APPENDIX

APPENDIX A

Method of analysis

In vitro digestibility using Daisy^{II} incubator

2.1 Materials

- DAISY^{II} Incubator - ANKOM Technology
- F57 Filter Bags - ANKOM Technology
- Digestion jar 1 L
- Thermometer
- Cheese cloths for filtering

2.2 Chemical used

1. **Buffer Solution A:** **g/liter**

KH_2PO_4	10.0
$MgSO_4 \cdot 7H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2H_2O$	0.1
Urea (reagent grade)	0.5

2. **Buffer Solution B:** **g/liter**

Na_2CO_3	15.0
$Na_2S \cdot 9H_2O$	1.0

3. **Rumen fluid inoculum**
4. **Neutral Detergent Reagents**

2.3 Methods

1. Weigh each F57 filter bag and record weight (W1). Zero the balance and weigh 0.25g of sample (W2) directly into filter bag. Place in the Daisy^{II} Incubator digestion jar (up to 25 samples per jar). Samples should be evenly distributed on both sides of the digestion jar divider. Include at least one weighed and sealed blank bag for correction factor (C1).
2. Pre-warm (39°C) both buffer solutions (A & B). In separate container, added solution B to solution A (1:5 ratio). The exact amount of A to B should be adjusted to obtain a final pH of 6.8 at 39°C. Add 1600 ml of combined A/B mixture to each jar containing the sample bags.
3. Place digestion jars with samples and buffer solution into **Daisy^{II} Incubator** and activate heat and agitation switches (red lights in switches indicate power). Allow temperature of digestion jars to equilibrate for at least twenty to thirty minutes. This time could be used for collection and preparation of rumen inoculum.

Preparation of Inoculum and Incubation:

1. Purge the blender container with CO_2 gas and continued during the transfer of the inoculum. Empty the rumen inoculum from the thermos through four layers of cheesecloth into a five-liter flask (pre-heated 39° C) and blend at a high speed for 30 seconds.
2. Measure 400ml of rumen inoculum in a graduated cylinder and add the inoculum to each jar contained buffer solution and samples. Purge the digestion jar with CO_2 gas for thirty seconds and secure lid. Repeat process for all digestion jars to be used.
3. Incubate for 48 hours to determine the In Vitro True Digestibility result. The **DAISY^{II} Incubator** will maintain a temperature of 39.5°C ± 0.5. At completion of incubation, remove jars and drain fluid. Rinse bags thoroughly with cold tap water until water is clear. Use a minimum of mechanical agitation.

Analytical procedures

The incubated nylon bag forage samples were analyzed for DM according to AOAC (1984). The NDF residues in forages and bag residues were determined using reagents and methods as described by Van Soest *et al*., 1991 with exceptions. These were that NDF was calculated as the residue in the in vitro bags after 60 min of immersion in boiling ND with sodium sulfite and amylase.

Calculation

$$NDFD\ (\%\ DM) = 100 \times [(W2 \times \%NDF_{Feed}) - (W3 - (W1 \times C1))]/(W2 \times \%DM_{Feed})$$

$$IVDMD\ (\%DM) = 100 - [(W3 - (W1 \times C1)) \times 100] / (W2 \times \%\ DM_{Feed})$$

Where: W1 = Bag tare weight

W2 = weight of sample

W3 = final weight (Filter bag + sample)

NDF_{Feed} = NDF contain in Feed (%DM)

DM_{Feed} = dry matter contain in feed

C1 = correction of factor (blank filter bag)

Organoleptic test (DLD, 2004)

Organoleptic observations mean using the sense organs (eyes, nose, taste, ears, touch) to evaluate the quality of the forage. This method is practical because it can be easily done, requires no special equipment, and is readily applied. It is the simplest method but it provides the least information. Organoleptic observation can be useful in some ways but cannot determine chemical composition.

Silage Characteristic		
1. Odour	Smell like preserved fruits	12 points
	Little pungent	8 points
	Very pungent	4 points
	Rancid smell or mold smell	0 points
2. Texture	Firmly, have many leaves and stems and without adulterated thing	4 points
	Firmly, leaves and stems are soften and mucus	2 points
	Firmly, leaves and stem are very soften and have adulterated things	1 points
	dirty	0 points
3. Colour	Greenish-yellow or Khaki	3 points
	Yellowish-green or bottle-green	2 points
	Hazel	1 points
	Niggerbrown or black	0 points
4. pH	3.5 – 4.2	6 points
	4.4 – 4.7	4 points
	4.7 – 5.1	2 points
	> 5.1	0 points

The sum of total scores will grade:

20 – 25	very good
15 – 19	good
6 – 14	fair
0 – 5	poor

APPENDIX B

Statistical analysis

Table 1 ANOVA: The chemical composition of the forages (Experiment 4.1.1)

	Source	SS	df	MS	F	Sig.
DM	Treatment	13,856.237	5	2,771.247	38,110.210	0.000
	Error	1.309	18	0.073		
	Total	13,857.546	23			
CP	Treatment	3.830	5	1.966	50.682	0.000
	Error	0.698	18	.039		
	Total	10.528	23			
EE	Treatment	3.119	5	.624	.748	.598
	Error	15.007	18	.834		
	Total	18.125	23			
CF	Treatment	46.669	5	9.334	11.238	.000
	Error	14.950	18	.831		
	Total	61.619	23			
NDF	Treatment	140.548	5	28.110	9.227	.000
	Error	54.834	18	3.046		
	Total	195.382	23			
ADF	Treatment	20.897	5	4.179	10.282	.000
	Error	7.317	18	.406		
	Total	28.214	23			
ADL	Treatment	8.240	5	1.648	.951	.473
	Error	31.203	18	1.734		
	Total	39.444	23			
Ash	Treatment	27.995	5	5.599	45.083	.000
	Error	2.235	18	.124		
	Total	30.231	23			

	Source	SS	df	MS	F	Sig.
Ash	Treatment	28.545	5	5.709	18.263	0.000
	Error	5.627	18	0.313		
	Total	34.172	23			

Table 2 ANOVA: Silage characteristic of pangola silage (Experiment 4.1.2)

	Source	SS	df	MS	F	Sig.
DMafter	Treatment	22.781	1	22.781	986.913	0.000
	Error	.139	6	0.023		
	Total	22.920	7			
%loss	Treatment	558.782	1	558.782	274.332	0.000
	Error	12.221	6	2.037		
	Total	571.004	7			
pH	Treatment	1.575	1	1.575	103.440	0.000
	Error	.091	6	0.015		
	Total	1.667	7			
Acetic acid	Treatment	1358.452	1	1358.452	95.226	0.000
	Error	85.593	6	14.266		
	Total	1444.045	7			
Butyric acid	Treatment	12.804	1	12.804	7.698	0.032
	Error	9.979	6	1.663		
	Total	22.783	7			
Lactic acid	Treatment	1635.028	1	1635.028	182.342	0.000
	Error	53.801	6	8.967		
	Total	1688.829	7			
Scores	Treatment	4050.000	1	4050.000	243.000	0.000
	Error	100.000	6	16.667		
	Total	4150.000	7			

Table 3 ANOVA: *In vitro* gas production characteristics of forages in buffered rumen fluid, organic matter digestibility (%) and metabolizable energy (ME) (Experiment 4.1.3)

	Source	SS	df	MS	F	Sig.
G24	Treatment	1920.346	5	384.069	149.089	0.000
	Error	46.370	18	2.576		
	Total	1966.715	23			
G48	Treatment	1571.026	5	314.205	20.502	0.072
	Error	275.862	18	15.326		
	Total	1846.887	23			
G72	Treatment	1630.244	5	326.049	11.289	0.205
	Error	519.873	18	28.882		
	Total	2150.117	23			
G96	Treatment	1782.103	5	356.421	10.023	0.259
	Error	640.083	18	35.560		
	Total	2422.186	23			
OMD	Treatment	749.746	5	149.949	547.443	0.000
	Error	1.643	18	0.274		
	Total	751.389	23			
ME	Treatment	22.997	5	4.599	692.508	0.000
	Error	.040	18	0.007		
	Total	23.037	23			

Table 4 ANOVA: *In vitro* dry matter and fiber digestion using Daisy^{II} incubator (Experiment 4.2)

	Source	SS	df	MS	F	Sig.
IVDMD 24h	Treatment	185.103	5	37.021	2.343	0.080
	Error	284.422	18	15.801		
	Total	469.525	23			

	Source	SS	df	MS	F	Sig.
NDFD 24h	Treatment	102.772	5	20.554	2.957	0.000
	Error	125.114	18	6.951		
	Total	227.886	23			
IVDMD 48h	Treatment	353.264	5	70.653	5.767	0.002
	Error	220.509	18	12.251		
	Total	573.773	23			
NDFD 48h	Treatment	38.189	5	7.638	1.325	0.298
	Error	103.785	18	5.766		
	Total	141.973	23			

Table 5 ANOVA: Degradation of forages in cattle using nylon bag technique (Experiment 4.3)

	Source	SS	df	MS	F	Sig.
24h	Treatment	692.541	5	138.508	4.214	0.010
	Error	591.680	18	32.871		
	Total	1284.221	23			
48h	Treatment	1476.016	5	295.203	13.634	0.000
	Error	389.738	18	21.652		
	Total	1865.754	23			
72h	Treatment	637.609	5	127.522	6.685	0.001
	Error	343.343	18	19.075		
	Total	980.951	23			
96h	Treatment	371.498	5	74.300	29.484	0.000
	Error	45.361	18	2.520		
	Total	416.858	23			
a	Treatment	79.547	5	15.909	704.358	0.000
	Error	.136	18	0.023		
	Total	79.683	23			

	Source	SS	df	MS	F	Sig.
b	Treatment	238.244	5	47.649	3156.496	0.000
	Error	.091	18	0.015		
	Total	238.335	23			
c	Treatment	.000	5	0.000	143.836	0.000
	Error	.000	18	0.000		
	Total	.000	23			
a+b	Treatment	243.153	5	48.631	10.915	0.000
	Error	80.196	18	4.455		
	Total	323.349	23			
ED0.02	Treatment	4257.160	5	851.432	170.611	0.000
	Error	89.829	18	4.990		
	Total	4346.989	23			
ED0.04	Treatment	7130.098	5	1426.020	595.365	0.000
	Error	43.114	18	2.395		
	Total	7173.212	23			
ED0.08	Treatment	8647.449	5	1729.490	1765.864	0.000
	Error	17.629	18	.979		
	Total	8665.078	23			

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Educations

- Wachirawit secondary School, Chiang Mai, Thailand, 2001-2007: Certificate of matayom VI
- Chiang Mai University, Chiang Mai, Thailand, 2008-2012: Bachelor of Science (Agriculture) in Animal Sciences

Scholarship

2014 Partial fund from Graduate School, Chiang Mai University, Thailand.

Presentation

2015 Oral presentation: *In Vitro* Digestibility and Ruminal Degradability of Fresh and Preserved Pangola Grass at 45 days of regrowth stage cutting (Outstanding research). *In:* the 4nd National Animal Science Conference of Thailand. At Prince of Songkla University, Songkla, Thailand.