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Research article**A Preliminary Study on Growth, Yield and Nutritive Value of Four Varieties of Alfalfa and the Utilization of Alfalfa Dehydrated Pellets in a Total Mixed Ratio in Meat Goat Diet****Supreena Srisaikhram* and Quanjai Rupitak****Faculty of Agricultural Technology, Burapha University, Sa Kaeo Campus, Sa Kaeo 27160, Thailand*

Abstract This study proposed to examine the alfalfa varieties suitable for further field experiments in Sa Kaeo province in order to cultivate alfalfa plants for a meat goat diet. Four varieties of alfalfa were collected from four distributors, source from the Republic of Turkey (coded as AC1), source from the United Kingdom (coded as AC2) and source from the United States of America (coded as AC3 and AC4, respectively). Their growth rate, yield and chemical composition were analysed in Sa Kaeo province. The results show that the plant height, the number of leaves per plant and the leaf width increased from 30 to 90 days after all the varieties were planted. There were significant differences between the varieties in their chemical composition after the first (90 days) and second (120 days) harvest times except for dry matter (DM) 90 days after planting. To determine the effects of increasing levels of alfalfa dehydrated pellets (ADP) in total mixed ration (TMR) on feed intake and productive performance of meat goat, a randomized complete block design experiment was conducted with 9 crossbred Native-Anglo Nubian goats, 3-4 months' old, with body weight ranging from 8-10 kg which were fed received 0%, 10% and 20% of ADP in a TMR. The results revealed that increasing levels of ADP with 10% and 20% in the diet had no effect on DM intake, average daily gain, nutrient digestibility and blood urea nitrogen.

Key words: Alfalfa, Plant height, Number of leaves, Total mixed ratio, Meat goats

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INTRODUCTION

Alfalfa is suited to temperate and Mediterranean climates. In tropical climates with high temperatures and high humidity, alfalfa is vulnerable to several diseases which do not occur in cold temperatures. Some alfalfa cultivars are tolerant to extremely cold winters, hot summers and drought due to its deep root system. Nevertheless, it cannot tolerate waterlogging or acid soils (Clements, 2019). Forage quality is important for animals as it results in raised animal performance and reduced supplement costs. Alfalfa stores carbohydrates (sugars and starches) in its crown and roots which is used for regrowth after cutting. When alfalfa height reaches 6 to 8 inches, carbohydrates are stored in the crown and root again. This cycle of carbohydrate preservation encourages the potential of alfalfa for regrowth (Undersander et al., 2011). Testa et al. (2011) showed that alfalfa had high protein but low yield at the beginning of the bud stage compared to the full flowering stage. According to Undersander et al. (2011), at early vegetative stages alfalfa has more leaf weight than stem weight which affects its quality. In Thailand, there have been many research projects on alfalfa conducted by the Department of Livestock Development, Ministry of Agriculture and Cooperatives. Sornprasitti and Witayanupapinyong (1991) and Phaikaew et al. (2005) conducted experiments with alfalfa in Pakchong district, Nakhon Ratchasima province, where the average temperature is as cold as a temperate country (17-35°C). Sornprasitti and Witayanupapinyong (1991) reported that fertilizers had no effects on yield and quality of alfalfa but when alfalfa was rainfed (3.8 ton/ha/year) it yielded less than alfalfa grown under irrigation (10.2 ton/ha/year). Phaikaew et al. (2005) introduced alfalfa from Saudi Arabia, Australia, Japan and the United States of America which was evaluated for 14 months. They reported that cultivar from Australia (Eureka) had the highest dry matter yield of 17.5 ton/ha and crude protein (CP) of 23.02% at first cut (90 days) and average protein of 19.8% after an interval of 40 days followed by the cultivar from Saudi Arabia (CUF101). The average dry matter yield of 24 cultivars was between 9.6–17.5 ton/ha and the average CP content was 18.6–23.5%.

The selection of different varieties will be important for alfalfa production in the future. In previous studies, the characteristics used for alfalfa selection included yield, yield composition and nutritive values (Avci et al., 2018; Cacan et al., 2018). A good forage quality is indicated by high protein and low acid detergent fiber (ADF). Protein is the main nutrient in ruminant animals and it has a negative correlation with ADF. Moreover, CP has a positive correlation with relative forage value (RFV) and relative forage quality (RFQ) which can be used as an index to identify suitable forage for livestock (Undersander et al., 2011). Alfalfa is known as the 'Queen of Forage' as it has high protein content between 11.3–25.9% (Testa et al., 2011; Zhang et al., 2014; Avci et al., 2018; Cacan et al., 2018), high dry matter intake (DMI) (3.4–5.9%) (Basbag et al., 2009; Cacan et al., 2018) and highly digestible dry matter (DDM) (57.4–75.8%) (Basbag et al., 2009; Avci et al., 2018; Cacan et al., 2018). Several studies have found variations in alfalfa quality in different environments and varieties as, for example, in CP yield (2.1–5.8 ton/ha) (Kavut and Avcioglu, 2015; Cacan et al., 2018), in neutral detergent fiber (NDF) (20.3–49.6%) (Basbag et al., 2009; Kavut and Avcioglu, 2015; Avci et al., 2018; Cacan et al., 2018). The ADF was reported to range from 16.8–40.4% (Basbag et al., 2009; Kavut and Avcioglu, 2015; Avci et al., 2018; Cacan et al., 2018). Tannin, a polyphenol-complex found in a wide range of many plant species (Terrill et al., 1992) is commonly consumed by ruminants. Leguminosae (Medicago) has been found to contain tannins. Tannin helps in better utilization of dietary protein; its effect may be either harmful in high concentrations of tannins. The ingestion of low to moderate concentrations of tannins may improve the digestive utilisation of feed and this important effect is reflected in animal performance mainly by reducing protein degradation in the rumen and subsequently a greater amino acid flow into the small intestine (Frutos et al., 2004). Tannins can be classified into two groups, according to their chemical structure: hydrolysable and condensed (Piluzza et al., 2014). Frutos et al. (2004) also stated that condensed tannins (CT) usually have a higher molecular weight (1,000–20,000 Da) than hydrolysable tannins (HT) (500–3,000 Da). The HT are in leaves and the bark of plants such as oak (*Quercus pyrenaica*) and chestnut (*Castanea sativa*) but are toxic in animals and inhibit digestion in cattle (Naumann et

al., 2017). However, Minz et al. (2013) reported that oak leaves (0.85%CT and 4.4%HT) improved nutrient utilization and growth performance in goats compared to grass hay. The CT are released during chewing or cutting of forage but concentration in the forage is low, as it is in *Lotus corniculatus* (bird's-foot trefoil) (2.0-4.7%CT) and alfalfa (0.5-0.9%CT). While moderate concentration of CT (2-4%) in dietary DM can bring benefits to the nutrition and health of ruminants (Cleef and Dubeux, 2019). The demand for alfalfa has increased in a short period recently of time. It has been imported in the form of crude powder and pellets for sale to ornamental animal breeders, such as rabbits, rats, horses, chinchillas, or imported as seeds for planting, in sprouts. The pellet form of alfalfa is often standardized to 17 or 18% protein content (Desialis, 2013). Alfalfa dries by dehydration which is the best method as it preserves its high protein, vitamins, and overall nutritive value (Renaud, 2002). Holstein calves supplemented with starter and different physical forms of alfalfa hay (chopped alfalfa vs pelleted alfalfa) have been studied for their performance. However, it was found that pelleted alfalfa hay did not result in any improvement in calf performance or health (Jahani-Moghadam et al., 2015). Ishaq et al. (2019) reported that pelleted-hay alfalfa feed increased the weight gain of sheep wether, feed efficiency and rumen bacterial richness, including common fibrolytic rumen inhabitants, more than long-stem (loose) alfalfa hay. However, pelleted-hay alfalfa is not yet well-known to cattle and goat farmers in Thailand. Thai customs (2017) reported that Thailand imported 159,420 Thai baht (THB) of alfalfa seed and 3,678,040 THB of alfalfa pellet in 2016, as there is no commercial alfalfa cultivation in Thailand due to a lack of knowledge about alfalfa adaptation in the different regions of Thailand.

The objective of this study was to investigate the growth, yield, and chemical composition of four alfalfa varieties in Sa Kaeo province in order to determine the best varieties of alfalfa to select for farmers to cultivate in the future. However, another option for meat goat farming is to encourage Thai farmers to grow or use this alfalfa as fodder, and its high nutritive value should be analyzed in a study of the use of alfalfa as a forage crop for ruminants. Despite the fact that alfalfa has been confirmed to be superior to other forage crops because of its high CP and energy, in general, alfalfa is not typically found in Thailand, moreover, alfalfa in the form of hay or dehydrated pellets which grow in Atlantic or Mediterranean environments are imported into Thailand at a high price, especially if it is of good quality. Nonetheless, it has been successfully used to a limited extent by Thai smallholders. To determine how alfalfa can best be used we will focus in this study on the most effective utilization of alfalfa nutrient using a simple method to investigate the necessary amount to include in supplements of alfalfa in the form of alfalfa dehydrated pellets (ADP) as required in a total mixed ratio (TMR) to enhance the growth performance of crossbred Native Anglo-Nubian goats.

MATERIALS AND METHODS

Four varieties of alfalfa

Four varieties of alfalfa seed were collected from distributors in the Republic of Turkey (coded AC1), in the United Kingdom (coded as AC2) and the United States of America (coded as AC3 and AC4), respectively. The experiment was conducted in a completely randomized design (CRD) at the greenhouse of the Faculty of Agricultural Technology at Burapha University, Sa Kaeo Campus, Sa Kaeo province, in Thailand. The mean temperature was 27.50-28.78°C (Thai Meteorological Department, 2019). Soil was collected before the experiment and analyzed for soil properties at the Laboratory, Department of Soil Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province in Thailand. Initial chemical characteristics of the soil were pH 7.0, organic matter 2.57%, total N 0.1%, available P 13.6 mg kg⁻¹ and exchangeable K 88.9 mg kg⁻¹. Soil pH was measured using a 1:1 soil-to-water ratio. Organic matter was analyzed by the Walkley and Black method. Total nitrogen (N) was analyzed by the Kjeidahl method. Available phosphorous (P) was analyzed by Bray-II.

Exchangeable potassium (K) was analyzed by using 1N NH₄OAc and AAS. The alfalfa seeds were grown in trays for 2 weeks and then the seedlings were transplanted to 5 pots (10 plants per pot). Pot diameter was 12 inches with height of 12 inches. Plants were watered every day. Fertilizer (15-15-15) was applied 4 times with 0.5 g/pot.

Data collection from Alfalfa plants

Growth data such as, plant height, number of leaves per plant, leaf width and length of leaf petioles were collected at 30, 45, 60, 75 and 90 days after planting. Alfalfa plants were harvested 2 times at first cut (90 days after planting) and second cut (120 days after planting) of cutting interval at 30 days. Plants were cut at 10 cm above soil for fresh weight per plant (FW) and then samples were dried at 60°C for 72 hours to obtain a dry weight (DW). Dried weight samples of the alfalfa plants were ground through a 1-mm screen in order to analyze the nutritive values using the standard method of the Association of Official Analytical Chemists (AOAC, 2016), which included dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and tannin at 90 and 120 days after planting. The nutritive values were determined by AOAC (2016) at the Feed Analysis-2 Laboratory, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province in Thailand (ISO/IEC 17025: 2005 under Bureau of Laboratory Accreditation). The CP was analyzed using an in-house method based on the AOAC official method (2016; procedure 2001.11). The ether extract (EE) was analyzed by the in-house method based on the AOAC official method (2016; procedure 2003.05)/(2016; procedure 954.02). The CF was also analyzed by the in-house method based on the AOAC official method (2016; procedure 978.10). Ash, NDF, ADF and ADL were analyzed following the AOAC method (2016; procedure 942.05; 920.08; 2002.04; 973.18 and 973.18, respectively). With regard to the alfalfa samples which were prepared for tannin analysis, whole plants were collected by harvesting at first and second cut at cutting intervals of 30 days. They were chopped into 3-4 cm length before being dried at 60°C for 48 h in a hot air-oven and later ground through a 1 mm screen. Determination of tannins (on a DM basis) was carried out by following Burns (1971) at the Feed analysis-2 Laboratory, Department of Animal Science, Faculty of Agriculture at Khamphaeng Saen, Kasetsart University, Khamphaeng Saen, Thailand.

Alfalfa dehydrated pellets (ADP)

Alfalfa commercial dehydrated pellets (ADP) were imported from distributor which were certified by quality analysis of xx laboratory. The ADP was sampled for chemical analysis five times in a dry condition and the pellet size of each piece was roughly between 1.5-2.0 cm, packed in a waterproof plastic bag.

Total mixed ratio (TMR) preparation

Feed ingredients including Napier grass (*Pennisetum purpureum*), ADP, ground corn, cassava chip, rice bran, molasses, soybean meal, urea and mixed minerals were mixed and used for the daily preparation of the total mixed ratio (TMR). The chemical composition of feedstuff, except for ADP, was calculated based on DLD (2004). The TMR diets were formulated to be 12%CP (CP-based TMR as calculation), with urea used to match the CP content between forages, while ADP and other ingredients were used for the diets (Table 4).

Animals, experimental design and diets

Nine cross-bred (Thai Native x Anglo-Nubian) male meat goats (approximately 3-4 months' old, of 8-10 kg in initial weight) were used. The animals authorized for use in this protocol were only used in the activities and in the manner described herein, as approved by the BUU-IACUC 010/2562 prior to its implementation. All goats were obtained from a farmer's goat meat farm in the neighborhood area of Ban Nong Yang, Watthana Nakhon district, Sa Kaeo province by the Department of Livestock Development, Watthana Nakhon district, Sa Kaeo province), no Brucellosis or blood parasites (*A. marginale*, *Babesia spp.*, *Microfilaria*, *Theileria spp.* and *Trypanosoma spp.*) were found after an examination certified by the Veterinary Research and

Development Center, Eastern Region. The goats were housed in individual wooden cages (80 cm x 150 cm x 130 cm, L x W x H) with elevated and perforated floors. The goats were assigned to a randomized complete block design (RCBD) and blocked by initial body weight (BW) into 3 treatments with 3 replicates of each. Prior to the start of the experiment, each goat was dewormed using Ivermectin injections and fed with the control diet for 14 days to adapt the goats to the experimental conditions. During the experimental period, all goats were hand-fed daily on a TMR diet *ad libitum* twice a day at 0800 a.m. and 1600 p.m. with an intake of the experimental diet (at least 10% refusals) and a different level of alfalfa in the form of the ADP in the TMR fed on 0, 10 and 20% (DM basis). Clean drinking water and mineral blocks were freely available to the animals at all times.

Sampling, measurements and chemical analysis

Dry matter intakes were determined by weighing daily feeds offered and 10% residual feeds were weighed from individual goats for two consecutive days weekly. Feeds were pooled and dried in an oven at 60°C for 48 h and then ground and milled through a 1-mm screen. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis (as feed basis). Faecal samples were collected separately per animal during the last 5 days of the experiment. The faecal samples were dried in an oven at 60°C for 48 h, milled through a 1-mm screen and analysed for DM, ash and CP using the standard methods of AOAC (2010), while NDF and ADF were analyzed following the method of Van Soest et al. (1991). The CP content was determined by the Kjeldahl method (procedure 984.13, AOAC, 2010), NDF and ADF were determined using the method described by Goering and Van Soest (1970). Chemical analysis was expressed on the basis of final DM.

During the experimental period of 60 d, live weight (LW) at the start and at the end of the experiment were recorded on the 30th and 60th day using hanging scales after a fasting period of at least 16 h to calculate the LW change (LWC), average daily gain (ADG) and the feed intake (FI) per BW. On the 60th day of the experiment, blood urea nitrogen (BUN) was collected via the jugular vein into a test tube at 0 (pre-feeding), 3 and 6 h post-feeding, test tubes were packed immediately in plastic containers with ice blocks and then centrifuged for 15 min at 6,000 rpm for 10 min at 4°C. Plasma was recovered for evaluation using a modified indophenol colorimetric method (Weatherburn, 1967).

Statistical analysis

All plant growth characteristics and chemical composition data were analyzed by using R statistics (R Core Team, 2019). The mean differences among varieties were compared by a least significant difference test (LSD). Correlation coefficients were calculated to determine the relationships between nine traits by using the Analysis ToolPak in the Microsoft Excel program. The FI, LW, nutrient digestibility and BUN of the meat goats were analyzed by ANOVA for RCBD using R statistics. Significant differences among treatments were assessed by Duncan's new multiple range test (DMRT). A significant level of $P < 0.05$ was used (Steel and Torries, 1980).

RESULTS

Growth characteristics of alfalfa

Figure 1 demonstrates the plant growth characteristics of alfalfa. The plant heights increased from 30 to 90 days after planting. The results indicate that the various mean plant height at 75 days after planting differed significantly ($P < 0.01$); whereas AC4, AC2, AC1 and AC3 had mean heights of 75.2, 71.6, 67.5 and 59.7 cm, respectively. The plant heights of AC2 and AC4 were higher than AC3 but they were not different from AC1 at 75 days after planting. Similarly, there was a significant difference ($P < 0.05$) of the mean plant height at 90 days after planting. The plant height of AC4 (106.4 cm) was higher than AC3 (91.0 cm) but not different from AC1 (97.4 cm) and

AC2 (98.5 cm). The regrowth of plants at the second cut (30 days later) showed that the height of the alfalfa plants was between 99 to 118 cm without any differences between the varieties. There were no differences in plant height between the first cut at 90 days and the second cut at 120 days. The number of leaves per plant and the leaf width increased from 30 to 90 days after planting. The number of leaves per plant showed significant differences between varieties at 75 and 90 days after planting. The number of leaves per plant of AC1 and AC2 were the highest at both periods. The number of leaves at 90 days after planting of AC2, AC1, AC4 and AC3 were 98.3, 86.3, 74.4 and 73.0 leaves, respectively. The leaf width increased from 30 to 90 days after planting. The leaf width showed significant differences between varieties at 30 ($P<0.05$), 45 ($P<0.05$) and 60 ($P<0.01$) days after planting. The leaf widths of AC2 and AC3 were the highest ($P<0.05$) at all three periods. At 30 days, the leaf widths of AC2, AC3, AC1 and AC4 were 2.4, 2.4, 2.0 and 1.8 cm, respectively. At 90 days after planting, the leaf widths of AC4, AC1, AC2 and AC3 expanded to 5.7, 5.6, 5.4 and 5.2 cm, respectively. The length of petiole showed significant differences between varieties at 30, 45 and 75 days after planting. The length of petiole was highest at 45 days after planting between 4.2 to 5.2 cm and then decreased from 45 to 75 days after planting. The petiole length at 90 days after planting did not differ between varieties in which AC4, AC2, AC3 and AC1 expanded to 3.8, 3.6, 3.5 and 3.4 cm, respectively. Although, AC1 and AC2 showed high plant height and a greater number of leaves, there were no differences in fresh weight or dry weight per plant between varieties both at first and second cuts at 90 and 120 days after planting (Table 1). Fresh weight was between 19 to 30 g/plant and dry weight was between 3 to 5 g/plant. Moreover, there was no correlation between the yield and all the growth characteristics (data not shown).

Chemical composition of alfalfa

Table 1 presents the nutritive value of four alfalfa varieties at first cut (90 days after planting) and second cut (120 days after planting). There were variations in the chemical composition of alfalfa for CP (14.3-20.1%), EE (2.1- 2.7%), CF (25.8-31.1%), ash (10.4-12.4%), NDF (38.1-41.5%), ADF (34.0-38.1%), ADL (6.1-8.3%), tannin (1.9-2.7%) and DM (91.9-93.6%). The CP was significantly different between varieties at 90 and 120 days ($P<0.001$). AC1, AC2 and AC4 alfalfa varieties had the highest CP content. Nevertheless, there was no difference in CP content between 90 and 120 days after planting. However, other chemical compositions differed significantly between varieties at 90 and 120 days, except for DM at 90 days after planting. ADF, DM and Ash of alfalfa harvested at the second cut (120 days) were significantly higher than at first cut (90 days) ($P<0.05$). The smallest amounts of NDF were obtained from AC1 and AC2 at first cut (38.5 and 38.3%, respectively) and AC1 and AC4 at second cut (38.5 and 38.1%, respectively). The smallest amounts of ADF were obtained from AC1 and AC2 at first cut (34.0 and 34.4%, respectively) and AC1 and AC4 at second cut (35.4 and 35.6%, respectively). AC1 and AC2 had largest amount of tannin at first and second cut. Table 2 shows the correlations between the chemical constituents. CF, NDF and DM showed a positive correlation with ADF and ADL. CP showed a positive correlation with ash but a negative correlation with CF, NDF, ADF and ADL.

Table 1. Chemical composition of alfalfa varieties.

Chemical composition	Cut	AC1 ¹	AC2 ²	AC3 ³	AC4 ⁴	P-value	%CV ⁵
FW ⁶ (g/plant)	first	29.76	29.02	27.32	19.61	ns	19.55
	second	22.62	22.48	21.37	18.51	ns	18.18
DW ⁷ (g/plant)	first	5.25	5.47	5.43	3.44	ns	17.84
	second	4.13	4.05	4.41	3.07	ns	18.04
DM ⁸ (%)	first	91.89	91.88	92.01	92.00	ns	0.12
	second	93.09 ^b	93.61 ^a	93.48 ^a	92.80 ^b	**	0.08
CP ⁹ (%)	first	20.11 ^a	19.77 ^a	17.42 ^b	19.03 ^a	***	0.85
	second	19.34 ^a	18.57 ^a	14.33 ^b	19.74 ^a	***	0.80
EE ¹⁰ (%)	first	2.68 ^a	2.67 ^a	2.57 ^a	2.13 ^b	***	1.94
	second	2.46 ^a	2.34 ^a	2.22 ^b	2.39 ^a	*	1.82
CF ¹¹ (%)	first	25.97 ^b	26.92 ^a	26.91 ^a	25.88 ^b	**	0.71
	second	27.21 ^b	26.94 ^b	31.12 ^a	26.91 ^b	**	1.75
Ash (%)	first	11.28 ^a	11.25 ^a	10.38 ^b	11.13 ^a	***	0.39
	second	12.31 ^a	12.18 ^a	10.78 ^b	12.35 ^a	***	0.42
NDF ¹² (%)	first	38.46 ^b	38.33 ^b	41.45 ^a	39.83 ^{ab}	**	0.95
	second	38.45 ^b	40.44 ^a	41.50 ^a	38.13 ^b	**	1.01
ADF ¹³ (%)	first	33.97 ^c	34.42 ^{bc}	35.77 ^a	35.17 ^{ab}	**	0.72
	second	35.42 ^b	36.93 ^{ab}	38.11 ^a	35.55 ^b	**	0.92
ADL ¹⁴ (%)	first	6.07 ^c	6.94 ^b	7.54 ^a	6.68 ^b	**	1.74
	second	7.05 ^b	7.84 ^a	8.25 ^a	7.04 ^b	**	1.85
Tannin (%)	first	2.57 ^{ab}	2.39 ^{ab}	2.13 ^{bc}	1.93 ^c	***	1.40
	second	2.51 ^{ab}	2.70 ^a	2.35 ^{ab}	2.13 ^b	***	1.67

Note: * significance at $P < 0.05$, **significance at $P < 0.01$, *** significance at $P < 0.001$. Different letters in the same row represent significant differences. ¹/Alfalfa from Republic of Turkey, ²/Alfalfa from the United Kingdom, ^{3,4}/Alfalfa from the United States of America, ⁵/Coefficient of variation, ⁶/Fresh weight, ⁷/Dry weight, ⁸/Dry matter, ⁹/Crude protein, ¹⁰/Ether extract, ¹¹/Crude fiber, ¹²/Neutral detergent fiber, ¹³/Acid detergent fiber, ¹⁴/Acid detergent lignin.

Table 2. Correlation coefficient among chemical constituents.

Items	CP ¹	EE ²	CF ³	Ash	NDF ⁴	ADF ⁵	ADL ⁶	Tannin
EE	0.45							
CF	-0.87**	-0.35						
Ash	0.52*	-0.08	-0.24					
NDF	-0.82**	-0.43	0.51*	-0.60*				
ADF	-0.84**	-0.62*	0.79**	-0.06	0.73**			
ADL	-0.80**	-0.42	0.74**	-0.13	0.72**	0.91**		
Tannin	0.09	0.37	0.10	0.39	-0.13	0.08	0.09	
DM ⁷	-0.47	-0.49	0.60*	0.48	0.29	0.80**	0.72**	0.43

Note: * significance at $P < 0.05$, **significance at $P < 0.01$. ¹/Crude protein, ²/Ether extract, ³/Crude fiber, ⁴/Neutral detergent fiber, ⁵/Acid detergent fiber, ⁶/Acid detergent lignin, ⁷/Dry matter.

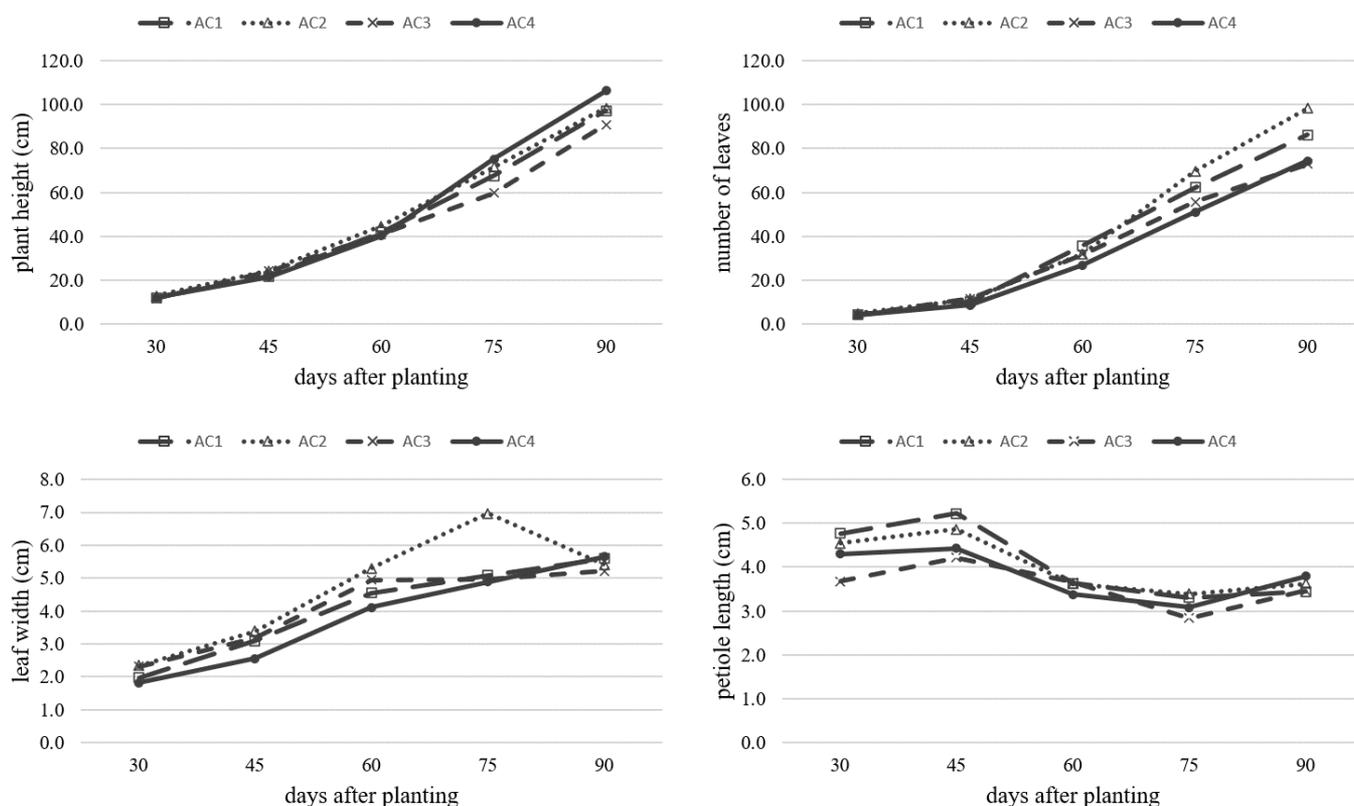


Figure 1. Plant height, number of leaves, leaf width and petiole length of the four alfalfa varieties at 30, 45, 60, 75 and 90 days after planting.

Feed ingredients and chemical composition

Table 3 shows the chemical composition of ADP products used in the present study. To evaluate the potential nutritive benefits of ADP for utilization as an ingredient in TMR for inclusion in a goat meat diet, the chemical composition data were analyzed and recorded from ADP beforehand. All ADP had quite similar content to DM, moisture, CP, EE, ash, CF, NDF, ADF and ADL. The CP in ADP ranged from 17.27 to 17.87% or approximately 17.63%CP throughout the year. Total energy of ADP during the period of a year was at 3,369 calories per gram in a one-time analysis.

Nutrient compositions of TMR in the experimental treatment diets are shown in Table 4. The DM content of the TMR diet used in this experiment averaged 90.45% of DM and the CP content ranged from 11.43 to 11.48%, while that of NDF ranged from 51.74 to 52.79%.

Feed intake, live weight, nutrient digestibility and blood urea nitrogen

The DMI, LWC, ADG, digestibility, and blood urea nitrogen of meat goats are shown in Table 5. Average DMI, DM, CP, NDF and ADF digestibility were not significantly different among the treatments. Initial and final live weights were similar ($P>0.05$) in all experimental treatment diet groups (Table 5). The LWC and ADG of meat goats at 0, 10 and 20% levels of ADP in TMR were no significant differences ($P>0.05$) among diets between the three treatment diets. No significant differences were found in blood urea nitrogen (BUN) concentration. The overall average value of BUN (average of pre- and post-feeding) of experimental goats ranged from 19.4-25.3 mg/dL.

Table 3. Chemical composition of alfalfa dehydrated pellets (ADP) used in the study.

Item	ADP ¹	ADP ²	ADP ³	ADP ⁴	ADP ⁵	ADP ⁶	ADP (Mean)
DM (%) ⁷	95.45	94.36	94.75	95.02	95.10	94.26	94.82
Moisture (%)	5.55	5.64	5.25	4.98	4.90	5.74	5.34
CP (%) ⁸	17.27	17.72	17.87	17.69	17.68	17.53	17.63
EE (%) ⁹	1.85	1.82	2.22	2.27	2.30	2.55	2.17
Ash	12.54	13.38	11.83	12.51	13.07	12.17	12.58
CF (%) ¹⁰	25.03	23.73	22.70	22.46	22.55	21.65	23.02
NDF (%) ¹¹	39.75	39.61	40.56	40.13	40.84	40.91	40.30
ADF (%) ¹²	28.11	31.57	29.76	30.00	30.11	28.88	29.74
ADL (%) ¹³	ND ¹⁴	6.90	6.45	6.73	6.63	6.02	6.55

Note :¹Alfalfa dehydrated pellets were certified by quality/analysis from xx laboratory, ^{2,3,4,5,6}Alfalfa dehydrated pellets sampled throughout 2018 for analysis at the Nutrition Laboratory at Burapha University, Sa Kaeo Campus and the Animal Nutrition Laboratory, Department of Animal Science, Faculty of Agriculture, Kasetsart University, ⁷Dry matter, ⁸Crude protein, ⁹Ether extract, ¹⁰Crude fiber, ¹¹Neutral detergent fiber, ¹²Acid detergent fiber, ¹³Acid detergent lignin, ¹⁴No data.

Table 4. Ingredients (kg fresh weight) and chemical composition of total mixed ratio of the experimental treatment diets on a dry matter basis.

Feed ingredients	Level of alfalfa dehydrated pellets in TMR ¹ (%)		
	0%	10%	20%
Napier grass	72	72	72
Alfalfa dehydrated pellets	0	4.3	8.6
Ground corn	5	5	4.5
Cassava chip	10.0	7.0	5.5
Rice bran	6.5	6.2	4.5
Molasses	1	1	1
Soybean meal	5.0	4.0	3.4
Urea	0.3	0.3	0.3
Mineral mix ²	0.2	0.2	0.2
Total (kg/100kg)	100	100	100
TDN ³	68	67	65
Roughage ratio (w/w) (DM ⁴ basis)	38.0	37.8	37.6
Chemical compositions (% DM basis)			
DM (fresh)	40.59	40.16	40.39
DM (air dry)	91.33	90.40	89.61
OM ⁵	91.93	90.88	90.27
Ash	8.07	9.12	9.73
CP ⁶	11.46	11.43	11.48
EE ⁷	1.81	1.73	1.94
NDF ⁸	51.74	52.58	52.79
ADF ⁹	26.71	27.28	28.03
ADL ¹⁰	9.49	10.08	10.16

Note :¹Total mixed ratio, ²Mineral mix contents per kg :120 g Ca; 140 g Na; 32 g P; 20 g S; 15.4 g Mn; 7 g Zn; 2 g Mg; 1 g Fe; 1.25 g Cu; 0.33 g Co; 0.03 g Se; 0.1 g, ³Total digestible nutrient by calculation, ⁴Dry matter, ⁵Organic matter, ⁶Crude protein, ⁷Ether extract, ⁸Neutral detergent fiber, ⁹Acid detergent fiber, ¹⁰Acid detergent lignin.

Table 5. Effects of different levels of alfalfa dehydrated pellets in a total mixed ratio on live weight change, average daily gain, feed intake, and nutrient digestibility in goats for 60 days.

Parameters	Level of ADP ¹ in TMR ² (%)			SEM ³	P-value ⁴
	0%	10%	20%		
Feed intake (g/day)					
Dry matter intake	997.23	1015.68	1059.89	25.36	0.4739
%BW	8.68	8.92	9.34	1.02	0.3529
g/KgBW ^{0.75}	159.58	163.82	171.18	6.75	0.3610
Body weight (Kg/head)					
Initial weight	9.80	9.50	9.40	0.31	0.2113
Final weight	11.51	11.39	11.37	0.21	0.1032
LWC ⁵ (Kg/head)	1.70	1.89	1.97	0.21	0.0633
ADG ⁶ (g/day/head)	28	32	33	2.36	0.0592
Digestibility, %					
DM ⁷	84.28	83.01	80.96	1.62	0.3228
OM ⁸	83.86	84.24	83.61	1.26	0.2313
CP ⁹	82.98	83.12	82.05	1.02	0.2194
NDF ¹⁰	74.48	71.35	70.83	1.41	0.3301
ADF ¹¹	54.56	54.40	56.04	1.27	0.4237
BUN¹² (mg/dL¹³)					
0 h, pre-feeding	20.3±0.2	20.1±0.4	19.4±0.5	1.14	0.5321
3 h, post-feeding	24.8±0.2	25.3±0.3	25.1±0.7	1.28	0.6551
6 h, post-feeding	21.1±0.4	20.7±0.7	19.8±0.6	1.30	0.6981

Note: ¹/Alfalfa dehydrated pellets, ²/Total mixed ration, ³/Standard error of mean, ⁴/Differences in probability values, ⁵/Live weight change, ⁶/Average daily gain, ⁷/Dry matter, ⁸/Organic matter digestibility, ⁹/Crude protein, ¹⁰/Neutral detergent fiber, ¹¹/Acid detergent fiber, ¹²/Blood urea nitrogen, ¹³/Milligrams per deciliter.

DISCUSSION

The variations in growth parameters including plant height, number of leaves, leaf width and petiole length imply that the varieties used for this study exhibited different adaptations to the environment of Sa Kaeo province. There are previous studies with similar findings on variations of growth parameters among alfalfa varieties, such as plant height (42.0 - 76.6 cm) (Phaikaew et al., 2005; Basbag et al., 2009; Chen et al., 2012; Cacan et al., 2018) and leaf width (0.4 - 1.4 cm) (Basbag et al., 2009). However, there were no differences in fresh weight and dry weight per plant between the varieties on both the first and second cut at 90 and 120 days after planting (Table 1). In contrast with many previous studies, this study showed different findings on the variations of yield among alfalfa varieties, such as fresh weight (108.3 - 258.0 g/plant) (Basbag et al., 2009), dry weight (21.7 - 56.6 g/plant) (Basbag et al., 2009), fresh yield (64.1 - 118.8 ton/ha) (Kavut and Avcioglu, 2015; Tuck et al., 2017), and dry yield (12.6 - 27.1 ton/ha) (Testa et al., 2011; Kavut and Avcioglu, 2015; Tuck et al., 2017). Moreover, there was no correlation between yield and the growth characteristics (data

not shown). This is the opposite of the results of Basbag et al. (2009) who found positive correlations between growth characteristics such as plant height, leaf width, leaf length, stem thickness, green weight and dry weight per plant. The results of our study are due to an occurrence of common leaf spot disease during the experiment. Leaf spot disease affects the leaves' loss and the yield and reduces quality (Undersander et al., 2011). This reveals that none of the four varieties of this study were resistant to common leaf spot disease in Sa Kaeo province. In addition, the average temperature of Sa Kaeo province might not suitable for alfalfa growth (22 - 35°C) compared to Pakchong district in Nakhon Ratchasima province (17 - 35°C). Therefore, we assume that AC1, AC2 and AC4 which had high plant height and leaf width would also have high fresh weight and dry yield under normal conditions if the plants had not been affected by leaf spot disease. Similarly, Cacan et al. (2018) found that plant height had positive correlations with fresh yield, dry yield, ADF and NDF but had a negative correlation with CP. ADF is a poor feed ingredient of the energy nutrient group. Its digestibility is low when ADF is high (Undersander et al., 2011).

Considering the correlation between the chemical constituents, CP showed negative correlation with CF, NDF, ADF and ADL. This is in accordance with the research of Zhang et al. (2014) which showed that CP had significant negative correlation with CF. Furthermore, they also found that CP had significant positive correlation with relative feed value (RFV) but CF had significant negative correlation with RFV. Thus, alfalfa variety with high CP and low CF content means that it is of good quality. Cacan et al. (2018) also found positive correlations between CP and RFV and negative correlations between CP and ADF and NDF. In this study, the AC1 variety had high CP and Ash content and low CF, NDF, ADF and ADL content. In contrast, AC3 had the highest CF, NDF, ADF, ADL but CP and Ash of AC3 were the lowest at both times of harvesting. Therefore, AC1 demonstrated better quality than AC3. In addition, the tannin content of the four alfalfa varieties was possibly due to enhancing nitrogen utilization in animals when added to feedstuffs. Martello et al. (2020) found that beef cattle were reared in 0.25 ha (per head) of *Urochloa brizantha* cv. Marandu found increased CP digestibility when of 0.1% in blend tannin (85% of CT and 15% of HT) and in dietary DM (10 g/head/day) together with 2% of urea in the DM.

The agronomical characteristics and typical nutritive quality of the four varieties of alfalfa plants included high plant height, high number of leaves, high CP, high NDF and ADF, and tannin. Similarly, the CP results of alfalfa planted in the present study were similar to those values previously reported (Dunham, 1998; DLD, 2004; Alhidary et al., 2016). These results imply that alfalfa plants can be used as a potential alternative protein which contain useful nutrient utilization and increase productivity of meat goat. Although all four varieties of alfalfa plant showed high protein in Sa Kaeo province, when raising animals in the long-term it is necessary to have large amounts of yields which can be utilized for grazing or hay production throughout the year. There were problems with disease in the early stages of growth. Moreover, the alfalfa plants were harvested by a cut-carry method to feed the animals. This resulted in an increase in the problems of smallholder farmers when growing cash crops because they do not have large areas in which to plant alfalfa, the climate conditions are unpredictable and they must provide tools and wages for workers to harvest each day and maintain production. These problems are difficult for Thai smallholders to solve in order to raise animals. Therefore, this experiment selected alfalfa in the form of pellets which are more practical and convenient for actual use for Thai farmers. Studies of the nutritional value of alfalfa in Thailand are still limited since alfalfa is considered as good quality animal feed legumes which is difficult to grow in Thailand and has a relatively high price. This research study was limited by the small proportion of ADP in TMR which was ordered at a low price for use in animal feed. The feeding strategy was to use the ingredients in TMR with an inexpensive source of non-protein nitrogen-containing urea and molasses to provide a source of energy for goats which was rich in water-soluble carbohydrates. This might have led to improved microbial protein synthesis in the rumen.

In the present study, the times of the cutting harvest and the stage of maturity of ADP were not stated and the CP in the ADP used in TMR was similar to pelleted alfalfa hay (mean = 5.8 mm.) as used by Jahani-Moghadam et al. (2015) which was very close to chopped alfalfa hay (Turner et al., 2005). CP from legumes naturally differs in being harvested at different ages, in line with our previous study (Srisaikhram and Lounglawan,

2018) in which the CP of legumes harvested decreased over longer intervals. It is possible that legume increases its stems and leaves more quickly resulting in high fresh and dry yields but low protein. This legume is similar to grass (*Pennisetum purpureum* cv. Mahasarakham) used by Chaikong et al. (2018) who reported that at early cutting intervals (4 to 6 weeks) was higher protein than 8 to 10 weeks, the grass developed stems and nodes but the size of the stems and leaves resulted in low yield. In addition, CP was higher in leaves than plants cut to ground level (Min et al., 2019).

The TMR with different levels of ADP in TMR had similar DM, OM, ash, CP, EE, NDF, ADF and ADL contents which averaged 90.45, 91.03, 8.97, 11.46, 1.83, 51.79, 33.29, 52.37, 27.34 and 9.91%, respectively. The final CP content in TMR in the experimental treatment diets was lower than expected for CP-based TMR as calculation, probably due to inconsistencies in TMR mixing or sampling as mentioned by Wahyuni et al. (2012). The fiber is affected by the roughage levels of grass and legume included in TMR (Table 4) and the use of Napier grass and ADP in this study increased the proportion of fiber in the TMR feed. Napier grass had low CP (5.04-5.57% CP; data not shown) compared to other studies. The low quality of the Napier grass planted on the university's farm (Sakeao soil) with no nitrogen fertilizer or irrigation may have resulted in less nutrients, as also described by Santos et al. (2016) who reported that an important factor for grass quality is the cutting age because, as crops age and become more mature, the protein content declines depending on the application of fertilizer and the soil conditions. These findings are also in agreement with those of Chaikong et al. (2018) who demonstrated that high protein content of *Pennisetum purpureum* cv. Mahasarakham at an early stage of cutting (4-6 weeks) under irrigation during the cold season is appropriate for feeding young animals that require high nutrients for productive performances. Therefore, good quality roughage sources is an alternative way to feed livestock and this can play a major role in determining goat productivity and efficiency of FI (Maksiri et al., 2017).

The DMI was not affected by increasing levels of ADP in TMR at 0, 10 and 20%. The lack of differences in the response often appears because all experimental diets have been designed to have similar CP content. Similar results were also reported for brown hemp or sunnhemp (SH) meal (*Crotalaria juncea*: a plant of the legume family (Fabaceae) which has been utilized as a protein source in the concentrate of Brahman×Thai-native cattle in the research of Srisaikham and Lounglawan (2021). In a recent study of male Santa Ines sheep, Surat Thani, Thailand, Mattaphong et al. (2019) indicated that there were no effects on DMI when the sheep were fed single *Leucaena leucocephala* silage compared to the other fermented TMR that was similarly formulated as both dietary treatments received CP content. Moreover, the dietary treatment of forage in its physical form, chopped alfalfa hay (19.8% CP) vs. pelleted alfalfa (20% CP), in TMR did not influence solid feed intake or total DMI of dairy calves in the study of Jahani-Moghadam et al. (2015). However, Alhidary et al. (2016) reported that the inclusion of alfalfa hay in addition to TMR increased FI in male Naemi lambs following Tufarelli et al. (2012) and Bodas et al. (2014) who reported that improved FI in fattening animals may be attributed to the manipulation of TMR with certain types of roughages. Normally, the environment, feed texture, animal housing conditions, efficiency of animal management, and health problems may affect the responses to different amounts of FI in small ruminants. The roughage consumption of goats is affected by various factors including fiber level in the cell walls (NDF and ADF) and the level of CP in the diet as mentioned by Polat et al. (2013). Several studies (Domingue et al., 1991; Hussain et al., 1996, and Goetsh et al., 2011) have reported that factors that affect FI in goats are positively correlated with the quality of roughage, or the type and size of roughage feeding (Semae and Kraiprom, 2018). This study found the average amount of tannin for all four alfalfa varieties at 90 and 120 days ranged from 2.26 to 2.42%, respectively, or with an approximate mean value of about 2.34%. The tannin intake of ADP in TMR consumption at 0, 10 and 20% of goats can be deduced from the calculation at 0, 2.37 g tannin/head/day (0.24% of DM intake) and 2.48 g tannin/head/day (0.25% of DM intake), respectively. However, tannin inclusion in ADP in TMR was considered to be low risk or detrimental for the consumption of these experimental goats. Zhu et al. (1992) found that much lower voluntary FI was drastically reduced after 25 h (from 18 to 2.5 g DM kg⁻¹ LW) when sheep were fed 8 g of tannic acid per kg LW. Relatively low tannin concentration (0.5% of DM intake) is sufficient to

destabilize the bloat proteins while concentration of high tannins (2-4% of DM intake) is needed for improvement of protein utilization (Addisu, 2016).

Although Dewhurst et al. (2003) and Niderkorn and Baumont (2009) reported that grass and legumes in mixed diets generally enhanced greater DMI in ruminants than grass alone or enhanced ADG in goats fed legumes forage SH or Bermuda grass (BG) vs. SH, mixed diet (Min et al., 2019). Min et al. (2019) mentioned that grazing goats seem to select leaves from SH forage, especially nutrients with CP content and rumen digestibility which have more leaves than plants cut to ground level, and resulted in greater ADG being observed in SH or BG vs. SH forage diets which is probably due to a higher CP intake. There were no effects in this study on final weight, LWC, ADG or on nutrient digestibility ($P > 0.05$), when feeding with increased ADP in TMR due in part to similar CP content in all formula feeds. The present study conforms to the general pattern, since the dietary treatments of goats fed TMR received similar DMI and/or CP content, thus final weight, LWC, ADG, and nutrient digestibility response should have similar changes. These results can partly be related to those of Semae and Kraiprom (2018), who confirmed that the difference in the CP content of feed affected the amount of protein the goats received, so that therefore it is a factor that affects goats which are fed on an unequal total protein intake. Male goats (50% Thai native x 50% Anglo-Nubian) fed concentrate at 2% of BW with 100% leucaena (*Leucaena leucocephala*) (21.23% CP, total DMI = 1,832.04 g/head/day) as a roughage source (*ad libitum*) gained heavier weight than those fed on 100% signal grass (*Brachiaria humidicola*) (4.26% CP, total DMI = 1,881.7 g/head/day), and 50% leucaena combined with 50% signal grass (16.56% CP, total DMI = 1,861 g/head/day). Consequently, digestibility rates for these male goats fed with 50% leucaena and 50% signal grass, and 100% leucaena were significantly higher on CP digestibility (85.03% and 82.44%, respectively) than fed on signal grass (74.82%). This result is due to the fact that leucaena has high protein and low fiber, whereas signal grass has higher fiber than leucaena, which causes low digestibility. However, if goats are fed on good quality forage or high protein with unequal treatment, it enhances digestibility of the animals that use such a nutrient, resulting in a higher growth of goats. The trial of Alhidary et al. (2016) found that a treatment with TMR plus alfalfa hay (AH) improved BW on a monthly basis or final BW, however, it was found that a higher total DMI in growing lambs fed TMR with different AH feeding amounts per day compared to the TMR monoculture group showed a significant difference ($P < 0.05$).

The BUN did not alter the negative value effects in this study, as the BUN concentration was close to the normal level (11.2-27.7 mg/dL) in normal goats (Lloyd, 1982). In general, it was observed that the BUN concentration of goats fed TMR tended to be higher after post-feeding than that at 0 h. This result is in agreement with that of Wahyuni et al. (2012) who reported that BUN of goats fed on the TMR pattern resulted in high average concentrations (21.84 mg/dL), which was similar to the results reported by Turner et al. (2005) when three breeds (BoerX, Nubian, and Spanish) of goat bucklings were offered *ad libitum* alfalfa (*Medicago sativa* L.), hay plus 16% CP corn (*Zea mays* L.)/cottonseed (*Gossypium* spp) -based supplement (21.1 mg/dL) and 20.0 mm/ml when dairy goats were fed alfalfa hay of similar nutritive value without grain supplementation (Gelaye et al., 1990). The trend of increasing BUN after post-feeding in this study can be attributed to both dietary protein intake and the possibility of increased protein being absorbed by the small intestine (Hong et al., 2003). However, differences in BUN concentrations can also vary according to the different varieties of breeds (Sahlu et al., 1993), for example, in breed x forage quality and interaction for plasma urea nitrogen levels (Hart and Sahlu, 1993).

CONCLUSION

This study indicated that alfalfa did not have the potential for cultivation in Sa Kaeo province. Although some varieties (AC1 and AC2) have high plant height, a large number of leaves and high protein content, all the alfalfa varieties only produced a low yield. In view of the low yield production of alfalfa, the best option that can be recommended for effective utilization of alfalfa nutrient for meat goat farming in

Thailand is to select alfalfa in the form of dehydrated pellets in the TMR diet which is more practical and convenient for smallholder farmers. ADP can be used by up to 20% as a feedstuff in the TMR diet without affecting the productive performance of meat goats.

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