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The authors advise that the first author's name is incorrect and should be S. Yammuen-art.

Effect of the ratio of maize cob and husk to napier Pakchong 1 silage on nutritive value and *in vitro* gas production of rumen fluid of Thai native cattle

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Abstract. The present study evaluated the chemical composition and *in vitro* ruminal digestibility of napier Pakchong 1 silage combined with maize cob and husk in different ratios. The napier Pakchong 1 grass was harvested at 45 days of maturity. The napier Pakchong 1 grass was ensiled with maize cob and husk at ratios of 1 : 5, 1 : 10 and 1 : 15. Three rumen fistulated Thai native cattle (White Lamphun cattle) with an average weight of 154 ± 4.7 kg were used to determine ruminal digestibility by *in vitro* gas-production technique. Gas production was recorded after incubating for 2, 4, 8, 12, 24, 48, 72 and 96 h. The microbial biomass yield was determined after incubating for 24 h. DM, NDF and ADF of maize cob and husk mixed with napier Pakchong 1 silage declined by increasing the proportion of napier Pakchong 1 grass, while gas production after 4–10 h of incubating maize cob and husk mixed with napier Pakchong 1 grass increased by increasing the proportion of napier Pakchong 1 grass. The metabolisable energy, organic matter digestibility and microbial biomass yield did not differ among the different ratios. The results of the study suggested a recommended ratio of maize cob and husk to napier Pakchong 1 grass of 1 : 10. The ensiling fermentation increased the proportion of protein in the roughage, which lead to increased *in vitro* gas production of roughage.

Additional keyword: ruminal digestibility.

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Introduction

Napier Pakchong 1 grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong 1) is a new hybrid napier created by crossing *P. purpureum* and *P. americanum*. The grass was developed by the Nakhonratchasima Animal Nutrition Research and Development Center, Thailand (Sarian 2013). It is claimed to be highly nutritious, with %crude protein range between 13.29% and 16.64% (Aganga *et al.* 2005). One hectare is capable of producing 480–500 t per year, enough to feed 50 dairy cows for the year (Tinsuntisook 2014). The dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations of napier Pakchong 1 grass are 18.2%, 7.9%, 73.1% and 45.7%, respectively, on a DM basis (Cherdthong *et al.* 2015).

So as to prepare for the seasonal scarcity of feed for livestock in the long dry season, the grass should be stored by silage making. However, because the concentration of water-soluble carbohydrates (WSC) of Pakchong 1 grass is too low for successful ensiling and the moisture content of the grass is high, it should be mixed with an absorbent, such as cassava chips, ground corn or hay. Maize is widely grown in north-eastern Thailand, with some also being grown in the central plains. Maize production byproducts, such as stover or cob and husk, can be used as roughage for ruminants. The DM, CP, ether extract (EE),

NDF and ADF concentrations of maize cob and husk are 94.7%, 6.76%, 1.13%, 82.79% and 42.94%, respectively, on a DM basis (Yammuen-art *et al.* 2012). For making silage, the recommended moisture content is 65–70%; higher moisture content may lead to prolonged fermentation, excessive protein breakdown, and energy loss (Seglar 2003). The DM concentration of maize cob and husk is too high for making silage. Therefore, ensiling napier Pakchong 1 grass with maize cob and husk might provide a suitable option to increase the dry matter and protein concentrations, which might lead to increased end-product fermentation, due to fermentation of the available nutrients. Recommended DM concentrations are 25–35% for silage (Seglar 2003); therefore, the ratio of silage from maize cob and husk (DM ~95%) to napier Pakchong 1 (DM ~20%) should be ~1 : 5–1 : 15. The objective of the present study was to evaluate the chemical composition and *in vitro* ruminal digestibility of napier Pakchong 1 silage combined with maize cob and husk at different ratios.

Materials and methods

Ensiling process

Napier Pakchong 1 grass was grown at Mae Hia Agricultural Research Demonstrative and Training Center, Faculty of

Agriculture, Chiang Mai University, Chiang Mai, Thailand. The grass was harvested at 45 days of maturity and chopped at 3–8-cm length. Maize cob and husk were collected from Mae Jam District, Chiang Mai, Thailand. The experimental design was a completely randomised design. The treatments were napier Pakchong 1 grass ensiled with maize cob and husk at ratios of 1:5, 1:10 and 1:15. The ingredients were mixed thoroughly. Approximately 1 kg of each replicate was collected as the representative of fresh roughage.

All experimental silages were replicated three times. Thereafter, the experimental silages were packed tightly in two-layered plastic bags and vacuumed. After 21 days of ensiling process, ~1 kg of each replicate was collected as the representative of silage roughage.

Analysis of chemical composition

The sample of fresh roughage and silage roughage were collected for chemical analysis. DM, CP, EE and organic matter (OM) were analysed according to the methodology of AOAC (2000). The NDF, ADF and acid detergent lignin were analysed by detergent methods (Van Soest *et al.* 1991).

Determination of ruminal digestibility by *in vitro* gas-production technique

Three rumen-fistulated Thai native cattle (White Lamphun cattle) with an average weight of 154 ± 4.69 kg were used to determine ruminal digestibility by *in vitro* gas-production technique. The cattle were fed 2 kg of concentrate and *ad libitum* maize cob and husk daily. Rumenal fluid was collected in the morning into a 1000-mL Erlenmeyer flask, and transferred into pre-warmed CO₂-filled thermos bottles. The fluid samples were combined with incubation medium before *in vitro* fermentation. The temperature of the rumen fluid was maintained between 37°C and 39°C throughout the preparation of the incubation medium.

The *in vitro* gas-production technique followed the methodology of Menke and Steingass (1988). Three replications of ~230 mg of feed samples were weighed into 100-mL calibrated glass syringes. The syringes were filled with 30 mL of rumen medium buffer solution consisting of 9.8 g of NaHCO₃ + 2.77 g Na₂HPO₄ + 0.57 g KCl + 0.47 g NaCl + 0.12 g MgSO₄·7H₂O + 0.16 g CaCl₂·2H₂O. The syringes were placed in a rotor inside the

incubator at 39°C. Gas production was recorded after incubating for 2, 4, 8, 12, 24, 48, 72 and 96 h.

The metabolisable energy (ME), OM digestibility (OMD) and short-chain fatty acids (SCFA) were calculated using the following equations (Blümmel and Ørskov 1993):

$$\text{ME (MJ/Kg DM)} = 2.20 + 0.136\text{Gv} + 0.057\text{CP} + 0.0029\text{CF},$$

$$\text{OMD (\%)} = 14.88 + 0.88\text{Gv} + 0.45\text{CP} + 0.651\text{XA}, \text{ and}$$

$$\text{SCFA (mol)} = 0.0239\text{Gv} - 0.0601,$$

where Gv = net gas production (mL/200 mg, DM) after 24-h incubation, CP = crude protein, CF = crude fibre and XA = ash.

The MBY was determined after 24-h incubation. The undigested residue was weighed to determine the apparent undigested residue. True digestibility was determined by refluxing the residue samples with neutral detergent solution (NDS) to remove the microbial biomass from the undegraded substrate, followed by filtering in pre-weighed Gooch crucibles (Harikrishna *et al.* 2012).

The MBY was modified to be expressed as mg/100 mg of truly digested sample, according to Blümmel *et al.* (1997):

$$\text{MBY} = (\text{truly digested sample} - \text{apparently digested sample}) / \text{truly digested sample}.$$

Statistical analyses

The means of all measured parameters were statistically analysed with ANOVA under a completely randomised design. Data were analysed using the model $Y_{ij} = \mu + T_i + \epsilon_{ij}$, where Y_{ij} is the observed response, μ is an overall mean, T_i is the treatment effect (ratio of napier Pakchong 1 grass), and ϵ_{ij} is the residual effect. Differences among means were tested using the adjusted Tukey test, with significance declared at $P < 0.05$ (Steel and Torrie 1980).

Results and discussion

Concentrations of DM, CP, NDF and ADF of maize cob and husk (Table 1) in the present study (86.62%, 2.74%, 62.18% and 41.09% respectively) were lower than those reported by Yammuen-art *et al.* (2012) (94.7%, and 6.76%, 82.79% and

Table 1. Chemical composition (% DM basis) of maize cob and husk mixed with different proportions of napier Pakchong 1 grass

MCH, maize cob and husk; NP1, napier Pakchong 1; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFa, free ash neutral detergent fibre; ADFa, free ash acid detergent fibre; NFC, non-fibre carbohydrate. Means within a row followed by different letters, either within fresh (lower-case letters) or silage (upper-case letters) treatments, differ significantly ($P = 0.05$)

Item	MCH	NP1	Fresh			s.e.m.	P-value	Silage			s.e.m.	P-value
			1:5	1:10	1:15			1:5	1:10	1:15		
DM	86.62	22.43	35.48a	31.47b	27.18c	0.942	0.001	35.22A	28.80B	26.27B	1.094	0.001
OM	97.32	81.75	85.80	86.87	85.87	0.726	0.531	94.80A	87.91B	90.81AB	1.421	0.024
CP	2.74	9.16	5.88	5.75	6.14	0.175	0.32	6.19C	6.76B	7.38A	0.07	<0.001
EE	0.27	3.71	1.57c	1.84b	2.27a	0.055	<0.001	3.04	2.65	3.16	0.151	0.097
NDFa	62.18	61.25	64.95	65.22	62.24	1.849	0.481	68.38A	60.83B	61.74B	1.918	0.042
ADFa	41.09	40.48	36.09	37.51	36.96	0.355	0.054	42.09A	35.29C	38.68B	0.801	0.001
Hemi-cellulose	21.09	20.78	28.86	27.69	25.28	1.893	0.430	26.28	25.54	23.06	1.374	0.273
Cellulose	37.83	33.49	17.81	18.84	17.46	0.757	0.441	26.85A	20.01B	22.88AB	1.473	0.028
NFC	32.13	7.63	19.38	18.90	21.27	1.740	0.614	11.99	19.29	17.25	2.030	0.078

Table 2. Gas production, estimated organic matter digestibility (OMD), metabolisable energy (ME) and microbial biomass yield (MBY) of maize cob and husk mixed with different proportions of napier Pakchong 1 grassSCFA, short chain fatty acid. Means within a row followed by different letters, either within fresh (lower-case letters) or silage (upper-case letters) treatments, differ significantly ($P = 0.05$)

Item	Fresh			s.e.m.	P-value	Silage			s.e.m.	P-value
	1:5	1:10	1:15			1:5	1:10	1:15		
<i>Gas production</i>										
2 h	1.72	1.61	2.39	0.383	0.358	2.67B	3.23AB	4.10A	0.300	0.04
4 h	3.11b	3.91b	5.13a	0.326	0.013	4.76B	5.89AB	6.68A	0.450	0.061
6 h	4.95b	7.01a	7.52a	0.361	0.005	6.62B	8.20AB	9.03A	0.481	0.031
8 h	6.80b	8.27b	10.14a	0.438	0.005	8.82	11.09	11.14	0.645	0.072
10 h	10.38b	11.15b	13.67a	0.717	0.040	12.08B	14.55A	12.20B	0.472	0.017
12 h	13.14	13.56	16.29	0.947	0.110	14.86	15.13	16.42	0.447	0.099
24 h	20.86	23.69	22.22	1.843	0.584	23.34	20.33	21.36	1.451	0.389
48 h	33.82	30.349	39.54	2.637	0.119	38.56	38.55	35.91	0.970	0.163
72 h	38.84	37.122	47.09	2.579	0.071	43.46	43.95	40.88	1.331	0.290
96 h	41.34	49.57	50.39	2.743	0.107	44.68	45.34	44.15	1.346	0.829
<i>MBY</i>										
Weight (g)	0.045b	0.046b	0.051a	0.001	0.004	0.049	0.172	0.051	0.075	0.457
Percentage	35.89	36.57	36.58	1.218	0.903	40.93	31.53	39.37	2.729	0.103
<i>Estimated parameters</i>										
ME	5.47	5.83	5.62	0.251	0.586	5.85	5.42	5.56	0.197	0.359
OMD	41.23	43.77	42.42	1.622	0.587	45.97	42.85	43.64	1.277	0.275
SCFA	0.439	0.506	0.471	0.044	0.584	0.498	0.426	0.45	0.035	0.389

42.94% respectively). CP concentration (9.16%) of 45-day-old napier Pakchong 1 grass in the present study was higher than that of the 3-month-old napier Pakchong 1 grass (7.9%), while NDF and ADF concentrations (61.25% and 40.48% respectively) were lower (73.1% and 45.7% respectively) (Cherdthong *et al.* 2015). This may have been due to the stage of maturity because the CP concentration of forages declined with maturity (Jacobs *et al.* 2009).

Dry matter of maize cob and husk mixed with Napier Pakchong 1 grass declined by increasing the proportion of napier Pakchong 1 grass in both fresh and silage forms. This decline was related to the increase in the proportion of water in napier Pakchong 1 grass. NDF and ADF of maize cob and husk mixed with napier Pakchong 1 silage declined by increasing the proportion of napier Pakchong 1 grass. This decline was related to the decrease in the proportion of fibre in napier Pakchong 1 grass. Fermentation clearly affected the NDF content of napier grass. The reduction of NDF may be explained by the hydrolysis of NDF-bound nitrogen during fermentation (Jaakkola *et al.* 2006).

The concentrations of CP, EE and cellulose of maize cob and husk mixed with napier Pakchong 1 silage were significantly higher than those of fresh forage, being in agreement with Danley and Vetter (1973), who reported that ensiled forage had a significantly lower soluble-carbohydrate concentration and significantly higher ADF, cellulose and lignin concentrations than did fresh forage. The higher fibre in ensiled conocarpus leaves was probably due to the decrease of soluble carbohydrate or nitrogen-free extract (NFE). The fibre fractions increased in ensiled conocarpus leaves as crude fibre increased and NFE decreased; this may have been due to the lignin (undetermined component) content (Al Koik *et al.* 2014). WSC are the main source of food for microorganisms during silage fermentation (Bayatkouhsar *et al.* 2012).

Gas production after 4–10 h of incubating maize cob and husk mixed with napier Pakchong 1 grass increased by increasing the proportion of napier Pakchong 1 grass, while gas production after 2–6 h and 10 h of incubating maize cob and husk mixed with napier Pakchong 1 grass increased as the proportion of napier Pakchong 1 silage increased due to the high concentrations of nutrients (Table 2). Gas production results from the fermentation of nutrients. Higher gas values indicate a better nutrient availability for ruminal microorganisms. Higher digestibility arises from an improved nutritional diet, as these sources have higher protein content, lower cell walls content and higher DM digestibility (Barros-Rodríguez *et al.* 2014). OMD, ME and MBY did not differ. The results indicated that fermentation acids in silage do not yield direct gas and, therefore, provide little energy for rumen microbial growth; the contribution of fermentation acids to diet and ME content in diet should be subtracted during the calculation of fermented ME. The present results of ME and OMD in silage agree with those of (El-Waziry 2012). Gas production after 2–10 h of incubation and MBY of maize cob and husk mixed with napier Pakchong 1 silage were significantly higher than those for fresh forage, being in agreement with Tikam *et al.* (2015), who reported higher values of gas-production parameters, with the exception of the rate of gas production in pangola silage compared with its fresh form, probably caused by the lower fibre (cell-wall) content and the higher digestibility of the insoluble fraction. Hemicelluloses might have been partially degraded during the ensiling process (McDonald *et al.* 1991).

Conclusions

The results of the present study indicated a recommended ratio of maize cob and husk to napier Pakchong 1 grass of 1:10. The

ensiling fermentation increased the proportion of protein in the roughage, which lead to increased *in vitro* gas production of roughage. OMD, ME and MBY did not differ between fresh and silage treatments. The ratio of maize cob and husk to napier Pakchong 1 grass had no effect on OMD, ME and MBY.

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