CHAPTER 4

Results and discussions

4.1 Nutrient composition of fresh and preserved pangola grass at 45 days of regrowth stage cutting

4.1.1 Chemical composition

Chemical composition was presented in **Table 4.1.** Differences in DM, CP, CF, NDF, ADF, ash and TDN were shown significantly. Pangola hay presented highest DM, followed by fresh napier, fresh pangola, pangola silage added molasses, fresh ruzi and pangola silage respectively. After preservation, pangola silage with no additive showed the lowest DM from 21.57 to 18.68 than others due to the lower lactic acid result in the higher losses of DM from the crop during

Table 4.1 The chemical composition of the forages

Items	**FN**	**FR**	**FP**	**PH**	**PS**	**PSM**	**SEM**	***P*-value**
DM	22.82^{b}	19.21^{d}	21.57^{c}	84.69^{a}	18.68^{e}	20.84^{c}	0.09	<0.05
Nutrient composition (% dry matter)								
CP	8.75^{b}	8.15^{c}	8.90^{b}	8.58^{b}	7.57^{c}	9.96^{a}	0.12	<0.05
EE	1.91	1.80	2.54	2.01	1.74	2.67	0.33	0.93
CF	26.72^{b}	26.91^{b}	28.16^{b}	30.91^{a}	28.03^{b}	27.40^{b}	0.41	<0.05
NDF	61.07^{bc}	59.25^{c}	62.99^{b}	67.13^{a}	64.78^{bc}	61.64^{bc}	0.67	<0.05
ADF	38.53^{ab}	37.97^{ab}	36.77^{c}	38.82^{a}	37.88^{ab}	35.84^{d}	0.58	<0.05
ADL	4.55	4.30	3.68	5.22	4.54	3.42	0.82	0.47
Ash	12.58^{a}	9.86^{d}	9.66^{d}	11.93^{b}	11.64^{b}	10.63^{c}	0.29	<0.05
TDN	58.62^{bc}	59.26^{bc}	59.49^{b}	58.30^{c}	59.71^{b}	61.70^{a}	0.48	<0.05

$^{a-f}$ within columns for each main effect, values followed by different letters are significantly different (P < 0.05).

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storage (Kung and Shaver, 2001). Pangola silage with molasses resulted decreasing DM to 20.84%, but increasing CP contents (P<0.05) from 8.90 to 9.96%, followed by fresh pangola, pangola hay, fresh napier, fresh ruzi and pangola silage respectively (8.90, 8.58, 8.75, 8.15 and 7.57% CP of DM, respectively). The DM increasing may from the DM content of molasses used, which consist with the results of Hinds *et al*. (1985) and Lattemae *et al*. (1985). In fresh grass group, fresh napier resulted the highest DM, followed by fresh pangola and fresh ruzi. That can be explained that due to difference of plant variety which has different appropriate stage cutting.

The reason of increasing in CP during ensiling is the fact that proteolytic activity during fermentation produces NH_3 (Kung *et al*., 2000). The other possible reason of increase in CP contents due to additives may be protein sparing activity. CP of fresh pangola in this study indicated a high forage quality when compared with previous reports (Archimède *et al*., 2000; Lee *et al*., 2000; Chaichaum *et al*., 2007; Tikam *et al*., 2010), was similar with Yeh (1990) studied in Taiwan, pangola grass that was harvested in fall season from Lin *et al*. (2004) report and higher than those reported by Animal Nutrition Division, Thailand (2006)(7.9 % CP) which might be due to climate and environment. Fresh napier had little lower CP than fresh pangola (8.75%), while fresh ruzi had significantly lower (8.15%)(P<0.05). The type of forage (also variation between grasses) was the main factors of variation in terms of explained variance (Aumont *et al*., 1995).

Non-statistically difference in EE contents, which ranged 1.80-2.67% of DM. The CF showed different that pangola hay was the highest value due to an increasing of NDF, ADF and lignin contents by the sundried preserved process. The NDF might be attributed to increased CHO fraction C (non-degradable and non-available fraction) due to heat process. The cell-wall components range 59.25-64.78 % NDF and 35.84-38.82 % ADF. The cell-wall carbohydrates (NDF and ADF) appeared to vary little despite the wide range of type of forages and type of preserved process.

The silages added with molasses had also significantly lower cell wall components (NDF, ADF) and trendy lower lignin than other groups. The reduction NDF content of pangola silage with molasses was clearly affected by the process of fermentation explained by the hydrolysis of NDF-bound N during fermentation (Jaakkola *et al*., 2006; Huisden *et al*., 2009). Tjandraatmadja *et al.* (1994) reported that pangola grass which had a high significantly different chemical composition prior to ensiling, with lower NDF and lignin content. The lower NDF and lignin parts presented a dominant homo-fermentative lactic acid bacteria population in silage which was fairly well preserved even without molasses. This can be explained in that the addition of 5 % of molasses may dilute the proportion of fibers. ADF content in pangola silage added molasses was increased when compared with fresh pangola that may be caused by the losses of other components during the fermentation (Jaakkola *et al*., 2006).

Pangola hay showed increasingly DM, NDF and ADF ($P<0.05$) after preservation. Due to higher temperature by sun drying increased lignification of the plant cell wall promoted more rapid metabolic activity. Photosynthesis is more rapidly converted to structural components, which reduces nitrate, protein and soluble carbohydrate contents and increase cell wall contents (Deinum *et al*., 1968, Van Soest *et al*., 1978). The lower values of chemical compositions (CP, EE and ash) from pangola hay compared to fresh pangola grass and pangola silage were probably due to chemical changes during the drying process. The drying resulted in losses of valuable nutrients during the drying process as reported by McDonald *et al.* (2002). Fresh napier and ruzi showed higher ADF and ADL than fresh pangola may due to factors general thickening of plant cell walls.

TDN that was calculated from chemical composition data presented the highest value in pangola silage added molasses, followed by pangola silage, fresh pangola. Pangola silage and fresh pangola showed high TDN prediction that may be due to difference of some chemical composition parts compared with pangola hay.

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4.1.2 Silage characteristics

The two groups of silage showed different characters **(Table 4.2)**. The major goal in silage making is to preserve silage material with minimum nutrient loss. Molasses helped silages of reasonably good fermentation quality. When compared to untreated molasses silages, silages added molasses was lower DM loss than silage without additive. Pangola silage had 13.40% DM losses during ensilage which is relatively high when comparing with well-fermented maize and grass silage (Köhler *et al.*, 2013). This DM of fresh pangola results showed lower than some suggestion that storage of forage as silage at a moisture content of 75-85 % (15-25% of DM) is unsuitable for good silage production (Schroeder, 2004). Wilting the forages above 30% DM could help to reduce these losses. The additive of molasses had been proven to generally decreasing organic matter losses (Yitbarek and Tamir, 2014).

Table 4.2 Physical characteristic of pangola silage in the experiment

Items	**PS**	**PSM**	**SEM**	***P*-value**
DM after	18.68^{b}	20.84^{a}	0.06	<0.05
DM loss %	13.40^{b}	3.55^{a}	0.06	<0.05
Organoleptic test[1]	good (16)	very good (25)	0.23	<0.05
pH	4.54^{b}	3.66^{a}	0.06	<0.05
Volatile fatty acid (%total)				
Acetic (a)	35.54^{b}	9.48^{a}	1.61	<0.05
Butyric (b)	3.87^{b}	1.29^{a}	0.64	<0.05
Lactic (c)	60.64^{b}	89.24^{a}	1.20	<0.05
fermentation quality[2]	45.5^{b}	90^{a}	2.04	<0.05

$^{a-b}$ within columns for each main effect, values followed by different letters are significantly different (P < 0.05).

[1] organoleptic quality (odour, colour, texture and pH), score 25-20=very well, 19-15=well, 14-6=fair, and <5=poor (Animal Nutrition Division, 2004)

[2] Fermentation quality, score 100-81= Grade 1 very well, 80-61= Grade 2 well, 60-41= Grade 3 quite good, 40-21= Grade 2 fair, and <20= Grade 5 bad (Zimmer, 1966)

The quality of pangola silage as evaluated by organoleptic test showed the adding of molasses can improve silage characteristic. Base on this evaluation, the both group of pangola silage were classified as well fermented (good and very good). The final pH was lower in pangola silage with molasses than without additive (*P*<0.05), 3.66 and 4.54 respectively. This results were similar to that recorded by Khuamangkorn *et al.*

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(2006), who noted pangola silage without additive has lower pH than pangola silage added 2, 4, 6 and 8% molasses (4.62, 4.13, 3.96 and 3.86 respectively). According to Phiri *et al*. (2007) tropical grasses and legumes are not natural ensilage material because at cutting they have low content of water soluble carbohydrates, which are essential to successful ensilage. Due to the low WSC content of pangola grass (4.79 % at 45th day of regrowth stage cutting) that low amount of available WSC may have restricted the growth of lactic acid bacteria, preventing a faster drop in pH in the inoculated silage. As pH lower than 4.2 had been reduced sufficiently to inactivate the hydrolysis of plant proteolytic enzymes (Skerman and Riveros, 1990) and inhibit clostridial fermentation (Leibensperger and Pitt, 1988). Generally, pH is one of the simplest and quickest ways of evaluating silage (Babayemi, 2009). It is generally accepted that in silage pH values should be <4.5 (McDonald *et al*., 1991).

The pangola silage with no additive showed higher acetic acid than another. Other authors had described the positive aspect of the formation of acetic acid by hetero-fermentative LAB (Cooke, 1995; Rooke, 1991 and Weinberg and Muck, 1996). A hetero-fermentative lactic acid bacterium, such as *Lactobacillus buchneri*, had been studied as an additive to improve the aerobic stability of silages (Muck 1996). *L. buchneri* produces high levels of acetic acid in silage. In addition, high metabolic activity of hetero-fermentative LAB led to high pH in silage. Butyric acid resulted higher in pangola silage without additive. A high concentration of butyric acid indicates that the silage has undergone clostridial fermentation, which is one of the poorest fermentations. These values were low; according McDonald *et al*. (1991) silage containing more than 10% butyric acid are poorly preserved.

Addition of molasses in pangola silage resulted in the higher lactic acid than silage without molasses (P<0.05), which is in agreement with the results reported in the literature (O'Kiely *et al*., 1989 and Bruins, 1990). This could be due to the fact that the material which is rich source of carbohydrate is easily fermentable. Increased lactic acid concentrations were associated with lower concentrations of acetic acid in the silages. Other results supported that adding water-soluble carbohydrate (WSC) sources such as

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molasses, glucose and cassava meal to fresh Elephant grass at ensiling has resulted in increased lactic acid production (Yang *et al*., 2004).

The higher lactic acid concentration is probably due to the higher WSC of molasses additive. This is possible due to the addition of fermentable carbohydrate in form of molasses, resulting in lactic acid being the major organic acid (89.24%). The lower lactic acid in silage with no additive may cause sample taken after considerable aerobic exposure that has degraded lactic acid (Kung and Shaver, 2001). As if Danner *et al*. (2003) presented about types of lactic acid bacteria. The inoculation with homo-fermentative lactic acid bacteria led to silages with high lactic acid contents, while inoculation with hetero-fermentative lactic acid bacteria resulted in high levels of acetic acid. Moreover, it has been indicated by Lima *et al*. (2010) that a good quality of silage is associated at least 70% but no more than 90% lactic acid and no more than 20% acetic acid. As the result, the fermentation quality scores from the scheme (Zimmer, 1966), which objectively assesses silage fermentation quality by means of contents of acetic acid, butyric acid and lactic acid using a point-base system, were ranked "fair" for pangola silage and "very good" for pangola silage with molasses.

4.1.3 *In vitro* gas production measurement

The *in vitro* gas production technique followed the methods by Menke and Steingass (1988) had the potential to indicate the *in vitro* total tract digestibility of feeds for ruminants. The parameters of gas production were presented in **Table 4.3** and the cumulative gas curve was given in **Figure 4.1**. There were different in the cumulative gas with greater values noted for pangola silage with molasses (58.27 ml), followed by fresh pangola and pangola hay (52.56 and 51.87 ml, respectively). The products of gas at 24 hours were shown different (P<0.05). The highest value showed in pangola silage added molasses (48.92 mL/200 mg DM), followed by fresh pangola, pangola hay and pangola silage. The higest gas at 24 h in this experiment was higher when compared with Tikam (2014) (45.3 mL/200 mg DM).

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Table 4.3 *In vitro* gas production characteristics of forages in buffered rumen fluid, organic matter digestibility (%) and metabolizable energy (ME)

Items	**FN**	**FR**	**FP**	**PH**	**PS**	**PSM**	**SEM**	***P*-value**
Cumulative gas (ml)								
G24	27.28^{e}	20.75^{f}	38.88^{b}	33.87^{c}	29.99^{d}	48.92^{a}	2.755	<0.05
G48	32.29	25.45	44.35	42.37	42.44	49.65	2.809	0.07
G72	38.35	28.22	49.00	46.80	46.33	53.43	3.131	0.21
G96	42.04	31.53	52.56	51.87	47.99	58.27	3.377	0.26
OMD (%)	53.32^{d}	43.41^{e}	62.90^{b}	62.16^{b}	58.76^{c}	67.90^{a}	2.385	<0.05
ME (MJ/kg)	7.74^{e}	6.38^{f}	9.57^{b}	9.02^{c}	8.51^{d}	9.86^{a}	0.418	<0.05

$^{a\text{-}f}$ within columns for each main effect, values followed by different letters are significantly different (P < 0.05).

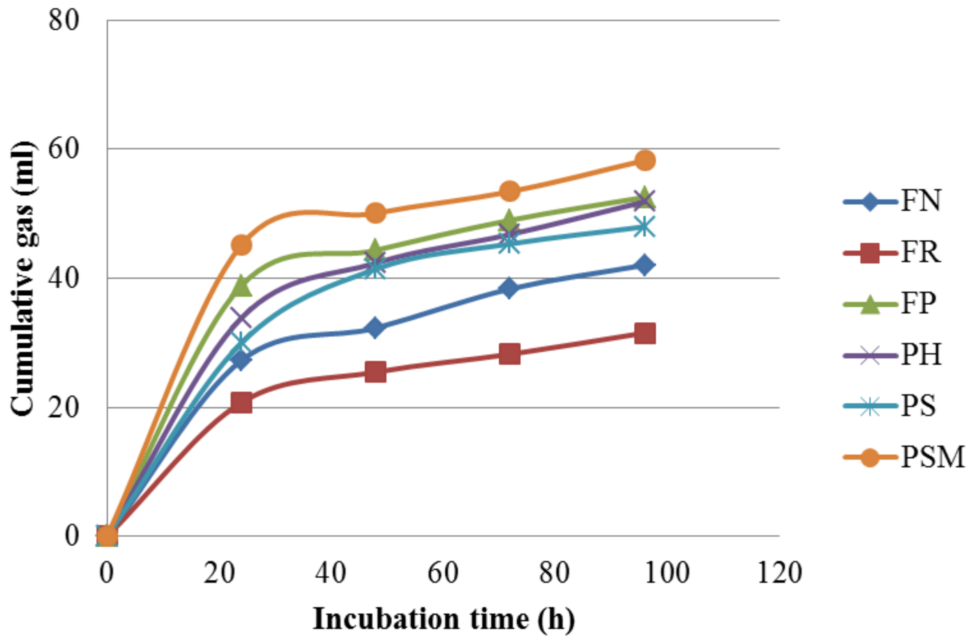

Figure 4.1 The cumulative gas production at 24, 48, 72 and 96 hours of forage incubation

Pangola hay showed lower gas products than other groups that confirmed the negative correlation between NDF level and gas production is consistent with other studies (Nsahlai *et al*., 1994; Larbi *et al*., 1998). Moreover, Menke *et al.* (1988) suggested that GP after 24 h incubation has a high positive correlation with OMD, net energy of lactation (NEL), ME and SCFA in nutritive value of feedstuff. The high GP at 24 hour resulted high OMD, NEL, ME and SCFA.

Estimated organic matter digestibility (OMD) and metabolizable energy (ME) were calculated by using gas accumulation at 24 h as described by Menke and Stengess (1988). OMD and ME resulted significantly different (P<0.05). The highest OMD and ME were observed in pangola silage with 5% molasses (67.90% and 9.86, respectively) supported with Tikam *et al*. (2010) that presented the additive of molasses improved ME. These results are in agreement with the findings reported by Ndlovu and Nherera (1997), Larbi *et al* (1998), Karabulut *et al.* (2007) and Abdulrazak *et al*. (2000) that cell wall contents are strongly negative related with gas production data. In addition, Karabulut *et al*. (2006) found a negative correlation between ME or OMD and cell wall contents (NDF, ADF and ADL). The ME content of fresh pangola in this trial was 9.57% lower than the value obtained by Lee *et al*. (2000) and Lin *et al*. (2004) in pangola grass that grown spring season and higher than in fall season.

4.2 *In vitro* digestibility using the DAISY^{II} incubator

The *in vitro* digestibility using Daisy^{II} ANKOM incubator was presented in **Table 4.4**. The IVDMD 24 h showed little different. The additive of molasses in pangola silage tended to increase IVDMD 24 h (73.35% from 70.95%). The use of molasses as a silage additive associated with a significantly higher DM digestibility compared to the silage without additive. The addition of molasses in pangola silage affected increasing NDFD 24 h values (P<0.05) compare to fresh pangola after 24 hours of incubation (39.95% from 35.12%, respectively), while pangola silage decreased (33.00% from 35.12%, respectively).

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Table 4.4 *In vitro* dry matter and fiber digestion using Daisy^{II} incubator.

Items	FN	FR	FP	PH	PS	PSM	SEM	***P*-value**
IVDMD 24h %	68.08	67.86	70.95	68.41	64.40	73.35	1.74	0.08
NDFD 24h %	34.26^{b}	34.18^{b}	35.12^{b}	35.02^{b}	33.00^{b}	39.95^{a}	1.05	<0.05
IVDMD 48h %	76.54^{a}	76.54^{a}	79.32^{a}	78.83^{a}	71.70^{b}	80.29^{a}	1.60	<0.05
NDFD 48h %	64.37	65.01	66.35	68.14	65.54	67.01	1.18	0.30

$^{a, b}$ Means within a row without a common superscript letter differ (P<0.05)

Moreover, molasses inclusion resulted the highest the IVDMD 48 h of incubation. This result was very similar to that recorded by Bureenok *et al*. (2012), who noted silage added molasses resulted in higher DM and NDF digestibility compared with silage without additive. This can be explained in that due to changing chemical composition of preserved pangola grass.

Due to cell-wall concentration affected large influence on forage digestibility. The increasing are in line with the observed lower NDF content of this silage, which are at least partly caused by addition of rapidly fermentable carbohydrate in molasses. That showed contrast result with pangola silage without additive that contained higher NDF which affected lower NDF degradability. There was no different IVDMD between fresh grass, pangola, napier and ruzi, but fresh pangola had higher value (79.32 vs 76.54%) than other group supported with Yunus *et al*. (2000) reported that napier shows a low digestibility and low CP. The increased NDF degradation which enhances the energy content in pangola silage added molasses might be attributed to the improvement in animals. However, there was no significantly different among the NDFD 48 h of incubation.

4.3 *In sacco* technique (Nylon bag technique)

The curve for disappearance for all treatments showed an exponential trend as described by the Ørskov and McDonald (1979) equation. The kinetic parameters of forage DM degradation of the 6 types were calculated using the NEWAY program was given in **Table 4.5**. Degradation of DM showed significantly different (*P*<0.05) at all of parameters. Fresh pangola presented rapidly high soluble fraction (a) among other

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groups (P<0.05). This result was little lower than Archimède *et al*. (2000) that estimated pangola grass at 42 days cutting stage with consumed by black-belly sheep (18.6 vs 20.9). Insoluble fraction but fermentable fraction (b) and degradability rate constant (c) resulted highest in pangola silage with molasses (P<0.05). The observed two parameters (b and c) significantly changed for the better by addition of molasses. That affected by the lower fiber contents, which was caused by addition of rapidly fermentable carbohydrate in molasses for bacteria.

Table 4.5 Degradation of DM using nylon bag technique

Items	**FN**	**FR**	**FP**	**PH**	**PS**	**PSM**	**SEM**	***P*-value**
Fermentation kinetic values								
a	15.9^{b}	14.1^{c}	18.6^{a}	14.4^{c}	11.1^{e}	11.3^{d}	0.78	<0.05
b	37.1^{e}	37.3^{e}	40.5^{d}	43.9^{c}	47.0^{b}	48.4^{a}	1.34	<0.05
c	0.010^{d}	0.013^{d}	0.022^{b}	0.017^{c}	0.026^{a}	0.027^{a}	0.003	<0.05
a+b	53.0^{b}	51.4^{b}	59.1^{a}	58.3^{a}	58.1^{a}	59.7^{a}	1.01	<0.05
Incubation times (hour)								
24	19.7^{b}	18.4^{b}	31.4^{b}	33.0^{b}	29.0^{b}	40.4^{a}	2.23	<0.05
48	51.1^{ab}	46.1^{b}	61.3^{ab}	57.1^{ab}	44.0^{b}	57.7^{a}	2.24	<0.05
72	68.3^{ab}	60.6^{bc}	70.3^{ab}	69.0^{ab}	59.1^{c}	73.7^{a}	2.18	<0.05
96	76.7^{b}	69.8^{c}	77.0^{b}	74.5^{c}	67.6^{d}	80.0^{a}	1.88	<0.05
Effective Degradability (%)								
0.02	39.3^{d}	42.3^{cd}	66.7^{b}	49.2^{c}	64.2^{b}	75.3^{a}	4.15	<0.05
0.05	28.8^{e}	27.2^{f}	63.5^{b}	33.8^{d}	62.8^{c}	66.0^{a}	5.21	<0.05
0.08	24.4^{d}	22.9^{e}	62.9^{b}	28.8^{c}	62.7^{b}	63.9^{a}	5.72	<0.05

$^{a-f}$ within columns for each main effect, values followed by different letters are significantly different (P < 0.05).

Throughout the incubation time, the pangola silages with molasses showed increasing disappearance of DM until 96 h (P<0.05). Moreover, it was found that degradability of insoluble but fermentable fraction (b) increased with fermented silage (from 40.5 to 47.0 and 48.4). Potential degradable fraction or disappearance rates at time (a+b) showed significantly different that may due to difference species. All pangola grass groups had higher DM degradability than fresh napier and ruzi. However,

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pangola silage without addition showed low gas products and low IVDMD by Daisy^{II} incubator, but did not affect the *in sacco* rumen DM, OM and NDF degradability. These findings agree with previous experiment Filya (2003) that indicated effects of strains of lactic bacteria. In addition, Effective degradability (ED) showed significantly difference (P<0.05) at all of rate out flow. Molasses addition resulted the highest at rumen passage rates 0.02, 0.05 and 0.08/h, followed by fresh pangola. The improvement in each parameter increased with molasses additive silage.

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