	60.04 ± 0.13	34.62 ± 0.53	9.40 ± 0.10^{a}
	LP5	12.09 ± 0.26	60.45 ± 0.50	34.41 ± 0.89	9.52 ± 0.53^{a}
	LP6	11.49 ± 0.38	59.65 ± 0.26	35.79 ± 0.15	9.40 ± 0.34^{a}
LB5	LP0	12.02 ± 0.24	59.88 ± 1.31	34.36 ± 1.67	7.24 ± 0.55^{ab}
	LP5	11.37 ± 1.09	60.56 ± 0.09	34.73 ± 0.17	9.10 ± 0.10^{a}
	LP6	11.28 ± 0.28	60.94 ± 0.58	35.26 ± 0.30	9.25 ± 0.81^{a}
LB6	LP0	11.82 ± 0.75	59.40 ± 0.24	33.46 ± 0.98	6.09 ± 0.07^{b}
	LP5	12.05 ± 0.29	60.85 ± 0.62	35.75 ± 0.06	9.41 ± 0.18^{a}
	LP6	12.10 ± 0.07	61.31 ± 0.24	35.54 ± 0.32	8.13 ± 0.10^{ab}
LB main effect					
LB0		11.94 ± 0.19	60.05 ± 0.21	34.94 ± 0.38	9.45 ± 0.15^{p}
LB5		11.55 ± 0.33	60.46 ± 0.42	34.79 ± 0.47	8.53 ± 0.44^{pq}
LB6		11.99 ± 0.37	60.45 ± 0.38	34.84 ± 0.51	7.88 ± 0.66^{q}
LP main effect					
LP0		12.02 ± 0.22	59.77 ± 0.37	34.15 ± 0.56	7.59 ± 0.64^{q}
LP5		11.83 ± 0.33	60.55 ± 0.18	34.96 ± 0.35	9.35 ± 0.27^{p}
LP6		11.62 ± 0.20	60.64 ± 0.36	35.45 ± 0.14	8.93 ± 0.26^{pq}
***P*- values**					
LB		NS	NS	NS	< 0.01
LP		NS	NS	NS	< 0.01
LP × LB		NS	NS	NS	< 0.05

$^{a, b, c, d; p, q, r}$ statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively.

4.4 Napier grass silage aerobic stability characteristics at 2, 4, and 6 days after exposed to air

The silage of each treatment combinations had exposed to air through 2, 4, and 6 days were opened from the seal plastic bag at 24 days of ensiled to evaluate the silage aerobic deterioration characteristics such as silage pH, dry matter loss, residual WSC, and microbial profiles (yeast, lactate-assimilating yeast, mold).

The treatment combinations effect had been able to improve the silage aerobic stability characteristic after silage exposed to air. The sole inoculation of *L. plantarum* had found there were the highest silage pH after exposed to air at 2 days than other treatment combination groups. This result was consistence with the other studies, the inoculation of *L. plantarum* has a lower acetic acid content, and there were a higher of residual WSC resulted to a higher aerobic detrimental effects than the other treatment combined with *L. buchneri* (Weinberg et al., 2002; Tabacco et al., 2011). Nevertheless, silage was exposed to air at 4 days, the inoculation at the level of 1×10^5 cfu *L. buchneri*/ g fresh forage weight (LB5) combined with the several levels of *L. plantarum* was found the pH slightly increases, and the groups that combined with 1×10^5 cfu *L. plantarum*/ g fresh forage weight (LP5) were found a higher silage pH than other.

Also, the inoculation at the highest level of *L. buchneri* combined with the several levels of *L. plantarum* was rarely found the increasing of silage pH after exposed to air through 6 days after exposed to air. Therefore, the results of the inoculation at the highest level of *L. buchneri* combined with the inoculation at several levels of *L. plantarum* (LP0LB6, LP5LB6, LP6LB6) have been able to improve the silage aerobic stability. And, the inoculation at a lower level of *L. buchneri* combined with several levels of *L. plantarum* had not been able to improve aerobic stability through 6 days after silage exposed to air.

Table 4.6 The results of treatment combinations on residual WSC, and pH (mean ± SE) in Napier grass silage exposed to air at 2 days[1].

Treatment[1]		**residual WSC (g/kg DM)**	**pH**
	LP0	25.86 ± 1.14^{a}	3.94 ± 0.02^{c}
LB0	LP5	19.05 ± 0.19^{bc}	8.25 ± 0.02^{a}
	LP6	25.97± 1.87^{a}	8.04 ± 0.00^{b}
	LP0	18.06 ± 0.37^{bc}	3.86 ± 0.05^{cd}
LB5	LP5	17.11 ± 0.89^{c}	3.82 ± 0.00^{d}
	LP6	22.84 ± 0.86^{ab}	3.82 ± 0.01^{d}
	LP0	20.95 ± 0.89^{abc}	3.87 ± 0.00^{cd}
LB6	LP5	20.47 ± 0.64^{bc}	3.92 ± 0.01^{c}
	LP6	17.80 ± 0.54^{bc}	3.89 ± 0.00^{cd}
LB main effect			
LB0		21.62 ± 1.77 ^{p}	6.75 ± 0.89 ^{p}
LB5		17.47 ± 0.88^{q}	3.83 ± 0.01^{r}
LB6		17.52 ± 1.68^{q}	3.90 ± 0.01^{q}
LP main effect			
LP0		19.10 ± 2.70	3.89 ± 0.02^{r}
LP5		18.47 ± 1.09	5.33 ± 0.92^{p}
LP6		19.05 ± 0.70	5.24 ± 0.88^{q}
***P*- values**			
LB		< 0.05	< 0.01
LP		NS	< 0.01
LP × LB		< 0.05	< 0.01

$^{a, b, c, d; p, q, r}$ statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^{5}, and 1×10^{6} cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^{5}, and 1×10^{6} cfu/ g fresh weight, respectively.

For the reason that, the inoculation at the highest level of *L. buchneri* combined with the several levels of *L. plantarum* had the lowest in the silage dry matter loss, and had the lowest in the silage pH values at day 6 after silage exposed to air than the inoculations at lower levels of *L. buchneri* as resulted from the inoculation at highest level of *L. buchneri* combined with several levels of *L. plantarum* have the highest concentration of acetic acid at opened the sealed plastic bag than other treatment combination. As recognized that, acetic acid had been able to inhibit the proliferations of yeast, and mold while the silage exposed to air (Muck et al., 2018a; Muck et al., 2018b). Thus, the inoculations at highest level of *L. buchneri* in Napier grass added molasses had able to improve the silage aerobic stability after the silage was exposed to air through 6 days.

And, at 6 days after exposed to air, residual WSC in the inoculation at the highest level of *L. buchneri* had been stable than the inoculation at a lower levels of *L. buchneri*. As, the growth of yeasts, and mold was inhibited by acetic acid. And was confirmed by the results of aerobic microbial profiles at 6 days after exposed to air (shows in appendix table 4), as there were a lower of yeast, lactate-assimilating yeast, and mold in the inoculation at the highest level of *L. buchneri*.

Table 4.7 The effect of treatment combinations on residual WSC, and pH (mean ± SE) in Napier grass silage exposed to air at 4 days[1].

Treatment[1]		**residual WSC (g/kg DM)**	**pH**
	LP0	26.58 ± 1.13^{a}	7.20 ± 0.11^{a}
LB0	LP5	17.71 ± 0.62^{b}	8.80 ± 0.04^{a}
	LP6	11.35 ± 0.91^{b}	8.67 ± 0.01^{a}
	LP0	21.31 ± 1.03^{ab}	4.53 ± 0.08^{c}
LB5	LP5	20.35 ± 2.45^{ab}	7.58 ± 0.35^{a}
	LP6	20.13 ± 1.32^{ab}	4.85 ± 0.01^{b}
	LP0	16.70 ± 1.37^{b}	3.89 ± 0.12^{e}
LB6	LP5	17.87 ± 0.15^{b}	3.97 ± 0.05^{d}
	LP6	18.21 ± 4.16^{b}	3.93 ± 0.01^{ed}
LB main effect			
LB0		18.54 ± 1.85	8.23 ± 0.33^{p}
LB5		20.60 ± 0.69	5.65 ± 0.62^{q}
LB6		17.60 ± 1.19	3.93 ± 0.01^{r}
LP main effect			
LP0		21.53 ± 1.85^{p}	5.21 ± 0.64^{r}
LP5		18.64 ± 0.94^{pq}	6.78 ± 0.92^{p}
LP6		16.56 ± 2.03^{q}	5.82 ± 0.92^{q}
***P*- values**			
LB		< 0.05	< 0.01
LP		NS	< 0.01
LP × LB		< 0.05	< 0.01

$^{a, b, c, d; p, q, r}$ statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^{5}, and 1×10^{6} cfu/g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^{5}, and 1×10^{6} cfu/ g fresh weight, respectively.

Table 4.8 The effect of treatment combinations on residual WSC, and pH (mean ± SE) in Napier grass silage exposed to air at 6 days[1].

Treatment[1]		**residual WSC (g/kg DM)**	**pH**
	LP0	26.97 ± 1.58[a]	8.28 ± 0.28[a]
LB0	LP5	19.60 ± 0.09[ab]	8.87 ± 0.00[a]
	LP6	18.30 ± 4.16[ab]	8.84 ± 0.10[a]
	LP0	16.90 ± 0.37[ab]	8.00 ± 0.00[a]
LB5	LP5	16.41 ± 2.97[ab]	9.10 ± 0.04[a]
	LP6	19.10 ± 3.17[ab]	8.00 ± 0.24[a]
	LP0	13.41 ± 1.84[b]	4.20 ± 0.09[b]
LB6	LP5	19.40 ± 2.51[ab]	3.70 ± 0.32[b]
	LP6	19.76 ± 1.71[a]	4.24 ± 0.27[b]
LB main effect			
LB0		21.62 ± 1.77	8.66 ± 0.14[p]
LB5		17.47 ± 0.88	8.34 ± 0.24[p]
LB6		17.52 ± 1.68	4.06 ± 0.14[q]
LP main effect			
LP0		19.10 ± 2.70	6.82 ± 0.83
LP5		18.47 ± 1.09	7.01 ± 1.10
LP6		19.05 ± 0.70	7.23 ± 0.90
***P*- values**			
LB		NS	< 0.01
LP		NS	NS
LP × LB		< 0.05	< 0.05

[a, b, c, d; p, q, r] statistically significant difference for means in the columns ($P < 0.05$). NS = non-statistically significant difference. [1]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively.

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CHAPTER V

CONCLUSIONS

(1) The sole inoculation at the highest level of *L. buchneri* had affected to the silage qualities since *L. buchneri* was able to degrade lactic acid into acetic acid. Consequence, there was the highest of silage pH. The higher in pH values had affected to the silage quality. Thus, the inoculation of both lactic acid bacterial species at different levels resulted to there was a lower fermentation quality than non-inoculation of both lactic acid bacterial species. However, the inoculation of both lactic acid bacterial species at different levels had not affected to the overall of fermentation quality as since the fermentation end products were ranged in the standard recommendation. (2) The inoculation at the highest levels of *L. buchneri* resulted to there was the highest silage dry matter loss. However, the combination at the highest levels of *L. buchneri* with *L. plantarum* had solved the silage dry matter loss. (3) The treatment combination of the highest level of *L. buchneri* with *L. plantarum* had been able to improve the silage aerobic stability through 6 days after silage exposed to air, as same as the sole inoculation of *L. buchneri* at the highest level. (4) Therefore, the results of this study indicated the inoculation at the highest level of both lactic acid bacterial species had appropriated to inoculant into Napier grass added molasses at 5% of fresh forage weight as had improved silage qualities, dry matter loss, and also had not affected to the silage aerobic stability when compared with the sole inoculation at the highest level of *L. buchneri*.

5.1 Suggestions

- As this study had performed in laboratory scale. Thus, the further study should confirm the result in farm scale.

- The further study should study the effects of inoculations at the highest levels of both lactic acid bacterial species into Napier grass added molasses at 5% of fresh forage weight on animal production efficiencies.

- The investigation of return of investment for the inoculation of both lactic acid bacterial species form this study is needed to confirm the efficiency of overall farm profitability.

APPENDIX

Table 1A The results of *L. plantarum* combination with *L. buchneri* on silage microbial profiles in Napier grass at day 24 of ensiled[1].

Treatment[2]		Lactic acid bacteria	Enterobacteria	Clostridium
		(log_{10} cfu/ g fresh)[1]		
LB0	LP0	8.48	6.21	6.83
	LP5	9.28	7.56	7.76
	LP6	8.40	7.82	7.15
LB5	LP0	8.54	5.00	7.87
	LP5	8.85	6.21	6.57
	LP6	8.38	6.38	7.42
LB6	LP0	8.38	7.46	6.46
	LP5	8.73	4.36	7.42
	LP6	8.51	4.48	7.03

[1]non-statistical significance test since has not replicated for each treatment combinations. [2]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively.

Table 2A The results of *L. plantarum* combined with *L. buchneri* at different levels of both on microbial profiles in Napier grass silage exposed to air through 2 days[1].

Treatment[2]		Yeast	Lactate-assimilating yeast	mold
		(log_{10} cfu/ g fresh)[1]		
	LP0	6.99	7.15	5.00
LB0	LP5	7.52	7.11	NF
	LP6	7.18	7.33	NF
	LP0	6.34	5.00	5.00
LB5	LP5	6.28	6.47	5.00
	LP6	6.48	6.20	NF
	LP0	6.35	6.00	NF
LB6	LP5	6.13	5.26	NF
	LP6	5.43	5.00	NF

[1]non-statistical significance test since has not replicated for each treatment combinations. [2]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. NF = not found.

Table 3A The results of *L. plantarum* combined with *L. buchneri* at different levels of both on microbial profiles in Napier grass silage exposed to air through 4 days[1].

Treatment[2]		Yeast	Lactate-assimilating yeast	mold
		(log_{10} cfu/ g fresh)[1]		
	LP0	7.54	6.04	4.53
LB0	LP5	7.15	6.95	ND
	LP6	7.23	7.47	ND
	LP0	7.34	6.36	5.08
LB5	LP5	7.23	6.89	4.90
	LP6	7.36	6.23	3.70
	LP0	6.08	4.40	NF
LB6	LP5	5.76	5.08	NF
	LP6	6.66	5.23	NF

[1]non-statistical significance test since has not replicated for each treatment combinations. [2]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. NF = not found.

Table 4A The results of *L. plantarum* combined with *L. buchneri* at different levels of both on microbial profiles in Napier grass silage exposed to air through 6 days[1].

Treatment[2]		Yeast	Lactate-assimilating yeast	mold
		(log_{10} cfu/ g fresh)[1]		
LB0	LP0	10.95	7.56	6.46
	LP5	10.34	9.70	ND
	LP6	10.20	9.53	ND
LB5	LP0	9.95	7.56	7.15
	LP5	10.28	9.95	6.52
	LP6	10.57	10.56	6.68
LB6	LP0	8.04	5.70	6.38
	LP5	8.43	8.48	5.04
	LP6	7.95	7.18	5.85

[1]non-statistical significance test since has not replicated for each treatment combinations. [2]LP0, LP5, and LP6 is the concentration of *L. plantarum* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively; LB0, LB5, and LB6 is the concentration of *L. buchneri* at 0, 1×10^5, and 1×10^6 cfu/ g fresh weight, respectively. NF = not found.

Table 5A Typical suggested concentrations of common fermentation end products in grass silages, adapted from Kung et al. (2018).

Fermentation end products	**Grass silage, 25-35 % of DM**
pH	4.3-4.7
Lactic acid, g/ kg DM	60-100
Acetic acid, g/ kg DM	10-30
Propionic acid, g/ kg DM	< 1
Butyric acid, g/ kg DM	< 5-10
Ethanol, g/ kg DM	5-10
NH_3-N, % of total N	8-12

Table 6A Typical suggested concentrations of common fermentation end products in grass silages, adapted from Agriculture and Food Development Authority (Teagasc).

Fermentation end products	**Low**	**high**	**Quality is best when**
pH	3.4-3.7	4.5-5.5	Medium to low
Lactic acid, g/ kg DM	5-50	90-120	High
Acetic acid, g/ kg DM	10	40-60	Low
Propionic acid, g/ kg DM	1	10-20	Low
Butyric acid, g/ kg DM	1	10-40	Very low
NH_3-N, g/ kg DM	4-7	15-25	Low

BIOGRAPHY

Mr. Pattarapong Jaiboonlue was born on May 18th, 1994 in Saraburi Province, Thailand. He graduated his bachelor degree from School of Animal Production Technology at Suranaree University of Technology, Thailand in 2017. After graduation, he obtained the scholarship from Suranaree University of Technology. And, he obtained the research fund from National Research Council of Thailand (NRCT) to pursue in his Master degree study in School of Animal Production Technology at Suranaree University of Technology, Thailand. His M.Sc. thesis title was "Effects of *Lactobacillus plantarum* and *Lactobacillus buchneri* on Napier grass silage qualities and aerobic stability". The results from part of this thesis had been presented as poster presentation in the 2nd international conference on Tropical Animal Science and Production (TASP 2019), July 9-12th, 2019 at Surasammakhan Hotel, Suranaree University of Technology, Thailand.