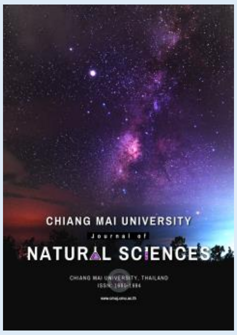

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Research article

Evaluation of Yield Production and Chemical Composition of Three Types of Forage Legumes at Different Cutting Intervals and Cutting Times to Assess Their Benefits as Ruminant Animal Feed

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Abstract The purpose of this study was to evaluate the potential of yield production and chemical composition response to cutting intervals of three forage legumes over a 5 month period of the dry season in Sa Kaeo province for use as an alternative forage for farmers. The experiment was conducted in a factorial arrangement in randomized complete block design. The first factor was the types of forage legumes, including alfalfa, hamata and stylo 'Tha pra' and the second factor was the cutting intervals at 30, 45, 60 and 75 days. The results for stylo 'Tha pra' showed that the average height, total weight per area, fresh weight (FW) and dry weight (DW) per plant were the highest (P <0.001). The cutting interval at 75 days provided the highest average height, FW and DW in all legume forage types. The FW and DW increased with increases in the cutting times (number of cuts) at all cutting intervals for all three forage legume types. The three different types of forage legumes and the different cutting intervals resulted in different chemical compositions (P <0.001) except for dry matter (DM) for which there was only a difference from the cutting interval. Each chemical composition showed an unequal response to the increase in cutting times.

Keywords: Chemical composition, Cutting intervals, Cutting times, Forage legumes, Ruminant feed, Yield production

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INTRODUCTION

The protein content requirements for ruminant production is more valuable in forage legumes than in forage grasses. The benefits of forage legumes are an increase in N availability in soil and the prevention of soil erosion (Schultze-Kraft et al., 2018). Forage legumes, which are suitable for cut and carry management by small farmers, were resistant to grazing or cutting, but able to regrow like alfalfa, stylo 'Tha pra', hamata and stylo 'graham'. Alfalfa (*Medicago sativa*) or lucerne is the most important forage legume to be cultivated in temperate climates for ruminant feed as it provides highly nutritious and palatable forage (Clements, 2019). Several studies showed that alfalfa had high protein content (16.8-25.9%) (Testa et al., 2011; Cacan et al., 2018; Sousa et al., 2020). Under irrigation, alfalfa provided a high yield of 12.6-15.7 ton/ha (Testa et al., 2011). In 2019, Thailand imported 27,513,853 THB (880,161 USD) of alfalfa pellets which was an increase of 7.5 times from the imported value in 2016 (Thai Customs, 2020). These were imported by pet businesses for use with rabbits, rats, and horses, or for human food (alfalfa seedling sprouts). This showed that there was an increasing demand for alfalfa pellets for domestic use. However, there is no commercial alfalfa cultivation in Thailand. *Stylosanthes guianensis* has been adapted to hot and humid climates and can grow on all types of soil being particularly well-adapted to poor acid soils. *S. guianensis* can produce dry matter (DM) up to 10 tons/ha, depending on soil fertility and moisture level ('t Mannetje, 2016). *S. guianensis* CIAT 184 is known as tha pra stylo in Thailand. Stylo 'Tha pra' produces a DM yield of 8.2 and 4.4 ton/ha at 60 and 75 days' cutting intervals, respectively (Kiyothong et al., 2002). DM yield of stylo 'Tha pra' was 4.7 ton/ha in the first year and was reduced to 2.0 ton/ha in the second year (Peters et al., 1994). *S. hamata cv. Verano* (hamata) is suitable for semi-arid to sub-humid tropical regions. It can be cultivated on sandy or clay loam soils and it adapts to low fertile and moderately acidic soils with low phosphorus (Edye and Topark-Ngarm, 2016). The DM yield of hamata was 2.3 ton/ha in the first year and increased to 3.5 ton/ha in the second year (Peters et al., 1994). The average nitrogen content of alfalfa, hamata and stylo 'Tha pra' was in the range of 2.5-4.0% (Clements, 2019), 1.8% (Edye and Topark-Ngarm, 2016) and 1.5-3.0% ('t Mannetje, 2016), respectively. Previous studies that investigated forage yield and quality found that they were affected by growth stages and cutting intervals. Testa et al. (2011) showed that early harvest at the flower buds' appearance of alfalfa had low yield with high protein content and digestibility while late harvest at full flowering led to high yield with low protein content and digestibility. With regard to cutting intervals, crude protein (CP) of alfalfa was reduced by increasing cutting intervals but DM yield of alfalfa at 40 days' cutting interval was higher than at 30 and 60 days (Chen et al., 2012). Kanjanapruthipong and Buathong (2014) also showed that DM of bana grass increases with increasing cutting periods, whereas CP and leaf proportions were reduced. Cutting intervals also affected seed production, for example, when stylo 'Tha pra' was cut at 60 days before flowering it produced a greater weight of 1,000 seeds than at 75 and 90 days (Kiyothong et al., 2002). Potential regrowth depended on the legume species as, *Aeschynomene histrix, Centrosema acutifolium and S. hamata* had a higher yield in the second year than in the first year. By contrast*, C. pascuorum* and *S. guianensis* had a lower yield in the second year than in the first year. Highly frequent cutting affected alfalfa by reducing plant vigor and yield compared to a low frequency of cutting. Orloff (2016) reported that the total seasonal yield in three years for cutting alfalfa 6, 7 and 8 times was 11.45, 9.92, and 9.32 tons per acre, respectively. Moreover, rough fescue and parry oat grass with a high number of cutting times had high CP but low yield, acid detergent fiber (ADF) and lignin compared to a low number of cutting times (Willms, 1991). While napier hybrid grass with a high number of cutting times had a high number of tillers per plant and leaf/stem ratio but low plant height, low total DM per plant, low leaf DM per plant and low stem DM per plant (Wangchuk et al., 2015).

The purpose of this study was to investigate yield production and chemical composition of the three forage legumes over a 5 month period with different cutting intervals in the dry season in Sa Kaeo province in order to encourage Thai farmers to cultivate them. This present study also hypothesized that the optimum number of cutting times in each cutting interval of these three forage legumes would involve a compromise between the quantity and quality of the livestock feed.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in a 3 x 4 Factorial arrangement in a randomized complete block design (RCBD) with 3 replications. There were 2 blocks per replication and each block was divided into 6 plots. The types of legume forages were alfalfa, hamata and stylo 'Tha pra' and the 4 cutting intervals were 30, 45, 60 and 75 days. Each cutting interval had a different cutting time (number of cuts). The experiment was conducted at Burapha University, Sa Kaeo Campus, Watthana Nakhon District, Sa Kaeo Province during October 2018 to July 2019. The temperature of the dry season (a period of low rainfall) in Sa Kaeo ranged between 17.2-39.2°C with an average temperature of 35.7°C (Thai Meteorological Department, 2019). The climate at the Burapha University Farm (13.7°N, 102.2°E, 89 m above sea level) is a tropical lowland climate. The soil is a clayey-skeletal, kaolinitic, isohyperthemic Typic Kandiustults Ultisol (USDA taxonomy) (Land Development Department, 2019). Soil was collected at 30 cm depth (Atis et al., 2019) and was analyzed for soil properties at the Laboratory, Department of Soil Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province in Thailand. The total nitrogen (N) was analyzed by the Kjeidahl method. Organic matter was analyzed by the Walkley and Black method. Available phosphorous (P) was analyzed by Bray-II. Exchangeable potassium (K), calcium (Ca) and magnesium (Mg) were analyzed by using 1N NH_4OAc and AAS.

Planting of Legumes

Three forage legume seeds were obtained from the Bureau of Animal Nutrition Development, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand. Seeds were grown in trays for 30 days and then the seedlings were transplanted to field plots (3 x 3 m). Each plot had 4 rows with 16 plants per row. The distance between the rows was 50 cm. Plants were watered every 4 days. At transplanting, 18.75 kg N/ha, 18.75 kg P/ha and 18.75 kg K/ha were applied; 28.75 kg N/ha was applied at 6 weeks after transplanting and every 40 days after the first cut. At 2 months after transplanting, all legumes plants were cut at 10 cm above the soil surface to a standard height. After that, plants were harvested at cutting intervals according to the experimental design. The number of cuts for the cutting intervals at 30, 45, 60 and 75 days were 5, 3, 2 and 2 times, respectively.

Growth characteristics and yield production

The growth characteristics of alfalfa, hamata and stylo 'Tha pra' plants were recorded at every cutting times for all treatments. The heights of the 10 plants in each plot of all treatments were collected manually from ground level to the tip of the highest leaf by using a measuring tape and then they were cut at 10 cm above ground level by using a wood stick as a marker. All plants in each plot were weighed for total fresh yield per plot and the total fresh yield per hectare was calculated. The samples of five plants per plot were weighed for fresh weight (FW) per plant for all treatments. These plant samples were oven-dried at 60°C for 72 h to obtain the dry weight (DW) per plant at the Agricultural and Technology Research Center, Faculty of Agricultural Technology, Burapha University, Sa Kaeo Campus.

Chemical analysis

After the fresh alfalfa, hamata and stylo 'Tha pra' plant samples were oven-dried at 60°C for 72 h to obtain a dried sample, they were were ground through a 1-mm screen in order to analyze the chemical composition using the standard methods of the Association of Official Analytical Chemists (AOAC, 2016). Dried, ground samples of each plants were divided into two portions; the first portion was used to determine the

DM content by oven-drying at 105°C for 16 h. An analysis of ash content was determined using the official AOAC method (2016; method 942.05) by ashing in a temperature-controlled furnace at 600°C for 3 h. The second portion was kept in a tinted, plastic zip lock bag and stored at 4°C for no more than one month prior to an analysis of the tannin as described by Burns (1971). Crude protein (CP) (method 2001.11), ether extract (EE) (denoting fat (crude)) (method 2003.05 and 954.02) and crude fiber (CF) (method 978.10) analyzed were determined using an in-house method based on the official AOAC method (2016), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed following the official AOAC method 2002.04; 973.18 and 973.18, respectively, at the Feed Analysis-2 Laboratory. Cellulose was calculated by ADF-ADL content (%), and the NDF-ADF content (%) was hemicellulose.

Statistical analysis

All plant characteristics and chemical composition data were statistically analysed using analysis of variance procedures for 3x4 Factorial arrangement in RCBD with the following statistical model:

$$Y_{ijk} = \mu + R_k + L_i + C_j + (LxC)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = the dependent variable, μ = the overall mean, R_k = the block effect, L_i = the type of forage legume effect, C_j = the cutting interval effect, $(LxC)_{ij}$ = the interaction between type of forage legume and cutting interval effect, and ε_{ijk} = the random residual error.

All data were analyzed by using R statistics (R Core Team, 2019). The mean differences among the treatments were compared by Duncan's multiple range test procedure.

RESULTS

Soil Conditions

The soil properties of the study area before and after the experiment are shown in Table 1. Soil after the experiment had a higher average soil EC, available P, exchange Ca and exchange Mg than before the experiment but low total N content and organic matter. In particular, the exchange Ca increased 2 times from before the experiment.

Table 1. Soil chemical properties before and after experiment.

Characters	Before Experiment	After Experiment
pH	7.07	7.05
EC[1] (dS/m)*	0.82	1.26
Organic matter (%)	2.57	2.34
Total N (%)	0.10	0.07
Available P (mg/kg)	13.64	19.60
Exchangeable K (mg/kg)	88.95	88.80
Exchangeable Ca (mg/kg)	2979.90	6185.89
Exchangeable Mg (mg/kg)	241.82	296.81

Note: [1]/ Electrical Conductivity; */ decisiemens/meter

Forage legume yield

The plant characteristics of all 3 types of forage legumes are shown in Table 2. There were no interaction effects on the surviving number of plants or total fresh yield.

The type of legume forage and cutting intervals effects on the surviving number of plant and total fresh yield were found. The average surviving number of hamata were 91.5% and stylo 'Tha pra' plants were 91.4% respectively, which were similar, while the average survival rate of alfalfa plants was 68.2%. The cutting interval at 30 days showed the least total fresh yield for all three legume forages although they were frequently harvested. The average total fresh yield of stylo 'Tha pra' was significantly higher than that of alfalfa but not different from that of hamata (P <0.001) (Table 2). Legume plants which were cut at intervals of 60 days showed the highest yield of 34.4 ton/ha.

The effect of legume types x cutting intervals interaction were significant for plant height, fresh weight and dry weight per plant (P <0.001) (Table 2). The results showed that each type of forage legume had an unequal response to increased cutting intervals. Stylo 'Tha pra' with a cutting interval at 75 days of treatment had the highest plant height, followed by stylo 'Tha pra' with a cutting interval at 60 days of treatment, which were 102.0 and 82.6 cm, respectively. With regard to the plant heights at each harvest time for every cutting interval (Figure 1), it was found that the height of alfalfa and hamata increased at the second cut of all the cutting intervals, but it did not increase in the case of stylo 'Tha pra'. Treatment of stylo 'Tha pra' with a cutting interval of 75 days showed both the highest FW and DW of 364.9 and 73.4 g/plant, respectively. All treatments of alfalfa produced the lowest FW (11.7-36.0 g/plant) and DW (2.1-10.7 g/plant). In Figure 1, it can be seen that FW increased according to the number of cuts in all treatments, except for stylo 'Tha pra' at a cutting interval of 75 days. The DW also showed the same results as for FW.

Chemical composition of forage legumes

The DM, CP, EE, CF, ash, NDF, ADF, ADL, cellulose, hemicellulose, and tannin of the alfalfa, hamata and stylo 'Tha pra' are presented in Table 2. It was found that there was a significant interaction between legume forage species, and the cutting intervals for CF, ash, NDF and cellulose. The different types of forage legumes effects on CP, EE, ADF, ADL and hemicellulose were found Which were due to the effects of the cutting intervals on CP, ADF, ADL, hemicellulose and tannin. The mean values of DM for alfalfa, hamata and stylo 'Tha pra' ranged from 95.4 to 95.6% for cutting intervals at 30, 45, 60 and 75 days which ranged from 95.1 to 95.9%. The longer period of cutting intervals from 30 to 75 days for all forage legumes significantly increased the mean values of CF, NDF, ADF, ADL, cellulose and hemicellulose but CP, EE, ash and tannin showed different values.

Alfalfa had considerable amounts of CP, ash, tannins and hemicellulose but smaller amounts of NDF, ADF, ADL and cellulose than those of hamata and stylo 'Tha pra' (P <0.05). Alfalfa had a constant tannin value throughout at the different cutting intervals (1.5%). Stylo 'Tha pra' had higher amounts of NDF, ADF, ADL than the other two types of legumes but the lowest CP, EE and hemicellulose. Stylo 'Tha pra' also had the highest CF but it was the same as for hamata. While hamata had the highest EE and cellulose but the least tannin compared to the other two types of legumes.

CP declined when the cutting intervals were extended (P <0.05). ADF, ADL and cellulose increased when the cutting intervals increased from 30 to 45 days, then reduced at 60 days and then increased again at a cutting interval of 75 days. CF, NDF, ADF, ADL, cellulose and hemicellulose had a high value for a cutting interval of 75 days. ADF for all the forage legumes increased protein content but this declined when the cutting intervals were extended (P <0.05). The tannin content of all forage legumes was similar for the different cutting intervals between 45 to 75 days, and a highly significant difference was found for the cutting interval at 30 days. The results showed that tannin accumulated in the early stages of growth of the legumes or cutting at 30 days, then decreased for late cutting intervals at 45, 60 and 75 days.

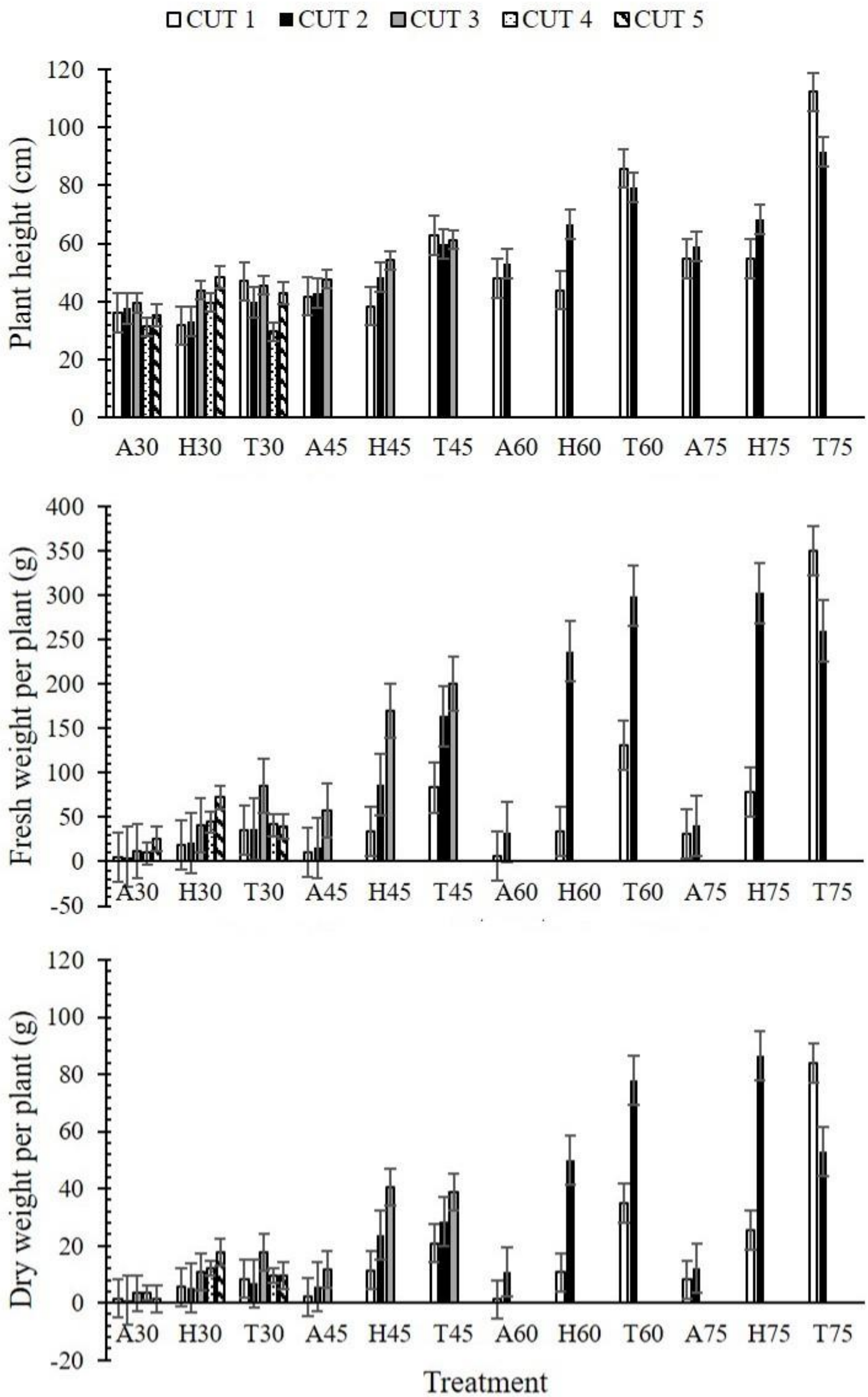

Figure 1. **Average height of plant, fresh weight per plant and dry weight per plant (mean ± SE) of 3 forage legumes with different cutting intervals and different cutting times.**

Table 2. Characteristics (mean) of three forage legumes at different cutting intervals.

Characters	Alfalfa				Hamata				Stylo 'Tha pra'				SEM	Pr>F		
	Cutting interval				Cutting interval				Cutting interval							
	30	45	60	75	30	45	60	75	30	45	60	75		L[1]	C[2]	LXC
Survival plants (%)	71.3	88.0	84.9	83.1	97.7	97.9	96.6	88.0	96.0	97.6	99.5	90.9	1.59	***	*	ns
Plant height (cm)	35.9	44.1	50.4	56.9	39.3	46.9	55.3	61.5	40.8	61.3	82.6	102.0	3.14	***	***	***
Yield (ton/ha)	2.0	3.9	3.3	3.7	18.9	18.6	53.5	26.1	20.0	29.5	46.2	53.6	4.05	***	*	ns
Fresh weight per plant (g)	11.5	27.7	19.8	36.0	39.6	97.2	104.9	190.2	48.0	148.9	215.2	304.7	15.78	***	***	***
Dry weight per plant (g)	2.1	6.5	6.1	10.7	10.7	25.1	31.2	55.9	10.6	31.1	56.4	73.4	3.96	***	***	***
DM[3] (%)	95.5	95.2	96.0	95.5	95.6	96.3	95.8	94.7	94.8	95.7	95.8	95.3	0.20	ns	ns	ns
CP[4] (%)	18.1	17.8	18.6	16.7	17.4	15.8	14.9	14.8	18.0	14.8	13.2	13.0	0.31	**	***	ns
EE[5] (%)	2.0	2.0	2.2	2.0	2.1	2.2	2.6	1.8	1.8	1.7	1.7	1.5	0.05	***	ns	ns
CF[6] (%)	23.0	25.4	23.9	27.0	29.0	32.8	33.0	36.3	26.0	33.1	34.0	38.4	0.62	***	***	**
ASH (%)	8.4	8.3	8.5	7.7	7.9	8.0	7.3	8.9	9.2	7.9	7.5	7.2	0.13	ns	ns	*
NDF[7] (%)	39.3	40.6	37.9	45.7	42.3	48.9	50.7	52.5	40.7	50.0	49.7	57.6	0.77	***	***	**
ADF[8] (%)	31.7	35.7	32.8	36.6	40.4	47.0	45.7	48.7	40.7	49.0	48.3	52.3	0.86	***	***	ns
ADL[9] (%)	7.2	8.2	7.5	8.9	8.1	10.2	9.5	9.8	11.7	13.6	12.8	13.7	0.32	***	**	ns
Cellulose (%)	24.5	27.6	25.3	27.7	32.3	36.8	36.2	38.9	29.0	35.4	35.5	38.5	0.63	***	***	*
Hemicellulose (%)	7.6	4.9	5.1	9.1	1.9	1.9	5.0	3.8	0.01	1.0	1.4	5.3	0.44	***	**	ns
Tannin (%)	1.5	1.5	1.5	1.5	1.6	1.1	1.0	1.2	1.7	1.3	1.5	1.1	0.05	ns	*	ns

Note: [1]/Forage legumes, [2]/Cutting intervals, [3]/Dry matter; [4]/Crude protein; [5]/Ether extract; [6]/Crude fiber; [7]/Neutral detergent fiber; [8]/Acid detergent fiber; [9]/Acid detergent lignin. */ significance at *P* <0.05, **/ significance at *P* <0.01, ***/ significance at *P* <0.001, ns non-significance (*P* >0.05).

Table 3. Average dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash and neutral detergent fiber (NDF) (%) of three forage legumes cut at different times and different intervals.

Item	Cutting Interval (days)	Alfalfa						Hamata						Stylo 'Tha pra'					
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean
DM[1]	30	95.93	96.17	94.99	94.93	-	95.51	97.08	95.92	96.36	95.19	93.48	95.61	96.49	95.72	94.13	94.30	94.50	95.03
	45	97.13	95.56	92.78			95.16	97.78	97.39	93.65			96.27	96.78	96.18	94.70			95.89
	60	96.17	95.87				96.02	95.81	95.75				95.78	96.34	95.33				95.84
	75	96.65	94.33				95.49	94.43	94.76				94.60	95.72	94.78				95.25
CP[2]	30	14.16	20.10	16.45	23.67	16.10	18.09	16.93	18.29	16.12	20.19	15.42	17.39	16.22	18.80	16.38	23.45	15.28	18.02
	45	17.08	16.79	19.57			17.81	15.72	15.54	16.12			15.79	13.39	15.59	15.51			14.83
	60	17.63	19.53				18.58	15.09	14.70				14.90	12.63	13.82				13.22
	75	18.50	14.99				16.75	16.52	13.03				14.77	13.55	12.40				12.97
EE[3]	30	2.06	2.35	2.15	2.06	1.33	1.99	2.35	2.29	1.77	1.90	2.01	2.06	2.02	1.45	1.47	2.09	2.03	1.81
	45	1.90	2.13	2.06			2.03	3.00	1.40	2.33			2.24	1.81	1.22	2.13			1.72
	60	1.93	2.52				2.23	3.24	1.88				2.56	1.67	1.66				1.66
	75	2.22	1.87				2.05	2.10	1.57				1.83	1.39	1.56				1.48
CF[4]	30	24.41	21.62	23.78	19.65	25.57	23.00	28.50	27.88	30.60	26.34	31.60	28.98	27.44	24.07	30.60	20.98	27.00	26.02
	45	27.71	26.41	22.07			25.40	30.85	34.20	33.27			32.77	31.91	34.46	32.82			33.06
	60	23.53	24.19				23.86	31.39	34.69				33.04	33.66	34.41				34.03
	75	23.40	30.66				27.03	32.53	40.15				36.34	36.37	40.50				38.44
Ash	30	8.86	8.78	8.00	8.10	8.09	8.36	8.27	8.26	7.82	8.65	6.57	7.91	8.86	9.13	9.59	10.58	7.73	9.18
	45	8.24	7.63	8.90			8.26	7.61	8.83	7.69			8.04	7.12	8.49	8.07			7.89
	60	8.19	8.85				8.52	7.67	6.90				7.28	7.16	7.81				7.48
	75	7.99	7.33				7.66	11.92	5.91				8.91	7.93	6.48				7.20
NDF[5]	30	37.23	36.66	39.59	37.78	45.10	39.27	40.66	47.21	44.86	35.00	43.57	42.26	40.37	42.47	44.55	32.42	43.53	40.67
	45	44.51	41.91	35.49			40.64	43.81	54.01	48.91			48.91	50.75	47.83	51.41			50.00
	60	33.59	42.15				37.87	52.81	48.56				50.68	49.74	49.65				49.70
	75	42.09	49.24				45.66	52.44	52.59				52.52	58.56	56.61				57.59

Note: [1]/Dry matter; [2]/Crude protein; [3]/Ether extract; [4]/Crude fiber; [5]/Neutral detergent fiber

Table 4 . Average acid detergent fiber (ADF), acid detergent lignin (ADL), tannin, cellulose and hemicellulose (%) of three forage legumes cut at different times and different intervals.

Item	Cutting Interval (days)	Alfalfa						Hamata						Stylo 'Tha pra'					
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	mean
ADF[6]	30	29.42	27.59	32.29	29.80	39.28	31.68	39.11	40.64	44.51	35.70	41.94	40.38	39.80	41.83	46.71	33.60	41.34	40.65
	45	37.57	35.65	34.03			35.75	42.86	49.66	48.63			47.05	46.93	47.98	52.12			49.01
	60	32.56	33.01				32.78	43.31	48.09				45.70	48.51	48.04				48.28
	75	31.49	41.68				36.59	45.61	51.86				48.73	49.74	54.76				52.25
ADL[7]	30	6.59	5.56	7.23	7.23	9.49	7.22	7.48	9.17	9.88	5.34	8.44	8.06	10.62	15.22	13.05	7.13	12.40	11.68
	45	8.86	7.75	7.88			8.16	9.51	10.48	10.73			10.24	13.24	11.13	16.46			13.61
	60	7.61	7.29				7.45	9.04	10.02				9.53	13.77	11.89				12.83
	75	6.75	10.96				8.85	8.60	11.06				9.83	13.48	13.97				13.73
Tannin	30	1.41	1.69	1.46	1.77	1.26	1.52	1.37	1.23	1.36	2.12	1.72	1.56	1.53	1.28	1.17	3.16	1.55	1.74
	45	1.34	1.47	1.62			1.48	0.96	1.18	1.15			1.10	1.24	1.33	1.25			1.27
	60	1.52	1.37				1.45	0.80	1.29				1.04	0.94	1.99				1.47
	75	1.79	1.16				1.47	1.32	1.13				1.22	1.11	1.19				1.15
Cellulose	30	22.83	22.04	25.06	22.58	29.80	24.46	31.64	31.48	34.64	30.36	33.51	32.32	29.19	26.62	33.66	26.48	28.94	28.98
	45	28.71	27.90	26.16			27.59	33.35	39.19	37.90			36.81	33.69	36.86	35.66			35.40
	60	24.95	25.72				25.33	34.27	38.07				36.17	34.74	36.16				35.45
	75	24.74	30.73				27.73	37.01	40.80				38.90	36.26	40.79				38.53
Hemicellulose	30	7.81	9.07	7.31	7.98	5.82	7.60	1.55	6.57	0.34	-0.70	1.63	1.88	0.57	0.63	-2.16	-1.18	2.19	0.01
	45	6.95	6.26	1.46			4.89	0.95	4.35	0.28			1.86	3.82	-0.16	-0.71			0.99
	60	1.03	9.14				5.09	9.50	0.47				4.99	1.23	1.61				1.42
	75	10.60	7.56				9.08	6.84	0.73				3.79	8.82	1.85				5.34

Note: [6]/Acid detergent fiber; [7]/Acid detergent lignin

Number of cuts and chemical composition

The chemical composition data for each number of cuts in all treatments are shown in Tables 3 and 4. The amount of CP, lignin and tannin slightly increased as the cutting times increased. Meanwhile the amounts of hemicellulose and DM gradually reduced in response to increases in the cutting times. All legumes had high DM at the first cutting times. At a cutting interval of 30 days, alfalfa, hamata and stylo 'Tha pra' resulted in high values of CP and tannin at the fourth cut. However, at a cutting interval of 75 days, all legumes had high CP content and hemicellulose at the first cut. Hamata showed high amounts of EE at the first cut while alfalfa showed high EE at the second cut at 30, 45 and 60 days. ADF and ADL were high at the second cut 75 days. Cellulose of all legumes was high at the second cut of 60 and 75 days' cutting intervals.

DISCUSSIONS

While forage legumes pasture increased N availability in soil (Schultze-Kraft et al., 2018) but frequent cutting of forage legume in this experiment resulted in low N from soil due to the cut-carry method used for forage which removed more soil nutrient from the soil than under pasture (Sigua et al., 2006). This indicates that forage legume uses the soil nutrient more for higher regrowth than for returning N back to the soil. The content of P after the experiment was higher than that before the experiment because forage legume requires low P (McCaskill et al., 2019). Therefore, the phosphorous from the fertilizer remained in the soil.

Alfalfa showed the lowest percentage of survival of all the plants. During the experiment, there was an occurrence of common leaf spot disease. This disease causes yield reductions and lowers forage quality through leaf loss (Undersander et al., 2011). The repeated cutting of alfalfa without a break significantly weakens the plant; this also reduces plant vigor and escalates the plant's susceptibility to diseases and insects (Orloff, 2016). But this disease was not found in hamata and stylo 'Tha pra'. Moreover, hot and humid conditions in the tropics causes several diseases in alfalfa (Clements, 2019). The total fresh yield of all three forage legumes was low at a cutting interval of 30 days with 5 cutting times. This is also consistent with a report that frequent cutting resulted in decreasing forage yield (Atis et al., 2019).

Stylo 'Tha pra' provided high total yields, which were different from the other 2 types (Table 2). This might be due to the facts that stylo 'Tha pra' is tolerant to drought and can adapt to all soil types (Cook et al., 2005). It was reported that S. guianensis remained greener throughout the dry season than *S. hamata* (Peters et al., 1994). Similarly, hamata, is suited to semi-arid sub-humid tropical conditions and is also well adapted to low fertile soil (Edye and Topark-Ngarm, 2016). Moreover, hamata can start flowering within 6 weeks of the beginning of the season and will continue throughout the growing season. The reproductive part (flowers and seeds) is larger than the vegetative part (leaves and stems) and provides the main yield for animal feed. On the other hand, stylo 'Tha pra' is a short-day plant which only flowers once a year. Thus, stylo 'Tha pra' has long periods of vegetative growth which result in a high fresh yield (Cook et al., 2005). Similarly, hamata shows longer periods of flowering (end of rainy season to early dry season) than stylo 'Tha pra' (dry season) (Peters et al., 1994), while Alfalfa is adapted to temperate and Mediterranean climates (Clements, 2019).

In this study, the plant height of forage legume increased when the cutting intervals increased (Table 2) which corresponds to what was reported by Manyawu et al. (2003) and Wangchuk et al. (2015). Moreover, the height at the second cut of alfalfa and hamata was higher than at the first cut for all cutting intervals (Figure 1) but not for stylo 'Tha pra'. Similarly, Ansa and Garjila (2019) reported that the height of elephant grass decreased with increased cutting times at cutting intervals of 5, 10, 15 and 20 days.

The age of plants at cutting has an impact on yield. The present study shows that FW and DW increased significantly with increases in the cutting age, which is consistent with the research of Hare et al. (2013). This is due to the fact that yield components such as plant height, stem length or number of leaves increases with increased harvest age. On the contrary, Njarui and Wandera (2004) reported that dry matter yield of

S. guianensis and *S. hamata* were not affected by cutting intervals (at 21, 42, 63 and 84 days). While alfalfa was significantly affected by cutting intervals (at 30, 40 and 60 days) which reduced yield and yield components. Alfalfa when cut at 40 days had a higher yield and shoot numbers than when cut at 30 and 60 days (Chen et al., 2012).

Considering the yield at each cutting time of all cutting ages and legume types (Figure 1), we found that fresh and dry weight per plant increased according to the number of cutting times except for stylo 'Tha pra' at a cutting interval of 75 days of which the yield per plant decreased at the second cut. There were no previous reports about yield per plant with different number of cuts and at the same cutting intervals. However, similar research by Peters et al. (1994) revealed that yield in the second year of *S. guianensis* was lower than in the first year whereas *S. hamata* produced a higher yield. In contrast, Srisaikham and Rupitak (2021) reported that at a cutting interval of 30 days, fresh and dry weight per plant of alfalfa decreased at the second cut.

The proximate chemical composition of all forage legumes has been used as a parameter to evaluate how the effects of different cutting intervals are linked to nutritional status changes in legumes. The chemical compositions of all forage legumes in this study showed that CP, ash and tannin decreased with increasing cutting intervals (30, 45, 60 and 75 d), whereas DM, EE, CF, NDF, ADF, ADL, cellulose, and hemicellulose increased with increasing cutting intervals. In general, the grass stage gives more yield with decreased nutritional value (Manyawu et al., 2003). The chemical compositions of EE, CF, NDF, ADF, ADL, and hemicellulose and cellulose increased with the cutting intervals in response to the positive effects of the renewal of the cut increased depending on the plant species, which is consistent with the report of Manyawu et al. (2003), who states that the DM, NDF and ADF of napier grass and hybrid *Pennisetums* increases with longer cutting periods (8 weeks), whereas the protein is reduced. Timpong-Jones et al. (2015) also reported that DM, NDF, ADF and cellulose content of cynodon grass increased with increased cutting intervals while CP decreased. This study found that the fat content (EE) increased with longer cutting intervals. On the contrary, Lounglawan et al. (2014), reported that varying cutting intervals did not affect the fat content of King napier grass.

We found that alfalfa had higher CP, ash, tannins and hemicellulose, especially at a cutting interval of 30 days. These results are consistent with those of several researchers (Hare et al., 2013; Min et al., 2019). Plants which are cut early usually result in a lower yield but higher protein than at later cutting intervals, thus this stage is suitable for the feeding of young animals that require high nutrients compared to more mature animals that require low nutrients as for non-lactating and non-reproducing animals (Hare et al., 2013). However, the nutrient value of forage protein is mainly determined by animal growth. Min et al. (2019) reported that the greater the average daily gain (ADG) of ruminant animals (goats) observed with sunn hemp forage diets (legumes) or bermudagrass was probably due to the higher CP intake of sheep and goats (Hegarty et al., 2010;), However, according to Min et al. (2016) this depends on a greater forage intake compared to a grass-based diet (Niderkorn and Baumont, 2009). However, the intake of forage increased linearly with decreasing legume content as well ($P < 0.01$). Crampton et al. (1960) suggest this may be due to the low palatability of legumes owing to their high tannin content (Cadisch et al., 1996).

Stylo 'Tha pra' has NDF, ADF, ADL in higher amounts than in other forage legumes. NDF is the most common measure of fiber used for animal feed analysis, but it does not represent a unique class of chemical compounds. NDF measures most of the structural carbohydrates in the components of a plant cell wall which is arranged in layers and contains, for example, cellulose microfibrils, hemicellulose, pectin, and lignin which need to be accurately measured to obtain the dietary fiber level (AOAC, 2002; Sticklen, 2008; Abdelrahman et al., 2018) which in turn affects the feed intake, passage rate and consequently the nutrient digestibility of ruminants. It is crucial to identify the proper dietary NDF level in a diet. Although fiber is considered to be an important ingredient in a ruminant animal diet since it is efficiently utilized by fiber-degrading bacteria in the rumen to produce beneficial end-products. These end-products are most important and beneficial to ruminants. They include volatile fatty acids (VFAs) comprising acetic acid (C_2), propionic acid (C_3), and butyric acid (C_4) and a few groups of iso-butyric acid (iso-C_4), iso-valeric acid (iso-C_5) and valeric acid, which are important energy sources for ruminants for maintenance, reproduction and meat and milk production. Few studies on comparisons between dietary fiber levels from tropical legumes fed to ruminants are found in the literature, especially in alfalfa in Thailand. Recent nutritional requirements reported a limited NDF intake for ruminants. The level of NDF in an animal ration influences the ruminant's intake of DM and the period of rumination. The concentration of NDF in feeds is negatively correlated with energy concentration. Noor et al. (2018) reported that the CF of millet was highest at a cutting interval of 75 days which corresponds to increases in plant height, number of leaves, leaf area, stem diameter and fresh and dry weight per plant while CP decreased. These results are in accordance with our results which revealed that ADF of all forage legumes increased while protein content declined when the cutting intervals were extended (P <0.05). The relative content of NDF, ADF and ADL is an important factor for fiber digestibility in ruminants before the utilization of the cell content (glucose or starch) and thus has potential effects on DM intake (DMI) and the various rumen fermentation parameters. Tafaj et al. (2007) reported that a linear decline in the DMI of ruminant animals was consequent upon an increased diet of NDF, thus the NDF in legumes is a major factor affecting feed intake and rumen fill in high-producing animals especially when using *ad libitum* roughage due to DMI at high NDF concentrations in diets is regulated by the rumen fill of ruminants (Mertens et al., 1994).

In the current study, longer cutting intervals (75 days) led to a dramatical increase in the content of CF, NDF, ADF, and ADL, particularly in stylo 'Tha pra' compared to other legumes, accounting for up to 45% of both NDF and ADF. The impact of feeding different levels of 30.88% NDF (medium level is recommended) as total mixed rations on growing Naemi male lambs compared with low and high NDF (25.67 and 55.93% NDF, respectively) was investigated by Abdelrahman et al. (2018), who found that growing lambs fed barley grain and alfalfa hay in a total mixed ration (TMR) with around 30% NDF improved their general performance. A significantly higher ADG (P <0.05) and lower value for the feed conversion ratio of lambs fed 30.88% NDF in TMR dietary regimes with iso-caloric and iso-nitrogenous content was compared with these other dietary groups. Alfalfa was found to have a higher level of hemicellulose and a lower level of cellulose than other legumes (P <0.05). Hemicellulose is a carbohydrate that can be calculated from the difference between NDF and ADF, while the amount of cellulose can be calculated from the difference between ADF and ash. Chemically, hemicelluloses are the second most important constituent of plants, and they are classified as polymers of sugars (e.g. the six-carbon sugars: mannose), galactose, glucose, and 4-O-methyl-d-glucuronic acid and the five-carbon sugars xylose and arabinose, unlike cellulose, which is made only from glucose (Asif, 2009; Bajpai, 2018). Ruminal bacteria can be directly ultilised by ruminally fermentable nonstructural carbohydrates (e.g. starch and sugars). Lignin strengthens plant cell walls and increased with age.

The highest average tannin content (Table 2) of alfalfa was found in the whole plant of DM (1.5% tannin), stylo 'Tha pra' (1.4% tannin), and the lowest in hamata (1.2% tannin). These results indicate the existence of tannin in individual legumes, such as alfalfa, stylo 'Tha pra' and hamata. There is a specific relationship between the elect-type regarding the same characteristic legume type. These findings show the indirect benefits of using legumes for ruminant feed with high tannin at 30 days, due to condensed tannin (CT) in some legume forage species which improved intake, growth, and the milk production of ruminants (Wang et al., 1996; Min et al., 2003; Hymes-Fecht et al., 2013). Moreover, legumes had anthelmintic effects (Terrill et al., 2007) when fecal nematode eggs in the infected sericea lespedeza hay fed male kid (Kiko x Spanish) goats were reduced (Moore et al., 2008). Huang et al. (2011a, 2011b) reported that legumes can inhibit methane (CH_4) production and is effective for improving ruminant performance (Waghorn, 2008). In general, many legumes contain condensed tannins (CT) (Broderick et al., 2017) which comprise one of two large groups of tannins (Yoshida et al., 2005). Moderate CT concentration (2-4%) in dietary DM leads to better utilization of dietary protein for the nutrition and health of ruminants (Cleef and Dubeux 2019). Observations of toxic effects in feeding studies have been reported using CT from sabiá (*Mimosa caesalpiniifolia* Benth.), while Alves et al. (2011) found that it reduced the consumption of sheep and goats by 13.3 and 13.4%, respectively. Although, surprisingly, the tannin content of forage legumes was found to be higher after 30 than 45, 60 and 75 days, the cutting intervals did not affect the amount of tannin in alfalfa in this experiment and the CT content and biological activity depend on harvested forages which vary between forage types (Acuña et al., 2008; Grabber et al., 2014), harvest time (Theodoridou et al., 2010), preservation method (Hagerman, 1988; Terrill et al., 1994) and growing conditions (Acuña et al., 2008; Grabber et al., 2014).

The average value of the chemical composition of forage legumes was calculated from the value for each cutting time. With regard to the cutting times, the CP of three legumes resulted in high values at the fourth cut of short cutting periods (at 30 days) while the CP of long cutting periods (75 days) resulted in higher content at the first cut. This contrasts with the results obtained by Vendramini et al. (2014), who found a decrease in the CP of the brachiaria grass hybrid at the second cut of short cutting periods (3 weeks) but an increase at the second cut of medium cutting periods (6 weeks). However, our study is consistent with that of Timpong-Jones et al. (2015) who found that the protein content of cynodon increased with an increase in the number of cutting times with short cutting intervals (6 weeks) but declined with longer cutting intervals (12 and 24 weeks). While NDF, ADF and cellulose content which are plant cell wall constituents decreased at cutting intervals of short periods. Ansa and Garjila (2019) showed that the leaf area of elephant grass decreased with an increase in the number of cutting times at short cutting intervals (5 days). Conversely, as plants age their leaves are older and require longer regrowth intervals (20 days). Those old leaves had low nitrogen content but high fiber content. Increasing regrowth intervals increased NDF and ADF, but reduced CP in stems and leaves (Hare et al., 2013). Therefore, increasing the number of cutting times resulted in high CP content for short regrowth intervals, while increasing the number of cutting times resulted in low CP content for long regrowth intervals.

CONCLUSION

The present study indicates that stylo ‘Tha pra’, cut at 75 days, had high yield production and chemical composition, followed by hamata cut at 60 days, although alfalfa had a high CP, and low yield unsuitable for the production of alternative forage types as ruminant feed in the Sa Kaeo province area. It was found that an increase in the number of cutting times had an effect on chemical composition for short cutting intervals (30 days) rather than long cutting intervals (75 days).

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