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Evaluation on dry forage yields and nutritional characteristics of introduced herbaceous legumes in Myanmar

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ABSTRACT

The study was carried out to evaluate the forage yields, nutritive values and *in vitro* fermentation parameters of herbaceous legumes. Five varieties of introduced herbaceous legumes; *Stylosanthes guianensis* cv. Ubon stylo, *Macrotyloma axillare* cv. Archer, *Centrosema brasilianum* cv. Ooloo, *Stylosanthes guianensis* cv. Stylo 184 and *Macroptilum bracteatum* cv. Cadarga were evaluated at the research farm, University of Veterinary Science, Yezin, Myanmar. No fertilizer and no irrigation were applied for cultivation to test drought resistance. Dry forage yield, nutritive values and gas production at four harvesting times were measured with 4×5 factorial arrangement (5 legumes and 4 harvesting time) in randomized complete block design. There was no interaction between legumes and harvesting time on forage yield, nutritive values and fermentation parameters but they were affected by main effects of legume types and harvesting time. Among the legume forages, the highest dry forage yields was found in Ooloo, Ubon stylo, and Stylo 184, and followed by the DM yield of Archer and Cadarga. The DM yield of second harvest was significantly higher ($p < 0.05$) than those of first, third and fourth harvest which were not significantly different from each other. As chemical composition, the DM content of Archer was lower ($p < 0.05$) than those of other varieties. Among the legumes forages, the lower CP content was found in Cadarga. The higher NDF was observed in Ooloo. Ooloo, Ubon stylo and Cadarga showed higher ADF in comparison with the other two varieties. Among the harvesting time, the lowest DM content was found at first harvest. The highest CP content was found at third harvest. The NDF content was not significantly different. The lowest ADF content was found in fourth harvest. According to the dry forage yield, Ubon stylo and Ooloo had highest dry forage yield and in term of nutritive values, Stylo 184 and Archer had higher nutritive values. As the main effect of forages, Stylo 184 and Archer had higher gas production in comparison with the other varieties. As the main effect of harvesting time, fourth harvest had highest gas production in comparison with other harvesting time. It could be better for cultivation by application of fertilizer and irrigation to get more forage yield and quality.

KEYWORDS: Forage yield, Gas production, Herbaceous legume, Nutritive values

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INTRODUCTION

The forage biomass containing large amount of complex carbohydrates, those mainly relied by ruminants as proper feeds are not digested by intestinal enzymes and cannot be used by monogastric animals. Crop residues and forages are inclusion in such kind of feed stuffs. The ruminants can use these forages efficiently and they have the ability to convert waste material to useful products (Speed & Pugliese, 1991).

As the forages and crop residues are poor in nutritive values, commercial concentrates were used for their livestock as supplementation, while also they are relying on home-made feeds such as acacia pods, maize and groundnut stover, crushed cowpeas mixed with maize, and crushed sunflower seed mixed with maize. There is needed to replace with good quality legumes to reduce the amount of concentrates, which are expensive for the smallholder farmers. One of the alternative ways to improve the utilization of such crop residue is by proper

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supplementation with leguminous forage because legumes can provide extra protein, as they have much higher levels of protein in their leaves and also provide essential minerals and vitamins for animal growth (Poppi & McLennan, 1995).

High quality sown forage such as leguminous fodder has been found to provide adequate supplementation in dry season and improve the productivity of grazing cattle. The legumes are fugitively recognized as protein banks and those can also be called under sown in food crops. It can be grazed, harvested and fed fresh or stored as hay or silage (Harricharaan *et al.*, 1988). As another option, cultivated forage legumes can provide high-quality feed, in cheaper price which can greatly enhance the ability to produce of traditional agricultural systems. Because of their higher nitrogen (N) content compared with grasses, it makes legumes to improve intake of forage, digestibility of fibre and reduce carbon dioxide (CO₂) and methane emissions, through a more proficient utilization of the energy content of the ingested forage in ruminants (Mannetje, 2000). As legumes have root nodules which can fix atmospheric nitrogen, they are mostly in the crucial role to reduce the input of nitrogen fertilizer to maintain sward yields at satisfactory soil condition (Michiels *et al.*, 2000).

A wide range of feedstuffs are applied in the feeding system in the tropical and subtropical, predominantly the crops and agricultural by-products, grasses, legumes, trees and shrubs. Because of rapid growth of grasses, plentiful biomass is produced after the onset of the rains, but protein concentration declines as grasses grow and mature. During the dry season, the crude protein (CP) concentration in the native grasses can drop below 3% dry matter (DM) (Adjolohoun *et al.*, 2008). Fodder tree is not sufficient and is of low quality in Myanmar (Myo & Tin, 2007). Shrubs represent an enormous potential source of protein for ruminants in the tropics (Devendra, 1992).

Browses which are rich in protein and minerals are used freshly lopped in the dry season or as dry leaves conserved for feed after collection. The larger cultivated area at the expense of grazing land produces more low quality crop residues and N supplementation in the form of browse can enhance the nutritive values of these crop residues. Herbaceous legumes can be intercropped with cereal crops as a relay within or as a rotation with cereals. Through bacterial activity in root nodules, atmospheric N fixation can access by herbaceous legumes and contribute to subsequent cereal crops. The growing of herbaceous legumes provides a cheaper and more suitable to drought resistant (Nulik *et al.*, 2013).

As Myanmar is one of the tropical countries, it is needed to solve the problem of feed shortage during dry season by cultivating good quality forages which are drought resistance. In Myanmar, Nang (2015) evaluated 16 kinds of grasses for selection of good quality grass in terms of yield and nutritive values. However, there is still limited information on evaluation of the legume forage species. Therefore, this study was conducted to evaluate dry matter yield and nutritive values of herbaceous legume forage varieties through analysis of chemical composition and *in vitro* gas method.

MATERIALS AND METHODS

Experimental Site and Experimental Design

The experiment was conducted at the research farm of University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar. For planting, a randomized complete block design with 4 blocks was used in this experiment. In each block, there were five introduced herbaceous legumes plots which were randomly assigned in a (4 × 5) factorial experiment. Rainfall, minimum and maximum temperature of experimental area were recorded monthly (June, 2014 to December, 2014) and harvesting times are shown in Table 1. Soil condition of the study area was observed and shown in Table 2.

The herbaceous legumes species used in this experiment were *Stylosanthes guianensis* cv. Ubon stylo, *Macrotyloma axillare* cv. Archer, *Centrosema brasilianum* cv. Ooloo, *Stylosanthes guianensis* cv. Stylo 184, and *Macroptilium bracteatum* cv. Cadarga.

Sample Collection and Measurements

All of the plants were harvested for four times at 8 weeks intervals. The harvestings were made every eight weeks interval for four times. The forage legumes were cut about 5cm to ground level and total fresh forage yield was recorded. Then, 500g of sample were taken from fresh yields of each plot. When weight of the sample was stable after sun-dried, the sample

Table 1: Monthly minimum and maximum temperature and rainfall during the experimental period in 2014 and 2015`

Month	Minimum temperature (°C)	Maximum temperature (°C)	Monthly rainfall (mm)	Harvesting time
June	28	32	50	-
July	28	32	116	-
August	28	30	326.4	1 st
September	29	32	183.3	-
October	29	31	61	-
November	28	31	0	2 nd
December	29	31	0	3 rd
January	29	32	0	4 th

Table 2: Soil test of the study area

Description	Value	Rating
pH	6	Moderately acid
EC	0.06dS/m	Non saline
Available N	85mg/kg	Medium
Available P	11mg/kg	Medium
Available K	92mg/kg	Low
Organic matter	1.5%	Low
Soil Textural Class		Loamy sand
Water soluble SO ₄ -S	0.2 mg/kg	Low
Exchangeable Ca	2cmol ₍₊₎ /kg	Low
Exchangeable Mg	1 cmol ₍₊₎ /kg	Medium
Exchangeable Na	0.1cmol ₍₊₎ /kg	Low
DTPA extractable Zn	1 mg/kg	Marginal
DTPA extractable Fe	12 mg/kg	Adequate
Cl ⁻	56 mg/kg	Non critical for salinity

were ground for analysis of chemical composition to determine the dry forage yields and nutritive values. *In vitro* fermentation parameters were measured by *in vitro* gas method described by (Menke & Steingass, 1988).

Determination of Nutritive Values through Chemical Analysis

Ground samples of feedstuffs were analyzed for dry matter (DM) and organic matter (OM) by the method described by (AOAC, 1999). Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were analyzed according to the method of (Goering & van Soest, 1970). All herbaceous legume samples were analyzed for nitrogen by using Kjeldahl method (Foss 2020 digester and Foss 2100 Kjeltac distillation unit) and CP was calculated as $6.25 \times N$ (AOAC, 1990).

In vitro Gas Method

Before the collection of rumen fluid, the fistulated bull (360 kg) was fed with the ration containing CP 13% (9 kg of rice straw and 2kg of groundnut meal) twice a day for 14 days as adaptation period. Rumen fluid was collected from the experimental animal before morning feeding. The procedure for *in vitro* gas production was as described by (Menke & Steingass, 1988). Rumen fluid was mixed with buffer medium at ratio of 1:2 (v/v) under a continuous stream of carbon dioxide (CO₂). The 200±10mg weight for experimental forage with four replicates was introduced into the 120ml calibrated syringe and followed by adding of 30ml inoculums. Blanks sample were also included for gas production. Incubation was carried out at 39°C using water bath. *In vitro* gas production was recorded at 1, 6, 12, 24, 48, 72 and 96 hours and cumulative gas production was calculated using the following equation.

Cumulative gas production (ml/200 mg DM) = $[(V - V_0 - G_0)/\text{Feed (mg)}] \times 200$ Where; V = Reading after incubation
V₀ = Reading just before incubation, G₀ = Gas produced in blank syringes

Statistical Analysis

The data were analyzed by analysis of variance in 4 × 5 factorial arrangement (4 harvesting times and five forage species) using SAS software, version (9.0) (2002). Duncan's Multiple Range Test (DMRT) was applied to compare the treatment means for different parameters at significant level of p<0.05.

RESULTS

Dry Forage Yield and Chemical Compositions of Selected Herbaceous Legumes

The effects of harvesting time on yield of forage legumes are shown in Table 3. No significant interaction (p>0.05) was found between forage species and harvesting time. Among the forage varieties, the highest dry forage yields of Ubon stylo (1.47t/ha) was not significantly different (p>0.05) from those

Table 3: Dry forage yield (t/ha) and Chemical composition of (%) of herbaceous legumes

Herbaceous legumes	Dry forage yield	DM	OM	CP	NDF	ADF
Ubon stylo	1.38 ^{ab}	25.53 ^a	91.17 ^{ab}	11.01 ^a	47.05 ^c	39.42 ^{ab}
Ooloo	1.47 ^a	27.19 ^a	88.28 ^b	10.57 ^{ab}	54.71 ^a	42.01 ^a
Archer	1.07 ^{ab}	15.76 ^b	90.09 ^{ab}	11.32 ^a	45.22 ^c	35.6 ^c
Stylo 184	0.74 ^b	24.83 ^a	91.64 ^a	11.37 ^a	44.63 ^c	36.1 ^{bc}
Cadarga	1.22 ^{ab}	28.57 ^a	88.59 ^b	9.62 ^b	50.43 ^b	38.86 ^{ab}
p value	0.001	0.000	0.044	0.003	0.000	0.000
Harvesting time						
First harvest	0.90 ^b	22.72 ^b	88.67 ^b	9.78 ^b	49.36	40.12 ^a
Second harvest	2.49 ^a	24.22 ^a	88.42 ^b	10.08 ^b	47.57	38.32 ^a
Third harvest	0.51 ^b	27.06 ^a	90.62 ^b	14.9 ^a	49.3	39.59 ^a
Fourth harvest	0.81 ^b	23.51 ^a	92.09 ^a	8.25 ^c	48.08	36.13 ^b
p value	0.0001	0.000	0.044	0.000	0.000	0.000
Forage×Harvesting time	0.61	0.9219	0.1179	0.0001	0.064	0.091

^{a, b, c}; p<0.05 significantly differences treatment means within the same column are indicated by dissimilar superscripts

of Ooloo (1.38t/ha), Archer (1.22t/ha) and Stylo 184 (1.07t/ha) however it was significantly higher than Cardarga (0.74t/ha) in dry forage yield. Ooloo, Archer and Stylo 184 and Cardarga were not different (p>0.05) in dry forage yield. Regarding to the harvesting time, the second harvest had highest total dry forage yields compared to the other harvesting times.

Chemical compositions of herbaceous legumes are shown in Table 3 and 4. There was no interaction between forage legumes and harvesting time on nutritive value except CP which show interaction between forage legumes and harvesting time.

In the aspect of the main effect of legume forage, the dry matter content of Ubon stylo, Ooloo, Stylo 184, Cadarga were not significantly different (p>0.05) from each other but they were significantly higher (p<0.05) than that of Archer. As effect of harvesting time, the mean dry matter content of selected legumes at second harvest, third harvest and fourth harvest were not significantly different (p>0.05) from each other but they were significantly higher (p<0.05) than that of the first harvest.

Comparison between forage varieties as main effect, the organic matter (OM) content of Stylo 184 was not significantly different (p>0.05) from those of Ubon stylo and Archer but it was significantly higher (p<0.05) than those of Ooloo and Cadarga. Ubon stylo and Archer, Ooloo and Cadarga were not significantly different (p>0.05) from each other. As harvesting time, the highest OM content found at fourth harvest was significantly higher (p<0.05) than those of first harvest, second harvest and third harvest.

In the aspect of neutral detergent fibre (NDF) between forage varieties, the NDF content was observed in Ooloo. Cadarga was significantly higher (p<0.05) than Stylo 184, Ubon stylo and Archer which were not significantly different (p>0.05) from each other. As the main effect of harvesting time, all of the harvesting time was not significantly different from each other. The acid detergent fibre (ADF) content of Ooloo, Ubon stylo, Cadarga were not significantly different (p>0.05) from each

other but Ooloo was significantly higher ($p < 0.05$) than Stylo 184 and Archer. Ubon stylo was not significantly different from those of Stylo 184 and Cadarga while it was significantly higher ($p < 0.05$) than Archer. As harvesting time, the ADF content of selected legumes at first harvest, second harvest and third harvest were not significantly different ($p > 0.05$) from each other but they were significantly higher ($p < 0.05$) than that of fourth harvest in ADF content.

There was significant interaction ($p < 0.05$) between forage species and harvesting time on CP contents indicating that harvestings time influenced on the CP content of forage species. At first harvest, the CP contents of Stylo 184 was not significantly different ($p > 0.05$) from that of Ubon Stylo and Cardaga but it was significantly higher ($p < 0.05$) from that Ooloo and Archer in CP contents. At second and third harvest, the similar CP contents were found in all forage legumes. At fourth harvest, Ubon Stylo and Cardaga showed similar CP contents with Ubon stylo and Ooloo but they gave higher CP contents compared to Archer.

Gas Production of Herbaceous Legumes

The gas productions of forage legumes are shown in Table 5. There was no significant interaction ($p > 0.05$) between legumes forage and harvesting time on gas production. The gas production was influenced by main effect of forage and harvesting time. From the incubation period of 24 to 96h, the higher gas productions were found in Archer and Stylo 184. After these two forages, Ubon stylo showed higher gas production only at 72 and 96h. As the main effect of harvesting time, the gas production of selected forage legumes in fourth harvest was significantly higher ($p < 0.05$) than that of first harvest, second harvest and third harvest which were not significantly different ($p > 0.05$) from each other.

Organic Matte Digestibility (OMD) and Short Chain Fatty Acid (SCFA) of Herbaceous Legumes

The organic matte digestibility (OMD) and short chain fatty acid (SCFA) of experimental herbaceous legumes are shown in Table 6 and 7. There was significant interaction ($p < 0.05$) between forage species and harvesting time on OMD (MJ/kg DM) contents indicating that harvestings time influenced on the OMD content of forage species. At third harvest, Archer had the highest ($p < 0.05$) OMD and followed by Ooloo and Stylo 184. The OMD (MJ/kg DM) of Ubon stylo and Cardaga were not significantly different ($p < 0.05$) from each other. At first harvest, second harvest and fourth harvest, the OMD of legume forages were not significantly different.

It was observed no significant interaction ($p < 0.05$) between forage species and harvesting time on SCFA (mmol/200mg DM). Harvesting and forage varieties, as main effect influenced on SCFA of Stylo 184 and Archer were significantly ($p < 0.05$) higher than those of Ubon Stylo, Ooloo and Cardaga. The SCFA (mmol/200mg DM) of Ubon stylo was significantly ($p < 0.05$) higher than those of Ooloo and Cardaga. As the

Table 4: Crude protein (%) of herbaceous legumes

Herbaceous legumes	First harvest	Second harvest	Third harvest	Fourth harvest
Ubon stylo	9.52 ^{ab}	10.09 ^a	16.64 ^a	7.80 ^{ab}
Ooloo	8.24 ^b	9.61 ^a	16.64 ^a	7.80 ^{ab}
Archer	6.78 ^b	10.57 ^a	11.21 ^a	5.39 ^b
Stylo 184	12.91 ^a	8.81 ^a	14.56 ^a	9.19 ^a
Cardaga	9.03 ^{ab}	8.78 ^a	11.71 ^a	8.98 ^a
P value	0.0373	0.9328	0.1303	0.0664

^{a, b;} $p < 0.05$ significantly differences treatment means within the same column are indicated by dissimilar superscripts

Table 5: Gas productions (ml/200mg DM) of forage legumes

Herbaceous legumes	Incubation period (h)				
	24	36	48	72	96
Ubon stylo	28.65 ^b	33.12 ^b	34.53 ^{bc}	37.03 ^{ab}	38.95 ^{ab}
Ooloo	24.28 ^c	28.24 ^c	29.77 ^d	32.66 ^c	34.46 ^c
Archer	31.30 ^a	35.56 ^{ab}	36.61 ^a	39.32 ^a	40.58 ^a
Stylo 84	32.00 ^a	36.05 ^a	37.61 ^a	40.35 ^a	41.65 ^a
Cadarga	26.00 ^c	30.28 ^c	31.77 ^{cd}	34.00 ^{bc}	35.21 ^{bc}
Harvesting time					
First harvest	26.31 ^b	31.20 ^b	32.10 ^b	35.06 ^b	36.84 ^b
Second harvest	28.13 ^b	32.00 ^b	33.51 ^b	35.97 ^b	37.13 ^b
Third harvest	27.00 ^b	31.23 ^b	32.76 ^b	35.38 ^b	36.75 ^b
Fourth harvest	32.00 ^a	35.83 ^a	37.25 ^a	40.02 ^a	41.33 ^a
p value	0.000	0.000	0.0001	0.0001	0.0001
Forage × Harvesting time	0.3396	0.4222	0.7319	0.6530	0.6592

^{a, b, c;} Mean value with different superscripts with the same column are significantly different ($p < 0.05$)

Table 6: Organic matter digestibility and short chain fatty acid contents of herbaceous legumes

Herbaceous legumes	OMD (%)	SCFA (mmol/dl)
Ooloo	41.75 ^d	0.53 ^c
Ubon stylo	45.12 ^c	0.63 ^b
Stylo 184	48.7 ^b	0.71 ^a
Cadarga	42.67 ^d	0.58 ^c
Archer	52.07 ^a	0.68 ^a
p value	0.0001	0.0001
Harvesting time		
First harvest	43.37 ^c	0.59 ^b
Second harvest	45.20 ^{bc}	0.62 ^b
Third harvest	45.83 ^b	0.60 ^b
Fourth harvest	49.85 ^a	0.70 ^a
p value	0.0001	0.0001
Forage × Harvesting time	0.000	0.181

^{a, b, c;} $p < 0.05$ significantly differences treatment means within the same column are indicated by dissimilar superscripts

Table 7: Organic matter digestibility (%) of herbaceous legumes

Herbaceous legumes	First harvest	Second harvest	Third harvest	Fourth harvest
Ooloo	40.271 ^a	43.401 ^a	46.171 ^{ab}	49.908 ^a
Ubon stylo	43.038 ^a	43.972 ^a	43.314 ^b	47.389 ^a
Stylo 184	41.750 ^a	43.084 ^a	44.515 ^{ab}	46.995 ^a
Cardaga	48.861 ^a	46.038 ^a	43.661 ^b	52.883 ^a
Archer	43.072 ^a	50.304 ^a	50.094 ^a	53.915 ^a
P value	0.5379	0.2268	0.1099	0.6723

^{a, b;} $p < 0.05$ significantly differences treatment means within the same column are indicated by dissimilar superscripts.

effect of harvesting time, the highest SCFA (mmol/200mg DM) of legumes was found at fourth harvest. First harvest, second harvest and third harvest were not significantly different ($p > 0.05$) between each other.

DISCUSSION

In this experiment, the dry forage yields of Ubon stylo (1.47t/ha) was not significantly different from those of Ooloo (1.38t/ha), Archer (1.22t/ha) and Stylo 184 (1.07t/ha) but it was significantly higher than Cardarga (0.74t/ha) in dry forage yield. However, the DM yields of all forages were lower than the other reports. (Cook *et al.*, 2005) reported that Stylo is a high yielding forage legume that can produce 10-20t DM/ha depending on soil fertility. However, in this study, Ubon stylo Ooloo and Stylo had mean values of 1.47, 1.38 and 1.07t/ha for 4 harvestings, respectively. The highest total dry matters yields of over 11.5t/ha were recorded in the N applied and the buffel and legume mixes of Ooloo, Milgarra and Maldonado (Shehu, & Akinola, 1995). The dry matter yield of Cardaga was 0.74t/ha and this result was lower than report of (Cox *et al.*, 2003). They reported that the production of Cadarga ranged from (1.0t/ha) in first year to (1.1t/ha) at second year. Archer is a summer-growing perennial best adapted to frost-free subtropical or tropical environment with 1000mm or more annual rainfall (Cameron, 1986; Oram, 1990). High yield of Archer can be achieved; a crop sown in December in north Queensland produced 3-4t/ha by (Staples, 1978). (Parbery, 1967) obtained 5-15t/ha yield of Archer on a Kununurra clay under irrigation. The reason for low DM yield of forages in the current study might be due to no application of fertilizer and irrigation. Therefore, no re-growth was found after 4th harvest (January 2015) in this experiment. (Jacobson *et al.*, 1996) reported that increased yield could be due to application of N fertilizer. This finding was supported by (Mahmut & Binali, 2011) who stated that increased DM yield of forages was because of application of N fertilizer. All of the former experiments mentioned applied fertilizer and irrigation.

When compared mean dry forage yields of introduced legumes between the harvest times in this experiment, the mean dry forage yield was higher in the second harvest than those of first harvest, third harvest and fourth harvest. This finding was supported to (Tudsri *et al.*, 2002) who stated that the high forage yield was found when the forages were cut at the end of rainy season. The increase in mean dry forage yield of introduced forage legume in the second harvest compared to the first and third harvest may be due to increasing number of tillers per plant at the highest rainfall during the period of second harvest (October). The production of legume biomass was significantly increased in the subsequent re-growths after first cut (Olivio *et al.*, 2009). Although higher quality forages can be obtained from earlier maturity harvests, repeatedly harvestings at immature stage can make reduction in stand longevity, vigor, and yield (Sheaffer *et al.*, 1997). Generally, it was discussed that climate, soil condition, plant spacing, plant stock used, management history and age of plant at harvest affect the forage yield (Skerman *et al.*, 1988).

In the results, average CP% of Stylo 184 had 11.37% and CP% of Ubon stylo and Ooloo at third harvest had 16.64%. It might be due to the suitable pH of soil condition for this species. (Cadisch *et al.*, 2000) reported that native habitat of Ooloo was acidic to very acidic (pH of 4.1-6.3) soils. (Peter *et al.*, 1998) reports that a range of 11.8%-19.6% of CP for Ooloo were found in large collections evaluated in Colombia and Nigeria during sampling at ages of 6-18 weeks, respectively. Blumenthal and Staples (1993) reported that Archer had 12-23% CP at the rainfall of 750mm to 1,700mm and temperature was ranged from 18 to 26°C. (Miller *et al.*, 1997) reported that Stylo 184 and Ubon stylo had 12-20% CP at the rainfall of 700-5,000mm per year and temperature had 23°C to 27°C in Colombia and French Guiana. In this study, CP% of Stylo 184, Archer and Ubon stylo had 11.37%, 11.32%, 11.01% respectively. The total rainfall was 736.7mm while the temperature was ranged from 28°C to 32°C. This environment was not as good as the other report so that the CP value was in the minimum range. On the other hand, the CP content of forages positively correlated with the soil fertilizer of N content of soil (Mahmut & Binali, 2011).

Moreover, in the current experiment, soil nutrient showed medium in N and low potassium, water soluble phosphate, organic matter and calcium. (Jutzi & Haque, 1984; Mohamed-Saleem & Von Kaufmann, 1985) reported that phosphate increase nodulation and hence increase N or crude-protein content, phosphate concentration or uptake by the plant. They interpreted that increasing N and phosphate fertilization probably effects on N (Mohamed-Saleem, 1985); (Messman *et al.*, 1991) and CP content of forage (Gillen & Berg, 1998). In this study, there were no irrigation and fertilizer in forage cultivation.

In the current data, CP% of legumes had more than 6-8%. (Minson, 1981) reported that CP content of forage was above the critical level for ruminants of 6-8%. Among the forage species, Stylo 184, Ubon Stylo and Archer had highest CP% than other species and among the harvesting time, third harvest had highest CP% than other harvesting time. It might be possible that forage quality was most likely influenced by environmental conditions and species composition. At the time of third harvest, the growth of forage is slower than other harvesting times as a result lower maturation in third harvest. The fourth harvest showed lower CP contents. It might be due to flowering stage of legume at fourth harvest. The crude protein yield generally decreased at flowering because of low protein concentration in the herbage (Bertilsson & Burstedt, 1983). The weather is known to influence forage quality (Thorvaldsson, 1987; Thorvaldsson, & Björnsson, 1990) along with management, harvest dates (Pelletier *et al.*, 2008) and applied fertilizer (Zemenchik *et al.*, 2002). The CP content was affected by seasonal variations, with a higher CP content in the wet season than in the dry season. Similar findings have been reported by (Hare *et al.*, 2004).

In this study, the NDF content of legume forages were ranged from 44.63 to 54.71%. (Singh & Oosting, 1992) pointed out that roughage feeds containing NDF values of less than 45% to be classified as high, those with values ranging from 45% to 65% as medium and those with values higher than 65% as

low quality. Therefore, the Ooloo and Cadarga were included in medium quality legume forages. The NDF content of forage varies widely, depending on species, maturity, and growing environment (Nelson & Moser, 1994; Buxton & Fales, 2004; Mahyuddin, 2007). Therefore, each plant species presents a unique NDF-ADF ratio in the feed. (Van Saun, 2006) is considered for legumes, <40% NDF content is classified as good quality forage, while more than 50% as poor quality forage. The Stylo 184, Archer and Ubon stylo could be assumed high quality forages because the NDF contents of these legume forages were lower than 50% and they also had lower ADF content. (Nastis, 1982; Papachristou *et al.*, 1993; Papachristou & Papanastis, 1994) reported that the gradual increase in the NDF contents was due to the progressive lignifications and fibre formation of cell walls consumable parts of woody shrub species depending on the season.

In this study, Stylo 184 and Archer had higher gas production than other species. It might be due to higher CP contents as well as lower fibre contents in these forages. (Buxton, 1996) reported that maturity strongly influences the digestibility of the forage and the higher degradability may be linked to its higher CP content which provides more N for microbial utilization (Abdulrazak *et al.*, 1997). McSweeney *et al.* (2001) reported that NDF and ADF were negatively correlated with gas production. (Haddi *et al.*, 2003) stated that the higher NDF and ADF contents could decrease the gas production. (De Boever *et al.*, 2005) reported that the higher NDF contents caused the lower gas production. High quality forage has high digestibility, low fiber content and high concentration of protein (McDonald *et al.*, 2002).

Among the harvesting time, the fourth harvest showed higher gas production due to lower ADF content and higher OM content although lower CP content at fourth harvest. (Makkar, 2012) stated that gas production is a result of fermentation of carbohydrates to acetate, propionate and butyrate. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation. (Afshar *et al.*, 2011) stated that high rate of gas production possibly influenced by carbohydrate fractions readily availability to the microbial population. In the results, Archer had the highest OMD and followed by Stylo 184 and Ooloo at third harvest while OMD of forage legume at other harvest were not different. As main effect of comparison in forage varieties, the higher SCFA was also observed in Archer and Stylo 184. The higher OMD and SCFA of forage were observed in fourth harvest. The higher value in OMD and SCFA might be related with the greater amount of 24 gas production. The production of SCFA which was based on carbohydrate fermentation was closely related with *in vitro* gas production from different class of feed (Blummel *et al.*, 1990). The molarity of ruminal short chain fatty acid was positively correlated with ruminal digestibility of feed (Kara, 2019). Availability of quality of forage to livestock is the main influencing factor for animal performance (Lazzarini *et al.*, 2009; Woolley *et al.*, 2009). Herbaceous and shrub legumes are promising sources of protein for supplementing diets of ruminants consuming low-quality forages (Hess *et al.*, 2003). As this study was conducted for the application of lowest input,

the DM yield and quality of forages are lower than other report. Therefore, the experiment on the application of fertilizer and irrigation to these herbaceous legumes should be carried out.

CONCLUSION

According to the findings, Ubon stylo, Ooloo, Archer and Stylo 184 showed higher DM yields and CP contents. The highest total CP content of forage legume was found in the third harvest. Stylo 184 and Archer showed the lower fibre content and higher gas production as well as higher OMD and SCFA. The lowest ADF content and highest gas productions of forage legumes were found in fourth harvest. Therefore, Archer and Stylo 184 could be assumed as good quality forage in terms of DM yields, high CP contents, low fibre contents and higher gas production in natural condition without fertilizer application and without irrigation. Application of fertilizer and irrigation would be preferable to get better forage quality and yield.

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