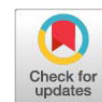


Research Article



Optimizing Yield and Nutritional Quality in Napier Grass (*Pennisetum purpureum*) Based on Harvest Interval

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Abstract | In Bangladesh and elsewhere in tropical countries, smallholder livestock farmers face a significant challenge to maintain quality of pakchong grass (*Pennisetum purpureum*) required to produce milk and meat from ruminants. This study was conducted to investigate the potential of pakchong at different harvest interval (HI). A 3×4 factorial experiment were carried out in a randomized complete block design at four harvest interval (25d, 35d, 45d and 55d), for a total of 36 plots, each 5×5 m² with 1m line spacing and 0.4m plant spacing. Dry matter yield (DMY) increased ($P<0.001$) at 55d HI than 25d HI whereas, crude protein (CP) content decreased ($P<0.001$) substantially from 19.52 to 9.51, degradability decreased from 62.9 to 51.7% and metabolizable energy (ME) from 8.74 to 7.06 MJ/kg DM. Plant height also found significantly ($P<0.001$) higher at 55d HI compared to 45d, 35d and 25d however leaf stem ratio significantly ($P<0.001$) increased in 25d HI followed by 35d, 45d and 55d. Furthermore, total gas production also found higher ($P<0.001$) at prolong HI. Negative correlation found ($r = -0.85$ to -0.90 ; and $P<0.001$) among CP, plant height, Acid detergent fiber (ADF) and Neutral detergent fiber (NDF), whereas significant positive ($r = 0.47$ and 0.18 ; and $P<0.01$) correlation among leaf weight and sheath weight. Data were also analysed to investigate seasonal impacts on yield and quality. Total DM yield and CP or ME contents of pakchong grass was similar ($P>0.06-0.08$) among seasons. These results suggest high quality pakchong grass is capable to supply CP requirement of high producing dairy cows (19.5% CP at 25d HI) or beef cattle (14.3% CP at 35d HI) with a great deal of energy (8.7-7.4 MJ ME/kg DM) respectively. Further research is required whether ruminants can be grazed rotationally by matching HI so that the cost of production could be reduced compared to the current practice of cut and carry system of pakchong grass.

Keywords | Biomass, Correlation, Degradability, *in-vitro*, Morphology, Season

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INTRODUCTION

Pakchong grass (*Pennisetum purpureum*) is more preferable roughage to farmers for their ruminants in

tropical and sub-tropical based on its biomass yield and nutritional quality for increasing ruminant's production towards ensuring animal protein sources (Hernandez *et al.*, 2019; Oosting *et al.*, 2014). Pakchong fodder has widely

spread across the country for last decade. Consequently, tremendous ruminant's population, milk and meat production has increased over the last half century from 6.5 mill to 57.14 mill, 1.34 lakh MT to 14.068 mill MT, 0.45 lakh MT to 8.71 mill MT while the population of buffalo increased from 0.08 mill to 1.52 mill, respectively (BBS 2020, DLS 2022-23). Moreover, about 80% of total forage ingested by ruminants are comprises of Napier pakchong in tropical and sub-tropical region (Kabirizi *et al.*, 2015). Pakchong fodder is important for livestock production but harvesting it late makes it low quality resulting did not provide much energy and crude protein (CP) to the animals. Generally, pakchong harvested at 60 days or more ages, provide poor nutritive value (<9.5% CP % and <8.5 MJ/DM ME) resulting failed to support animal productive performances (Muvinga *et al.*, 1992; Brown *et al.*, 1988). Additionally, higher level of Neutral Detergent Fiber (NDF) also reduced feed intake of dairy animals. Conventionally harvested (≥ 60 day's age) Napier contains more NDF ($\geq 72\%$) with lower CP ($\leq 8.5\%$) which suppress the DM intake (2.1%) resulting decreasing milk yield and losses live weight (36 g/day) (Islam *et al.*, 2024). Gwayumba *et al.* (2002) also reported that Napier grass harvested at 70 to 91 days interval had very lower amount of crude protein (6.8-8% of DM) and about 7.5 MJ ME per kg DM. Therefore, quality Napier (Increased CP and ME) with decreased NDF should be supplied to dairy animals for increasing dry matter intake beyond 2% to sustain milk yield and meat production. Moreover, Napier grass supplied to dairy animals harvested at 28 days or greater interval is failed to meet up the requirement of ME and CP for moderate milk yield and meat production (Islam *et al.*, 2024) because grasses offered to animals at early stage of growth had higher fiber digestibility which increase feed intake and animals productivity. Islam *et al.* (2023) also stated that, Napier pakchong grass has potential that, the crude protein content can be increased from 9.6 to 25.7 % of dry matter (DM), metabolizable energy can be 8.7 mega joule to 10.8 mega joule per kg DM with changing of defoliation leaf. Which suggested that, Napier pakchong can be yielded adequate CP and energy for optimizing dairy performances of ruminants. Quality Napier pakchong also induced more than 25000 liter milk and moderate meat production per animal per hectare in tropical and subtropical region (Farina *et al.*, 2011). However, Napier pakchong grass has the potentiality

to transform plant protein to animal protein all around the world. The important determinant of dairy animal's production is feed intake. Aroeira *et al.* (1999) stated that, dairy cows fed with solely Napier grazed or harvested at 30 days interval no need to provide concentrate feed except dry season for moderate milk and meat productions in subsistence farming. Productivity and nutritional quality of pakchong fodder highly influenced by cutting age, early age cutting fodder yield more crude protein and lower crude fiber. Morphological characteristics and nutritional quality of pakchong fodder have been studied at generative stage, while little study on early growth stage and different harvest interval. However, there was not enough study about early harvesting yield and quality of Pakchong over the year to mitigate the gap between yield and quality. Therefore, this study was conducted to determine the harvest interval and seasonal effects on pakchong forage and investigate the yield, quality, *in-vitro* degradability and their relationship at different growing stage.

MATERIAL AND METHODS

EXPERIMENTAL SITE AND SOIL CONDITION

The experiment was carried out at Buffalo Research Farm, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, situated about 30 km northwest of central city of Bangladesh. The location were existed in $23^{\circ}42'0''$ north and $90^{\circ}22'30''$ east and the average temperature (30.11°C) was found 2.37% higher compared to the average temperature of Bangladesh. Precipitation rate was 74.11 mm with 120.12 rainy days yearly and the average humidity is 70% (Miah *et al.*, 2025). The soil of experimental plot was clay in textured, under the Madhupur Terrace agro-ecological zone of Bangladesh (Hasan *et al.*, 2019). The soil was normal or non-saline but acidic (pH=5.3) in condition. Total nitrogen, phosphorous and calcium were found in medium range, whereas carbon was higher in the soil (Table 1).

EXPERIMENTAL LAND PREPARATION AND MANAGEMENT

Napier pakchong (*Pennisetum purpureum*) fodder was cultivated in the experimental plot and the plot size was 25 m^2 ($5\text{m}\times 5\text{m}$). Before transplantation of the fodder, the land was properly ploughed. The fertilizer doses were applied during land preparation, where 6 t/ha cow dung

Table 1: Soil composition of experimental plot.

Statistics	Soil elements							
	pH	EC (dS/m)	Carbon (%)	Total Nitrogen (%)	Phosphorous (cmol/kg)	Potassium (cmol/kg)	Calcium (cmol/kg)	Magnesium (cmol/kg)
Mean	5.3	0.072	0.87	0.09	22.81	0.09	3.31	0.12
SD	0.29	0.01	0.13	0.01	2.69	0.01	0.23	0.02
Remarks	Acidic	Non Saline	High	Medium	Medium	Low	Medium	Low

was applied as a basal dose, (TSP) Triple super phosphate and MP (Murate of Potash) were used 150:75 kg/ha. After each harvest 50 kg/ha urea fertilizer was top dressed uniformly and irrigation was applied. Line to line distance were kept 1m and plant to plant distance were 0.4m and 1m alley were maintained around each plot. Two stem with double nodes were transplanted into the plot. First harvest was completed after 60 days of transplantation for better tiller.

EXPERIMENTAL DESIGN

The experimental design was randomized complete block design (RCBD). Harvesting interval considered as treatment (4 harvesting interval 25 days, 35 days, 45 days and 55 days), each harvested at 3 season (3 season- rainy, winter and summer). This experiment was conducted at 4×3 factorial arrangement in a randomized complete block design (RCBD) with 3 blocks randomly, arranging 36 plots. Harvesting interval considered as treatment, each harvested at 3 season.

HARVESTING PROCESS, MORPHOLOGY IDENTIFICATION AND SAMPLE ANALYSIS

Pakchong fodder was cultivated and first 60 days were considered as an establishment period of fodder plot. Subsequently, forage was harvested at 25 days, 35 days, 45 days and 55 days interval for year round (Rainy season- June to September, Winter season- October to January, Summer season- February to March). Forage was harvested at 5cm above the ground level. After each cut the fodder was fertilized and managed properly. A total of 14 harvest at 25 days, 10 harvest at 35 days, 8 harvest at 45 days and 6 harvest at 55 days were completed after establishment period in a year. Plant height, stem length, leaf length, stem diameter, sheath length, leaf yield, Sheath yield and Stem yield were measured after each harvest at Feed Biotechnology laboratory and stem diameter were determined from lower parts, middle parts and upper parts of the stem after removing leaf and sheath by measuring tape digital slide calipers. Plant height and other morphological characteristics were counted from 30 individual plants of each plot. Sample were collected randomly and analyzed as per AOAC (2005) for DM, Ash, CP, Neutral detergent fiber (NDF) and Acid detergent fiber (ADF). DM was determined by oven drying samples at 105 °C for 6 hr. and ash was determined at 550 °C for 8 hour in Muffle furnace. Crude protein was determined by Kjeldahl method and ADF and NDF were determined by Gerhardt method in Feed Biotechnology laboratory, Animal Nutrition laboratory, BLRI, Dairy Science and Poultry Science laboratory, GAU and Quality control laboratory, DLS.

COLLECTION OF RUMEN FLUID

Rumen liquor was obtained from a previously cannulated

animal in Bangladesh Livestock Research Institute (BLRI) dairy farm. Collected rumen liquid was promptly combined, transferred to a pre-warmed (39°C) vacuumed flask, purged with CO₂, and then promptly capped to preserve anaerobic conditions. The flasks were conveyed to the laboratory in a thermostatic container to maintain a temperature of 39°C. The rumen fluid was filtered through pre sterilized four-layer cheesecloth while continuously flushing with CO₂ to reduce exposure to oxygen (O₂).

IN-VITRO GAS PRODUCTION KINETICS

The gas production kinetics of different ages Pakchong were measured using automatic gas production measurement technique which has been developed by Ankom Technology® (Macedon, NY, USA; ANKOMRF gas production system) (Ankom, 2011). Each module comprises a septa glass bottle (actual capacity: 313 ml) equipped with an ANKOM pressure sensor module (pressure range: -69 to +3447 kPa; resolution: 0.27 kPa; accuracy 0.1% measured values), featuring a microchip and a radio transmitter. The measured gas pressure was converted into moles of gas produced using the ideal gas law (Equation 1) and then converted to milliliters (ml) of gas produced by Avogadro's law (Equation 2).

$$n = p(V/RT) \dots (1)$$

Where n = gas produced in moles (mol); p = pressure in kilopascals (kPa); V = headspace volume in the glass bottle in liters (L); T = temperature in Kelvin (K); R = gas constant (8.314472 L·kPa/K/mol).

$$\text{Gas produced in mL} = n \times 22.4 \times 1000 \dots (2)$$

The incubation of the target sample was carried out in a module containing 1g of respective feedstuff, 80 ml of buffer medium and 20 ml of rumen fluid as inoculum. The composition of the buffer medium was Resazurin 0.1% (w/v) solution, *in-vitro* buffer solution (NH₄HCO₃, NaHCO₃), *in-vitro* micro mineral solution (CaCl₂·2H₂O, MnCl₂·4H₂O, CoCl₂·6H₂O, FeCl₃·6H₂O), *in-vitro* macro mineral solution (Na₂HPO₄ anhydrous, KH₂PO₄ anhydrous, MgSO₄·7H₂O), Reducing Solution (Cysteine HCl, 1N NaOH, Na₂S·9H₂O) as described by Goering and Van Soest (1970). All the glassware, solution and inoculum were maintained at 39°C before placing them into the respective module. Finally, all the materials (target sample, buffer medium, inoculum) were placed into the respective module, purged with CO₂ further and placed them in a shaking incubator with a temperature and rotation speed of 41°C and 100, respectively. The fermentation was carried out for 48 hour. The ME was estimated by using following equations: ME (MJ/kg DM) = 21.574 - 0.207 NDF (Minson, 1984).

DETERMINATION OF *IN VITRO* DRY MATTER DEGRADABILITY (IVDMD)

After 24 hours of fermentation under the specified conditions, each sample was filtered to separate the solid and liquid parts. The solid portion was then washed thoroughly with distilled water to remove any leftover fermentation medium. Next, the cleaned solids were dried in a forced-air oven at 105°C until constant weight was achieved. The DMD was calculated by comparing the final dry weight to the original sample weight. DMD was quantified using the following equations adapted from Zhang *et al.* (2018):

$$\text{IVDMD (g/kg of DM)} = [1 - W_1 * (V_1/V_2)/W_2] * 1000$$

Where, W2 and W1 indicate the DM weight (g) of Pakchong before and after 24h of fermentation, respectively, while V1 and V2 represent the volume (ml) of buffered rumen fluid in the bottle before and after sampling.

STATISTICAL ANALYSIS

Considering the harvest interval age as treatment, biomass yield (Fresh, DMY and CPY), nutritional composition (DM, Ash, CP, ADF and NDF) and morphological data were analyzed in two-way ANOVA of Randomized complete block design using R studio (4.2.2). Mean difference were measured by using Duncan Multiple Range Test (DMRT). Differences of mean value were considered significant if p value is less than 0.05. Correlation among morphological characteristics and nutritional parameters were determined through Pearson's correlation and the differences at p<0.05 considered as significant correlation, p<0.01 considered as highly significant correlation.

RESULTS

MORPHOLOGICAL CHARACTERISTICS OF PAKCHONG FODDER

The results (Table 2) revealed that both season and harvest interval (HI) significantly (p<0.01) influenced the growth characteristics pakchong fodder. Plant height increased (p<0.01) progressively with longer harvest intervals and reached maximum at 55 days harvest interval followed by 45 days, 35 days and 25 days. Regardless of the harvest interval, plant height exhibited higher in rainy season compared to summer and winter season. Similarly, the number of leaves and other morphological characteristics including leaf length, sheath length, stem length and stem diameter were all enhanced at the 55 day harvest interval (p<0.05) compared to 45 days, 35 days and 25 days interval. Additionally, seasonal effects were also showed, with these parameters showing significantly higher (p<0.05) values in the rainy season, indicating that favourable moisture and temperature conditions during this period promote better vegetative growth.

LEAF-STEM YIELD AND RATIO OF PAKCHONG AT DIFFERENT HARVEST INTERVAL

The results (Table 3) indicated that harvest interval and season both had significant (p<0.05) effects on the yield components of pakchong. Leaf yield was highest (p<0.05) at 25 days harvest interval gradually declining with longer intervals (Table 3). In contrast, sheath yield showed higher at 45 days, while stem yield increased its maximum at 55 days indicating the increasing structural biomass production over time. With respect to season, leaf and sheath yields were found higher during winter season whereas, stem yield was higher in rainy season. Notably, Leaf stem ration was significantly (p<0.05) higher at

Table 2: Morphological characteristics of Pakchong at different harvest interval seasonally.

Season, Harvest Interval (HI) and their interaction		Parameters					
		Plant height (cm)	Leaf Number (nos)	Leaf length (cm)	Sheath length (cm)	Stem length (cm)	Stem diameter (cm)
Season	Summer	109.50 ^b	9.50	57.42 ^b	18.63 ^b	26.94 ^a	6.23 ^b
	Rainy	116.37 ^a	10.85	64.45 ^a	21.03 ^a	25.51 ^a	7.08 ^a
	Winter	105.45 ^c	10.25	54.53 ^c	14.15 ^c	19.07 ^b	6.11 ^b
Harvest Interval (HI)	25 days	61.49 ^d	7.20	44.43 ^d	13.43 ^c	16.35 ^d	5.29 ^d
	35 days	88.65 ^c	8.65 ^c	57.79 ^c	16.27 ^b	21.81 ^c	5.82 ^c
	45 days	115.71 ^b	9.50 ^b	64.45 ^b	18.37 ^a	26.89 ^b	6.87 ^b
	55 days	146.61 ^a	11.50 ^a	71.67 ^a	19.23 ^a	38.68 ^a	8.11 ^a
	SEM	1.15	0.08	0.81	0.52	0.82	0.09
Level of Sig.	Season	<0.001	0.06	0.02	0.03	0.01	0.03
	HI	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Season*HI	0.62	0.04	<0.001	0.02	<0.001	0.01

^{a,b,c} Mean in the same column with different superscripts differ significantly (p<0.05); Highly significant (p<0.05); NS= Non significant (p>0.05).

Table 3: Leaf, sheath, stem percentage and ratio.

Season, harvest interval (HI) and their interaction		Parameter			
		Leaf yield (g/100g)	Sheath yield (g/100g)	Stem yield (g/100g)	Leaf stem ratio
Season	Summer	43.64 ^b	16.32 ^b	40.04 ^b	1.08 ^b
	Rainy	41.62 ^b	14.07 ^c	44.31 ^a	0.93 ^c
	Winter	45.07 ^a	17.55 ^a	37.38 ^c	1.21 ^a
Harvest Interval (HI)	25 days	64.95 ^a	10.82 ^b	24.23 ^c	2.68 ^a
	35 days	50.93 ^b	14.15 ^a	34.90 ^c	1.46 ^b
	45 days	40.67 ^c	16.02 ^b	43.29 ^b	0.93 ^c
	55 days	37.37 ^d	13.57 ^c	49.05 ^a	0.76 ^d
	SEM	0.52	0.30	0.73	0.07
Level of Sig.	Season	<0.001	<0.001	<0.001	0.02
	HI	<0.001	<0.001	0.01	<0.001
	Season*HI	<0.001	<0.001	0.02	0.01

^{a,b,c} Mean in the same column with different superscripts differ significantly ($p < 0.05$); Highly significant ($p < 0.001$); NS= Non significant ($p > 0.05$).

25 days harvest interval and declined as harvest interval increased, indicating younger pakchong yielded more biomass to leaves than stem. Across season, leaf stem ratio was higher ($p < 0.05$) in winter season than summer and rainy respectively. Interaction between season and harvest interval significantly influenced all yield components, the combined role of environmental conditions and harvest interval in determining Pakchong fodder productivity (Table 3).

EFFECTS OF HARVEST INTERVAL ON FORAGE PRODUCTION AND NUTRITIVE VALUE

The research result revealed that both season and harvest

interval had significant ($p < 0.05$) effects on yield and nutritive value of Pakchong fodder (Table 4). Significantly ($p < 0.05$) higher dry matter yield found with longer harvest interval and reached maximum at 55 days, while fresh biomass yield and crude protein yield varied significantly over the season. Similarly, dry matter content, ash, crude fiber, acid detergent fiber, neutral detergent fiber were highest at 55 days harvest interval reflecting advanced plant maturity. In contrast, crude protein content was significantly higher at 25 days and decreased with delayed harvest interval. The interaction between season and harvest interval also significantly ($p < 0.05$) affected both fresh biomass yield, crude protein yield.

TOTAL GAS PRODUCTION AND DEGRADABILITY

Season had significant effect ($p < 0.05$) on the 24 hour gas production (GP_{24}) and effective dry matter degradability of pakchong fodder (Table 5). GP_{24} found higher ($p < 0.05$) during summer and winter season than rainy season, while effective dry matter degradability was found higher ($p < 0.05$) in rainy season followed by summer and winter season. Irrespective of season, GP_{24} was higher ($p < 0.05$) with plant maturity or longer harvest interval, reached maximum at 55 days of harvest interval and lower ($p < 0.05$) in 25 days harvest interval, whereas *in vitro* dry matter degradability (IVDMD) showed opposite trend and found higher ($p < 0.05$) at 25 days of harvest interval, with advancing harvest interval IVDMD declined. Similarly, estimated metabolizable energy (ME) was found higher in pakchong harvested at 25 days compared to 35 days, 45 days and 55 days, respectively. An interaction effect were observed between season and harvest interval on GP_{24} gas production and metabolizable energy.

Table 4: Harvest interval and seasonal effects on biomass yield and nutritive value of Pakchong.

Season, harvest interval and interaction		Parameters							
		DM (g/100g)	CP (g/100g)	Ash (g/100g)	ADF (g/100g)	NDF (g/100g)	Total FBY (t/h/y)	Total DMY (t/h/y)	Total CPY (t/h/y)
Season	Rainy	16.92	13.48	9.59	38.45	68.27	166.44 ^a	28.26 ^a	3.63 ^a
	Winter	17.13	13.22	9.71	38.83	68.65	159.06 ^c	27.39 ^b	3.45 ^c
	Summer	16.90	13.56	9.74	38.59	68.55	162.18 ^b	27.56 ^b	3.54 ^b
Harvest Inter- val (HI)	25 days	12.58 ^d	19.52 ^a	7.84 ^c	33.98 ^d	62.99 ^g	167.93 ^b	21.504 ^g	4.25 ^a
	35 days	14.82 ^c	14.30 ^b	8.84 ^b	36.05 ^c	66.56 ^c	155.66 ^d	23.07 ^{ef}	3.30 ^d
	45 days	17.26 ^b	12.46 ^c	10.28 ^a	37.60 ^b	68.59 ^c	161.56 ^c	27.89 ^d	3.47 ^c
	55 days	20.72 ^a	9.51 ^d	10.84 ^a	38.93 ^a	69.65 ^a	155.90 ^d	32.29 ^b	3.07 ^e
	SEM	0.57	0.84	0.70	0.58	0.67	4.28	1.88	0.93
Sig. level	Season	0.05	0.06	0.33	0.10	0.06	<0.001	0.08	0.04
	HI	<0.001	<0.001	0.01	0.001	0.002	<0.001	<0.001	<0.001
	HI*Season	0.91	0.08	0.98	0.74	0.04	0.51	0.89	0.05

DM=Dry matter; CP=Crude protein; CF=Crude Fiber; ADF= Acid Detergent Fiber; NDF= Neutral Detergent Fiber; FBY= Fresh Biomass Yield; DMY=Dry Matter Yield; CPY=Crude Protein Yield. ^{a,b,c} Mean in the same column with different superscripts differ significantly ($p < 0.05$); Highly significant ($p < 0.001$); NS= Non significant ($p > 0.05$).

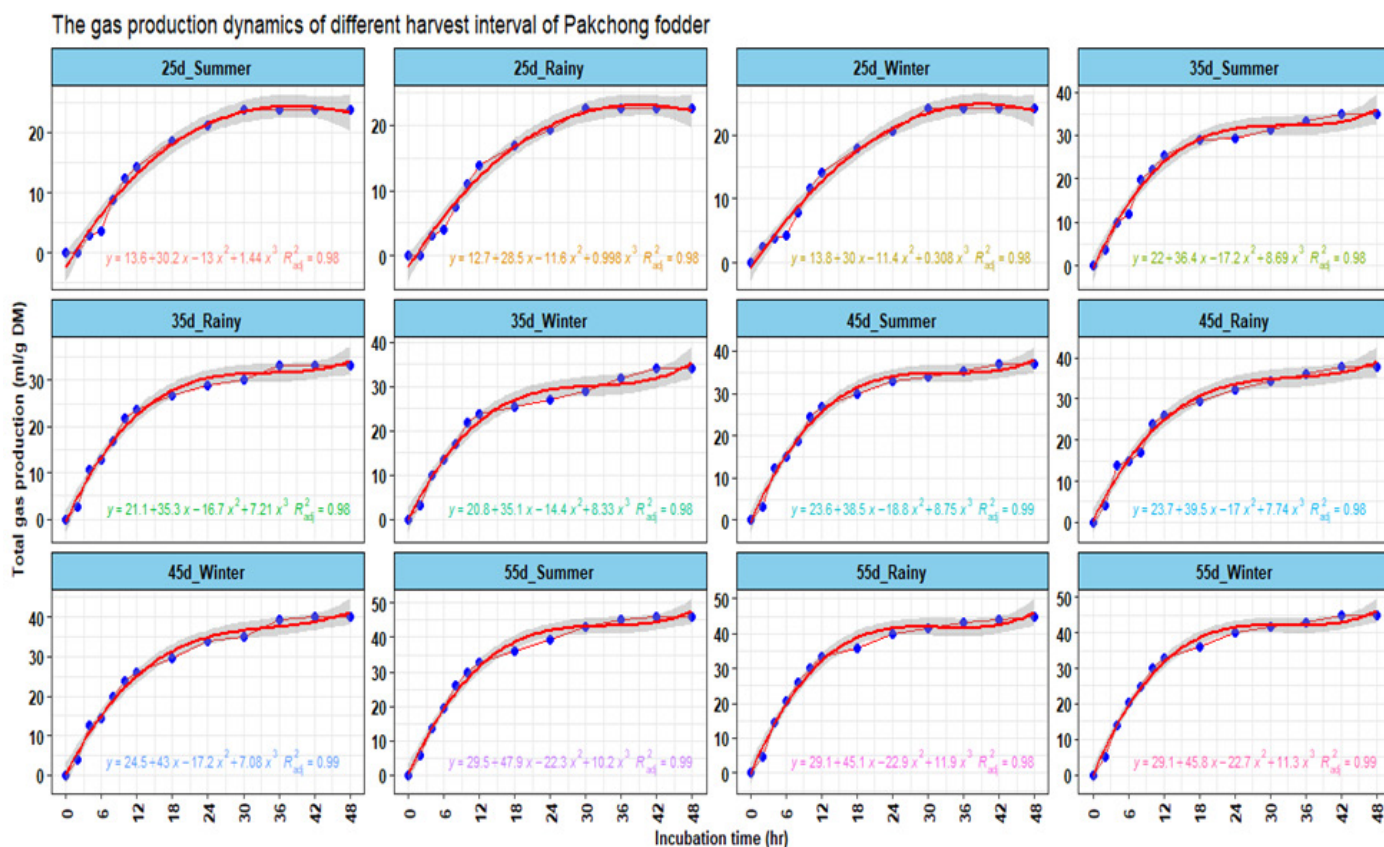


Figure 1: *In vitro* gas production dynamics of Pakchong following 48h of incubation.

Additionally, Figure 1 illustrated the gas production profiles of Pakchong fodder harvested at different intervals and seasons exhibited a clear non-linear (curvilinear) fermentation pattern, described by second order polynomial (quadratic) regression models. In all harvest interval and across seasons gas production increased rapidly during the initial incubation phase (0-12h) followed by a gradual decline in the rate of increased as incubation progress, finally reached a plateau stage between 30-48 hour. The characteristics of curve reflected the typical dynamics of microbial fermentations and the substrate for fermentation were utilized rapidly at the beginning after which gas production showed due to substrate depletion and reduced microbial activity. The quadratic equations for each harvest interval and season showed strong coefficients of determination ($Adj R^2 = 0.98-0.99$) indicated an excellent fit of the polynomial model to the observed data. The positive linear term in all curves described the initial upward fermentation trend while the negative quadratic term reflected the curve trajectory and eventual plateauing of gas production. This confirmed that the fermentation process is inherently non-linear and quadratic model is appropriated for determining the rapid-slow fermentation transition. Additionally, Seasonal effects was found. Gas production was significantly higher at rainy and winter season compared to the summer season at same harvest intervals. Similarly, Pakchong fodder harvested at longer intervals tended to produce more total volume of gas.

However, despite variations in measure, all curves showed similar quadratic shape, indicated the overall fermentation kinetics remained consistent on harvest interval.

Table 5: *In vitro* gas production of pakchong at different harvest interval.

Season, HI and their interaction		In vitro digestibility		
		GP ₂₄ (ml/g DM)	IVDMD (%)	Estimated ME (MJ/Kg DM)
Season	Summer	30.89 ^b	57.13 ^b	7.75
	Rainy	29.53 ^c	59.07 ^a	7.62
	Winter	31.87 ^a	55.66 ^c	7.56
Harvest Interval (HI)	25days	20.39 ^d	62.92 ^a	8.74 ^a
	35days	28.62 ^c	59.58 ^b	7.51 ^b
	45days	33.11 ^b	55.34 ^c	7.17 ^{bc}
	55days	40.47 ^a	51.70 ^d	7.06 ^c
	SEM	0.18	0.37	0.02
Level of sig	Season	0.04	<0.001	0.06
	HI	<0.001	<0.001	0.02
	Season*HI	<0.001	0.98	0.04

GP= Gas production, IVDMD= *in vitro* dry matter degradability; ME= Metabolizable Energy. ^{a,b,c} Mean in the same column with different superscripts differ significantly ($p < 0.05$); Highly significant ($p < 0.001$); NS= Non significant ($p > 0.05$).

CORRELATION BETWEEN MORPHOLOGY AND NUTRITIVE QUALITY OF PAKCHONG

Correlation analysis (Figure 2) revealed that dry matter (DM) had significant positive associations ($r = 0.41$ to 0.88 , $p < 0.001$) with plant height, leaf length, stem length, acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash content. Conversely, dry matter content was also strongly and negatively correlated with crude protein ($r = -0.91$, $p < 0.001$). Crude protein showed a moderate positive relationship with leaf yield ($r = 0.47$, $p < 0.001$), while strong negative ($r = -0.80$ to -0.90 , $p < 0.001$) correlation to plant height, leaf length, stem length, ADF, NDF and ash. Both, ADF and NDF were positively correlated ($r = 0.45$ to 0.82 , $p < 0.001$) with plant height, stem length and leaf length. Ash content also showed positive correlations ($r = 0.45$ to 0.80 , $p < 0.001$) with plant height, stem length, leaf length, ADF and NDF. Additionally, plant height and stem length also positively correlated ($r = 0.51$, $p < 0.001$) to each other.

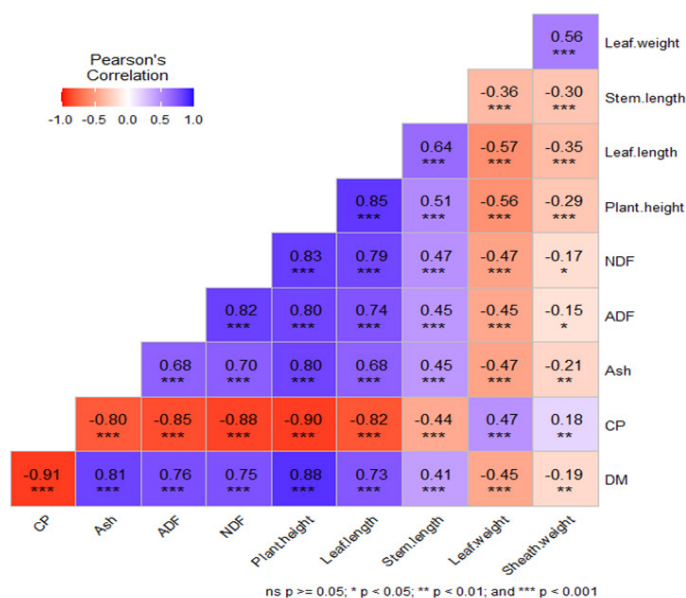


Figure 2: Correlation between morphology and nutritional composition.

DISCUSSION

MORPHOLOGICAL CHARACTERISTICS OF PAKCHONG FODDER

The results (Table 2) showed that both season and harvest interval (HI) had significant ($p < 0.01$) effects on plant height of pakchong fodder. Irrespective of season higher plant height found ($p < 0.01$) at 55 days (146.61 cm) harvest interval followed by 45 days (115.71 cm), 35 days (88.65 cm) and 25 days (61.49 cm). Ahamed *et al.* (2021) reported that plant height of pakchong fodder increased progressively with cutting interval, significantly higher plant height at 60 days cutting interval. Additionally, Roy *et al.* (2021) also stated that, pakchong plant height significantly differ from season to season at 50 days cutting interval (135.80

cm). Furthermore, Walaiphan *et al.* (2019) observed that pakchong fodder plant height increased with cutting interval (120 cm to 150 cm). Onjai-Uea *et al.* (2022) also reported that plant height of pakchong significantly influenced by plant space and age, who observed 148.51 cm plant height for pakchong cultivar and 137.72 cm harvested at 60 days. However, the present study was agreed to previous study and demonstrated that both season and harvest interval (HI) are key determinants of the vegetative growth of Pakchong fodder. Additionally, Plant height exhibited higher in rainy season (116.37 cm) compared to summer (109.50 cm) and winter (105.45 cm) season. The current study showed similar results with Roy *et al.* (2025) who exhibited that Napier pakchong height was higher in rainy season compared to summer and winter season. Holm *et al.* (1979) stated that pakchong fodder grown better height in rainy season because of moist and favourable climatic condition. Genetically, pakchong is a tallest plant (Sarker *et al.*, 2019) had better regrowth capability that was shaped and inherited through selection and slightly affected by environmental influences. Leaf number was found maximum ($p < 0.05$) at rainy season. Irrespective of season leaf number was found higher ($p < 0.05$) in 55 days than 45 days, 35 days and 25 days respectively. Leaf length, sheath length, stem length, and stem diameter were highest ($p < 0.05$) at the 55-day harvest interval, while all these traits were significantly greater ($p < 0.01$) during the rainy season than in summer or winter. Roy *et al.* (2021) showed that season had significant effects on pakchong leaf length (87.86 cm at 50 days). The current study revealed lower leaf length compared to previous study. Pakchong fodder leaf length increased with lowering plant spacing. The previous study was conducted with 50 to 50 cm spacing, whereas the current study showed 100 to 40 cm spacing. Assuero and Tognetti (2010) stated that tillering and growth of grasses is regulated by an interaction of genetic, physiological and environmental factors. In aligned with this, leaf length, sheath length, stem length and stem diameter found to be increased during rainy season due to long daytime temperature and humidity that favour Napier grass growth over winter season. Growth traits, including plant height, leaf number, leaf length, sheath length, stem length, and stem diameter, consistently increased with longer harvest intervals with the 55-day interval producing the most pronounced growth. Pakchong is highly adaptive quality to tropic and sub-tropic climate and have better growth potential compared to other available cultivar in Bangladesh.

LEAF-STEM YIELD AND RATIO OF PAKCHONG AT DIFFERENT HARVEST INTERVAL

The leaf to stem ratio considered as critical determinant to evaluate the forage nutritive value and grazing quality (Annicchiarico, 2007). Leaf-stem ratio (Table 3) was significantly ($p < 0.05$) higher at 25 days (2.68) harvest

interval compared to 35 days (1.46), 45 days (0.93) and 55 days (0.76). Onjai-Uea et al. (2022) stated that in Napier pakchong grass, the leaf-stem ratio decreased with prolonged harvesting age, with the leaf fraction showed a marked reduction between 45 and 75 days. Pakchong harvested at 25 days had more leafy part that contained more nutrients and less fibrous than stem fractions (Tyas et al., 1998). Zailan et al. (2016) explained that more leaf-stem ratio indicated good quality forage which was aligned with the current studies. So, the current study was fully agreed with the previous study, early harvesting of pakchong yielded more leafy part. Moreover, season affected the leaf-stem ratio and it was higher ($p < 0.05$) in winter (1.21) season than summer (1.08) and rainy (0.93) season respectively. Roy et al. (2025) showed similar results previously, who reported that Napier fodder had higher leaf-stem ratio in winter season than summer and rainy season. Halim et al. (2013) also reported that the Napier leaf stem ratio varied from 0.57 to 1.63. The current study showed increased leaf-stem ratio than previous study due to early harvest interval. Previous studies had demonstrated that the leaves of forage legumes provided higher nutrient concentrations than stems, which were generally of inferior quality (Rao and Northup, 2012). Accumulately, Leaf, sheath, stem yield and ratio had significantly affected by interaction of season and harvest interval. Liman et al. (2022) reported that significantly higher leaf content was found at 40 days of cutting compared the 50 days and 60 days. Additionally, increasing leaf to stem ratio increased crude protein yield indicated more nutrition because nutritional components are densely distributed in the leaf. Nutritional quality or leaf crude protein decreased while starting generative stage. Wangchuk et al. (2015) also stated that leaf stem ratio is higher at early stage indicated more leaf yield. The current study had completely agreed with previous studies. Additionally, Budiman et al. (2012) also reported that leaf yield decreased at 12 weeks of age. Additionally, average stem diameter increased ($p < 0.01$) at rainy season followed by summer and winter season respectively and also 55 days harvest interval compared to 45 days, 35 days and 25 days respectively. Liman et al. (2022) also stated that stem yield induced with increased cutting age for the elongation of stem and plant maturation. Ahamed et al. (2021) also observed significant effects of cutting interval on leaf yield and leaf stem ratio per tiller. Harvest interval and season reflected to shift in biomass production driven by plant maturity and environmental conditions. Short harvest intervals (25 days) favored leaf production and a higher leaf-stem ratio, indicated that younger plants prioritize photosynthetically active tissues over structural support.

EFFECTS OF HARVEST INTERVAL ON FORAGE PRODUCTION AND NUTRITIVE VALUE

The research results also showed (Table 4) that, the

harvesting interval significantly ($p < 0.05$) increased DM at 55 days (20.72g/100g) compared to 45 days (17.26g/100g), 35 days (14.82g/100g) and 25 days (12.58g/100g), respectively. Regardless the harvest interval, DM content did not varied significantly ($p > 0.05$). Sarker et al. (2021) stated that dry matter content of different napier cultivars in drought and non-drought region varied from 11% to 17% harvested at 60 days. ADF content found higher at 55 days (38.93g/100g) harvest interval than 45 days (37.60g/100g), 35 days (36.05g/100g) and 25 days (33.98g/100g), respectively. In terms of NDF found significantly higher at 55 days of harvest interval (69.65g/100g) followed by 45 days (68.59g/100g), 35 days (66.56g/100g) and 25 days (62.99g/100g). Ash content also found increased ($p < 0.05$) with 55 days of harvest interval (10.84g/100g) compared to 45 days (10.28g/100g), 35 days (8.84g/100g) and 25 days (7.84g/100g). Nonetheless, season had found no effects on dry matter content, acid detergent fiber, neutral detergent fiber and ash content. Onjai-Uea et al. (2022) found higher DM, CF and Ash at 75 days compared to 60 days and 45 days. Ansah et al. (2010) stated that dry matter yield of Napier varied from 25 t/h/y to 45 t/h/y. Current study had consented to Tessema et al. (2010) who also reported that DM yield, CF, ADF and NDF was higher in Napier pakchong grass in later harvesting due to faster growth rate which accumulated more dry matter. Baath et al. (2020) also reported previously that, levels of ADF and NDF increased steadily with the progression of the growing season, accompanied by an accumulation of cell wall constituents that peak at plant maturity. Sarker et al. (2019) also found similar results harvested at 60 days of pakchong. Furthermore, Sollenberger (2002) described that, higher dry matter, ADF, NDF, lignin were related to harvesting day, while decreasing nutritional qualities. Fiber contents tends to increase with growing maturity (Lounglawan et al., 2014). Moreover, Haryani et al. (2021) also reported that fiber content is lower in 6th week age pakchong compared to 8th weeks and also reported that 5.9% fiber content increased in 6th to 7th week of growing age and 3.4% in 7th to 8th week of growing age. So, lengthy harvest interval of pakchong increased these parameters due to its growing maturity. The present research also showed the similar results with previous study. Remarkably, crude protein content and yield increased ($p < 0.05$) at 25 days (19.52 g/100g and 4.25 t/ha/y) harvest interval compared to 45 days (12.46 g/100g and 3.47 t/ha/y), 35 days (14.30 g/100g and 3.30 t/ha/y) and 55 days (9.51 g/100g and 3.07 t/ha/y), respectively. Roy et al. (2021) reported that, dry matter content and crude protein yield varied seasonally, dry matter content found higher in Rabi season and crude protein yield found significantly higher in Kharif 1. In addition, harvest interval had significant effects on crude protein content and yield. Furthermore, the current research showed that harvest interval or

pakchong age had significant effects on crude protein. [Ahamed *et al.* \(2021\)](#) reported that pakchong had higher crude protein harvested at 40 days (11.23%) than 50 days (9.49%) and 60 days (8.01%). [Onjai-Uea *et al.* \(2022\)](#) also stated that, 45 days of harvesting of pakchong grass yield more CP compared to 60 days or 75 days. This changes occurred due to stage of plant maturity. The plant switched from vegetative growth phase to reproductive growth phase. In that phase, plants had more stem resulted higher fiber content but a lower crude protein level. Additionally, [Budiman *et al.* \(2012\)](#) reported that crude protein of Napier grass decreased with increasing of defoliation intervals. Not only this perennial forage, [Adjei and Fianu \(1985\)](#) reported that, with extended of harvesting ages (60, 90, and 120 days), the average crude protein (CP) content in the leaves and stems of forage legumes dropped from 22.5 to 17.5% and from 11.9 to 9.4%, but the average crude fiber content increased correspondingly from 20.0 to 26.8% and 27.1 to 31.9%. [Mohammad *et al.* \(2022\)](#) also mentioned that mean value of crude protein found highest at 45 days compared to 60 days and 75 days of cutting interval and the crude protein content decreased due to plant maturity. The current research revealed the similar results with previous study. Nutrient composition changed due to several factors such as climate, season, soil fertility, fertilizer application, soil type, weather and irrigation ([Schut *et al.*, 2010](#)). Furthermore, [Wadi *et al.* \(2004\)](#) described harvesting age as critical factor for crude protein, although dry matter increases CP content decreases. So, crude protein content had been used to determine the forage quality as key factor. Moreover, CP content is a critical factor for voluntary feed intake for ruminants, less than 7% CP in ruminants diet reduced voluntary feed intake as well as digestion ([Nori *et al.*, 2009](#)). Minimum requirement of CP for adult cattle and heifer is 10%, higher percentage requirement for growing cows or lactating stage ([Thayalini *et al.*, 2019](#)). The current study showed higher crude protein at 25 days interval of harvesting may fulfill the requirement for all growing stage.

The research result revealed that both harvest interval (age) and season had significant ($p < 0.01$) effects. Irrespective of season, harvest interval had significant effects on fresh biomass yield (FBY), crude protein (CP) contents, ash content, acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter yield, CP yield of pakchong grass ([Table 3](#)). Significantly ($p < 0.05$) higher fresh biomass yield were observed at 25 days (167.93 t/ha/y) than 45 days (161.56 t/ha/y), 55 days (155.90 t/ha/y) and 35 days (155.66 t/ha/y). Dry matter yield was found significantly higher at 55 days (32.29 t/ha/y) compared to 45 days (27.89 t/ha/y), 35 days (23.07 t/ha/y) and 25 days (21.504 t/ha/y). Due to increasing the harvest interval pakchong started their generative activities and plant elongation, maturation may

be the factor for increasing the fresh biomass yield. [Roy *et al.* \(2021\)](#) showed that pakchong produced 336.8 t/ha/y fresh biomass and 45.3 t/ha/y dry matter yield at 50 days harvest interval which was higher than current study. The reason of lowering the fresh biomass yield than previous study was plant spacing. [Rengsirikul *et al.* \(2011\)](#) stated that biomass yield of Napier varied significantly based on location, management strategies, season and variety. Dry matter content increased with increasing of plant growing age which lead to increasing dry matter yield. Harvest interval could affect the higher plant height, dry matter yield, photosynthetic activities, increasing tiller and mature leaf number. So this factor could be attributed to increase dry matter yield with increasing harvest interval. [Onjai-Uea *et al.* \(2022\)](#) stated that, pakchong produced more dry matter at increased of plant growing age but poor nutritional quality who reported 20.16 t/ha/y at 45 days and 22.98 t/ha/y at 75 days. Previous sole study ([Roy *et al.*, 2025](#)) on Leaf stage (LS) based management of Napier grass and demonstrated that LS could be used as a field friendly criterion to monitor yield and quality of Napier grass. This LS also could be matched easily with harvest interval. Increased the leaf stage (LS) from 6 to 14 reduced the crude protein (CP) level from 184 to 118 g/kg DM and lowered the metabolizable energy (ME) from 10.4 to 7.3 MJ/kg DM. This indicated that harvesting Napier grass at an earlier LS could enhance its nutritional quality. However, improved quality come with a trade-off, as dry matter yield was reduced by nearly 50% when harvested at the lower LS or early harvest interval. The combined effect of season and harvest interval on yield and quality revealed a trade-off between biomass yield and forage quality driven by plant maturity and environmental conditions. Longer harvest interval markedly increased dry matter yield, reduced forage digestibility and nutrient availability. Conversely, shorter harvest (25 days) interval suggested significantly higher crude protein. The inverse relationship between harvest interval and crude protein yield highlighted the fundamental quality and quantity compromise in forage management. [Roy *et al.* \(2025\)](#) reported that cattle fed the 6 LS pakchong showed markedly superior performance compared with those received the 14 LS pakchong showed higher average daily gain (610 vs. 270 g/day) and a more efficient feed conversion ratio (6.4 vs. 16.2). These differences highlighted a clear trade-off between forage yield and nutritional quality. Overall, the findings indicated that harvesting pakchong grass at 6 LS or 25 days harvest interval optimized feed nutritive value, enhanced cattle growth and improved feed conversion efficiency.

TOTAL GAS PRODUCTION AND DEGRADABILITY

Pakchong fodder harvested at 55 days (40.47 ml) produced significantly ($p < 0.05$) higher gas at 24 hours of fermentation followed by 45 days (33.11 ml), 35 days

(28.62 ml) and 25 days (20.39 ml). Nevertheless harvesting interval, season also affects ($p < 0.05$) the gas production at 24 hours. Maximum gas production at 24 hours found at and winter (31.87 ml) compared to summer (30.89 ml) rainy (29.53 ml) season. Roy *et al.* (2025) agreed to current results, who reported that 24 hour gas production had higher value at summer season and lower at rainy season. Additionally, Napier cultivar produced not less than 30 ml gas at 24 hour of fermentation, Pakchong produced 35.1 ml gas at 24 hour of fermentation harvested at 50 days interval. Significant ($p < 0.001$) *in vitro* dry matter degradability (IVDMD) of pakchong was discovered at 25 days (62.92%) harvest interval followed by 35 days (59.59%), 45 days (55.34%) and 55 days (51.70%). The current study results agreed to Tessema *et al.* (2010) who explained that Napier grass increased the fibre content that contributed to decrease in digestibility. Kabuga and Darko (1993) also reported that dry matter degradability decreased with increasing harvesting age of grass species in the tropics. Moreover, Taliafero *et al.* (1975) described that older fodder had lower *in vitro* dry matter degradability. In addition, Pakchong fodder harvested at 25 days (8.74 MJ/kgDM) revealed maximum estimated metabolizable energy (ME) than 35 days (7.51 MJ/kgDM), 45 days (7.17 MJ/kgDM) and 55 days (7.06 MJ/kgDM). Roy *et al.* (2025) previously stated that pakchong had 7.83 ME (MJ)/kg DM harvested at 50 days of harvest interval. Bashar *et al.* (2024b) also observed that pakchong fodder had greater dOM and ME than other varieties. Additionally, 1 kg of milk yield decreased for dropping 1% of dOM in grass (Peryaud and Delagarde, 2013). Warly *et al.* (2004) reported that the organic matter digestibility of tropical grasses varied for harvesting age, cutting interval and cutting height between 51% in *P. purpuphoides* and 64.4% in *P. purpureum*. Islam *et al.* (2022) also reported that 0.83kg milk yield increased with 1% of crude protein in Napier grass. Tauqir *et al.* (2009) reported that Napier grass contained higher fermentable carbohydrate that can be added more carbohydrate supplement to increased quality of silage and animal production. The current study also found the similar results that pakchong fodder harvested at prolong harvest interval showed lower degradability. Additionally, pakchong fodder harvested at four different harvest interval showed that cumulative gas production was increasing at 0-12 hours (Figure 1). Zailan *et al.* (2016) explained that 80% gas production occurred within 24 hour of incubation period, Gas production increased rapidly from 2 to 48 h of incubation and then plateaued between 48 and 96 h. The current study also agreed to Kamalak *et al.* (2005) also. After 30 hours it had turned into plateau state in 25 days of harvest interval and 36-48 hours for other harvest interval. Ansah *et al.* (2013) reported that Napier grass remained 48 hour in the rumen. Fodder of *Pennisetum* genus exhibited higher gas

production with advancing plant maturity (Nguyen *et al.*, 2024).

CORRELATION BETWEEN MORPHOLOGICAL CHARACTERISTICS AND NUTRITIONAL QUALITY OF PAKCHONG

Correlation of different morphological characteristics and nutritional composition had presented in Figure 2. While, the plant height, leaf length, stem length had found positively ($p < 0.05$) strong correlation with dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash content, whereas strong negative correlation with crude protein (CP). Crude protein had found moderate correlation with leaf yield, whereas had strongly negative ($p < 0.01$) correlation with plant height, leaf length, stem length, ADF, NDF and Ash. Additionally, plant height had positively ($p < 0.05$) correlated with stem length. The current research results had agreed to Ahamed *et al.* (2021) who found positively and strongly correlation among growth parameters and nutritional parameters. In addition, Cajarville *et al.* (2015) also reported the similar results between plant morphologies, *in vitro* gas production and WSC.

CONCLUSIONS AND RECOMMENDATIONS

The current study proved that harvest interval strongly affect the fresh biomass yield, dry matter yield, crude protein yield, *in vitro* gas production, degradability and correlation between plant morphologies and nutrients. With the plant maturation, dry matter yield increased and forage quality decreased significantly. Thus, a farmer may monitor pakchong grass quality directly after counting the last harvesting time and can determine harvest or grazing time. Finally, this study suggested the harvest interval of pakchong at 25 days with optimum field management to harvest more nutrients for animals. Further research could be carried on harvesting type whether rotational grazing aligned with optimal harvest intervals can maintain forage yield, regrowth viability and nutritive value comparable to cut-and-carry systems, improve animal intake, growth or milk yield and reduce labor and feeding costs.

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This research presents the thorough and comparative assessments of Pakchong fodder across multiple harvest interval and season, markedly revealed significant variation in yield, nutritional quality and *in vitro* degradability and total gas production dynamics. The results identified that Pakchong harvested at 25d contain higher crude protein, energy content, degradability and lower *in vitro* gas production, thereby providing valuable guidance for improving qualities of Pakchong fodder in tropical livestock production.

AUTHOR'S CONTRIBUTION

MAA reviewed the literature, monitoring the activities, data curation and performed to finalize the draft. MM reviewed the literature, conceptualized and conducted the research experiment, performed lab analysis, data curation, analysis and writing the original draft. NS monitoring the activities, data curation and performed to review and editing the final draft. MIAS reviewed the literature and lab analysis. GKD conceptualized the research experiment, supervised the research activities, funding acquisition and editing the final draft. All authors have read and agreed to the published version of the manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declared that no generative AI or AI-assisted technologies were used in the preparation of manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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