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IMPACTS OF DIFFERENT HARVESTING INTERVALS ON YIELD, NUTRITIVE VALUE, AND *IN VITRO* DRY MATTER DIGESTIBILITY IN FORAGES

RIFAT ULLAH JAN[1,2], SHAH ZEB AHMAD[1,] MUHAMMAD IFTIKHAR[1], MUHAMMAD IRFAN KHAN[1], MUHAMMAD HAMMAD ZAFAR[1], MUHAMMAD TAHIR KHAN[3], HOSAMELDEEN MOHAMED HUSIEN[5,6], HUANYONG HAN[4], ZANG YU[1] AND MENGZHI WANG[1*]

[1]College of Animal Sciences and Technology, Yangzhou University, Yangzhou, Jiangsu, 225009, China
[2]Livestock and Dairy Development Department (Research Wing), Khyber Pakhtunkhwa, 25000 Pakistan
[3]Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Science, University of Agriculture, Peshawar, 25130, Pakistan
[4]State Key Laboratory of Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural Reclamation Sciences, Shihezi, Xinjiang, 832000, China
[5]Laboratory of Metabolic Manipulation of Herbivorous Animal Nutrition, College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China
[6]College of Veterinary Medicine, Albutana University, Rufaa, 22217, Sudan

*Corresponding author's email: mengzhiwangyz@126.com

Abstract

This study determined the effects of different harvesting intervals (45, 60, 75, and 90 days) on the dry matter (DM) yield and nutritive value of forage products from 7 tropical grasses during a field trial under subtropical conditions in Peshawar, Pakistan: *Pennisetum glaucum, Setaria anceps, Panicum coloratum, Pennisetum purpureum, Sorghum almum, Panicum maximum,* and *Pennisetum orientale*. The trial used a randomized complete block design (three blocks) arranged as a factorial (species × harvest interval). Factorial analysis revealed a significant main effect of harvesting interval on dry matter yield ($p<0.001$); however, the interaction between species and interval for yield was not significant ($p = 0.739$). Forage quality declined with advancing maturity, as evidenced by decreases in crude protein and *In vitro* dry matter digestibility (IVDMD), and by increases in crude fiber and cell wall fractions. The species × interval interaction was significant for dry matter (DM), ether extract, crude fiber, and IVDMD, indicating that the species differed in their rate of quality change with harvest timing. Relative feed value (RFV) ranged from approximately 52 to 74 and generally declined as the harvesting interval increased from 45 to 90 days. Therefore, RFV was used as a comparative ranking index rather than as an absolute forage grade. In general, higher digestibility and RFV values were observed in the earlier intervals (45–60 days), whereas delayed harvesting increased dry matter yield. Intermediate intervals (60–75 days) were frequently a manageable compromise for some species, but the best interval was species-specific.

Key words: Harvesting frequency; Forage quality; Biomass; Relative feed value, IVDMD

Introduction

Forage production is crucial for sustainable livestock systems, particularly in developing countries, where smallholder farms frequently depend on home-produced feedstuffs because of limited access to commercial feed. The livestock sector contributes more than 60.84% to agricultural value addition in Pakistan and approximately 14.36% to the country's GDP, as reported by the Government of Pakistan (2023). Disruptions in world trade resulting from COVID-19 have contributed to an increase in the prices of feed and fertilizers, thus further magnifying feed insecurity in numerous countries. An overall global overview recently showed that inflation of cost price, increased investment and supply volatility, as well as reduced possibility to obtain feed, have aggravated the difficulties for sustainable and cost-effective livestock production (OECD & FAO, 2023). Evidence-based forage management strategies are needed to enhance yield and nutritional quality in resource-limited settings. Animal production is directly related to the availability and quality of forage, which makes up the bulk of the feed intake of buffaloes, cattle, goats, and sheep.

Tropical forage grasses have been used extensively because of their high production rates, biomass yield, and adaptability to varying agroclimatic conditions. These characteristics are ideally suited for subtropical weather in Pakistan. However, a trade-off usually exists between yield and nutritive value: as grasses mature, structural carbohydrates (hemicellulose and cellulose) and lignin accumulate at the expense of crude protein (CP), digestibility, mineral content, etc., which could limit rumen fermentation activity and nutrient supply. Such patterns have also been reported under agroecological stress conditions, such as warming trends, late harvesting, and soil nitrogen limitation (Notenbaert *et al.,* 2021).

In addition to the previously mentioned complementary management measures, one of the most important forage quality/yield trade-offs is the frequency of harvests or harvesting interval. Both biomass yield and nutritive value are influenced by harvesting frequency. Shorter intervals, such as 45 days, may retain higher values for digestibility

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and CP% through reduction in tissue lignification; however, they may be limited in cumulative biomass, likely reflecting shorter regrowth periods (Pedreira *et al.,* 2023). Longer cycles generally accumulate more mass but at lower nutritional quality because of higher fiber and lignin content (Lelis *et al.,* 2025; Tulu *et al.,* 2023).

Globally, several studies have explored the effects of harvest date on tropical forage performance under various environmental and management conditions. Studies conducted in India (Kumar *et al.,* 2024) and Ethiopia (Tadele *et al.,* 2026), and the Caribbean (Carvajal-Tapia *et al.,* 2022) demonstrated that longer harvesting intervals may improve DM yield while potentially decreasing CP content and digestibility. However, the majority of this work was carried out on a single species, Pennisetum *purpureum,* reducing the potential for cross-species comparisons. In comparison, the current experiment tested 7 of the most common grasses adapted to tropical conditions under the same environment, allowing a direct species-specific and comparative interpretation about the yield vs. quality trade-off. This is particularly true for Pakistan, where numerous forage species are employed, but the systematic, multi-species data under uniform environments are scarce. More recent work also indicated that a multivariate, multi-harvest approach permitted a better interpretation of species responses and recommendations at the site level (Wang *et al.,* 2024; Mustafa & Farooq, 2025).

In Pakistan, most forage studies have focused on fertilization and the performance of single species, with little attention to the stage of harvest and its effect on nutritive value. This hampers the development of effective management options, especially for small landholder farming under low-input conditions. Owing to the large seasonal rainfall, hot climate, and limited availability of land in the area, interventions that balance both forage quantity and nutritional value are challenging. Recent studies have also supported the opinion that optimization of planting density and harvest interval could positively affect overall system efficiency and productivity in mixed crops (Wei *et al.,* 2025; Dzvene *et al.,* 2024).

Harvesting frequency affects fiber fractions, including acid detergent fiber (ADF) and neutral detergent fiber (NDF), which influence energy availability and voluntary intake (Oluwadele *et al.,* 2024; Wekgari *et al.,* 2024). Relative feed value (RFV), derived from ADF and NDF, can serve as a comparative forage-quality index, although it is not routinely applied in local assessments (Lo *et al.,* 2024; Carvajal-Tapia *et al.,* 2022). Consequently, harvest scheduling based on fiber-based indices can support feed planning and improve system performance. Enhanced tropical forage management can contribute to climate-resilient agroecological livestock systems (Bonilla-Cedrez *et al.,* 2023; Liu *et al.,* 2025).

This study aimed to determine the impact of varying harvesting intervals on biomass and nutritional indices, including CP, ADF, NDF, IVDMD, and RFV, across seven widely used tropical grasses selected based on local adaptation, economic value, and use in ruminant feeding systems in subtropical Pakistan. These grasses represent a diverse mix of high-yielding, drought-tolerant, and nutritionally superior varieties commonly utilized by smallholder farmers. These findings are expected to provide species-specific guidelines for optimizing forage harvest schedules under subtropical climatic conditions.

Materials and Methods

Study area and duration: A field experiment was conducted from August to December at the Pakistan Forest Institute (PFI), Peshawar (34.01°N, 71.52°E), in Northwestern Pakistan. Further analyses were conducted at the University of Agriculture, Peshawar, and the College of Animal Science and Technology, Yangzhou University, China. The region has a mild subtropical climate, a hot summer, a mild winter, and monsoonal rainfall. The area is situated at an elevation of 359 meters above sea level, and the mean summer temperature varies between 30 and 39°C, while the average annual precipitation ranges between 450 to 550 mm. The soil was sandy loam with a pH close to neutral (pH ~7.5), low organic matter content (0.8%), and moderate drainage. Such environments are generally suitable for the growth and development of tropical forage grasses; therefore, it is possible to investigate their production and nutritional patterns under different harvesting intervals.

Experimental design and forage species: Seven tropical grass species were selected based on local adaptation, economic importance, and contribution to ruminant feeding in the subtropical region of Pakistan. The selected grasses include a wide range of high-yielding, drought-tolerant, and nutritionally important species that are traditional crops grown by small landholder farmers. The species included *Pennisetum glaucum, Pennisetum purpureum, Pennisetum orientale, Panicum coloratum, Panicum maximum, Setaria anceps*, and *Sorghum almum*.

All plots were established in the previous growing season and were allowed a full growth period before harvesting treatments were applied, allowing the reported responses to be due to mature stands rather than differences associated with establishment.

Seeds were collected from the Forage Research Institute, Sargodha, and the germplasm section of the Pakistan Forest Institute. A randomized complete block design (RCBD) with three replications per treatment was used to reduce environmental variation. Plot randomization was conducted using a randomized number table to assign species × harvesting interval combinations within each block.

Each species was sown at a density of 8 kg ha^{-1}, with rows spaced 25 cm apart on 2 m × 3 m plots. Standard agronomic practices, including irrigation and manual weeding, were uniformly applied to all treatments.

Fauji Fertilizer Company Limited (FFC) products, including Sona Urea at 110 kg ha^{-1} and DAP at 100 kg ha^{-1}, were applied according to local agronomic recommendations. The fertilizer application was kept uniform across all plots to ensure that the observed differences were mainly associated with species and harvesting interval rather than unequal nutrient supply.

Harvesting and sample collection: Harvesting using a sickle was done manually at the specified intervals. Biomass was measured at a consistent height of 5 cm above the ground to reflect grazing or forage-harvest conditions. Harvesting was done in the morning, between 08:00 and 10:00, to minimize diurnal fluctuations in moisture content.

Approximately five inner rows were sampled from each of the 2 m × 3 m plots to determine biomass; each plot contained 35–40 individual tillers, representing a sample without edge effects. The total fresh weight was determined using a digital scale. Composite biomass (approximately 5 kg per plot) was packed in stamped polyethylene bags and immediately processed and chemically analyzed at the Animal Nutrition Laboratory, University of Agriculture, Peshawar, Pakistan.

Laboratory Analysis

Dry matter and ash content: Subsamples were oven-dried at 65°C for 72 h and ground using a Wiley mill with a 1-mm screen. DM was measured in duplicate from each sample by drying at 103°C overnight (McCleary, 2023)

Ash content was determined in duplicate by combusting subsamples in a muffle furnace at 550°C for 4 h, following (McCleary, 2023), to ensure repeatability and precision.

Crude protein (CP): CP was determined according to the method described by (McCleary, 2023) using Kjeldahl analysis. Digestion, distillation, and titration of samples were performed to determine the total nitrogen (%), and CP was calculated as follows: CP (%) = total nitrogen (%) × 6.25.

Fiber analysis (NDF and ADF): Neutral detergent fiber and acid detergent fiber were analyzed according to the McCleary (2023) using an ANKOM200 Fiber Analyzer. Fiber contents were corrected for ash and reported on a 100% dry matter basis. These values are necessary for estimating forage quality and energy content.

***In vitro* dry matter digestibility (IVDMD):** Digestibility was determined according to the method described by (Sun *et al.,* 2025) as a two-stage system using an ANKOM Daisy II incubator system. Ground forage samples (0.5 g) were placed in nylon bags and incubated in buffered rumen fluid at 39 ± 0.5°C for 48 h; these were washed, dried, and reweighed to measure IVDMD as the loss of dry matter per cent.

Relative feed value (RFV): The relative feed value (RFV) was computed as a composite forage quality index, combining digestibility estimates with potential voluntary intake based on forage fiber fractions. The standard RFV index uses NDF and ADF to support normalized comparisons among species and harvesting intervals. The RFV was calculated using the equation described by (Guamán Rivera *et al.,* 2023).

RFV was calculated and derived from ADF and NDF as an index that combines predicted digestibility with intake. Digestible dry matter (DDM, %) was predicted using the following equation: DDM = 88.9 − (0.779 × ADF%). Dry matter intake (DMI, % of body weight) was estimated as DMI = 120 / NDF%. RFV was then computed as RFV = (DDM × DMI) / 1.29. Given that RFV was originally developed for temperate forages, it served as a comparative ranking tool across species and harvesting intervals.

$$\frac{\text{DDM} * \text{DMI}}{1.29} \quad \frac{120}{\text{NDF \%}} \text{(\% DM)}$$

Although RFV was originally developed for temperate forages, it was applied here as an exploratory comparative index for tropical grasses. The previous studies show that fiber-based indices, which are the result of ADF and NDF, could be used to monitor nutritive variations among forages (Pérez-Reverón *et al.,* 2024). RFV is thus only seen as a relative ranking of species and harvesting periods and not as an absolute market grade. It would be better calibrated against standards of forage quality relevant locally, and should be considered in future studies, especially in production systems in the subtropics.

Statistical analysis

All experimental data were analyzed using Statistix 8.1 (Analytical Software, 2005) in a randomized complete block design (RCBD) with three blocks (replications) as a factorial experiment (species × harvesting interval). The model included block, species, harvesting interval, and the species × harvesting interval interaction. If the interaction was significant ($p \leq 0.05$), simple effects were evaluated by comparing harvesting intervals across species. Mean separation was performed using Tukey's HSD test at $p \leq 0.05$, which controls for family-wise error in multiple comparisons. The full factorial RCBD ANOVA results for species, harvesting interval, and species × interval interaction are presented in Table S1.

Table S1. Factorial RCBD ANOVA p-values (Species, harvesting interval, and species × interval).

Trait	Species (p)	Interval (p)	Species × Interval (p)
Dry matter yield kg ha^{-1})	<0.001	<0.001	0.739
DM (%)	<0.001	<0.001	0.003
Moisture (%)	0.805	0.115	0.895
Ash (%)	0.747	0.371	0.059
Crude protein (%)	0.096	<0.001	0.988
Crude fiber (%)	<0.001	<0.001	<0.001
Ether extract (%)	<0.001	<0.001	0.010
Nitrogen-free extract (%)	<0.001	0.025	0.809
NDF (%)	0.297	<0.001	0.959
ADF (%)	0.204	<0.001	0.763
ADL (%)	0.293	0.852	0.520
Hemicellulose (%)	0.485	0.602	0.725
Cellulose (%)	0.207	0.051	0.354
Lignin (%)	<0.001	<0.001	0.974
IVDMD (%)	<0.001	<0.001	<0.001
DDM (%)	0.204	<0.001	0.763
DMI (% BW)	0.365	<0.001	0.944
RFV	0.210	<0.001	0.981

Note: Simple effects were interpreted where the species × interval interaction was significant ($p \leq 0.05$). Mean separation was performed using Tukey's HSD test at $p \leq 0.05$

Results

Dry matter yield: The longer the harvesting period, the higher is the yield of dry matter among species (Table 1). An example is that *Pennisetum purpureum* showed the highest mean yield at 90 days of 18,350kg/ha, while in *Panicum coloratum*, the lowest mean yield was recorded at 45 days (4,340kg/ha). The factorial response surface design showed harvesting interval to have a significant effect on yield ($p<0.001$), but no significant species × harvesting interval interaction ($p = 0.739$), indicating an overall similar pattern of responses across species. Mean separation between intervals was not always found significant between species ($p>0.05$).

***In vitro* dry matter digestibility (IVDMD):** IVDMD decreased with an increasing harvesting interval across all species (Table 2). At 45 days, the highest IVDMD values were observed in *Sorghum almum* (66.75%) and *Pennisetum glaucum* (64.24%). At 90 days, the IVDMD was lowest in *Setaria anceps* (28.33%) and Pennisetum glaucum (28.53%). The species × harvesting interval interaction for IVDMD was significant ($p<0.001$), indicating that species differed in the rate of decline with increasing harvesting intervals.

Dry matter content: Dry matter content increased with increasing harvesting interval across all species (Table 3). For example, Pennisetum purpureum increased from 19.57% at 45 days to 25.01% at 90 days. At the final interval, *Panicum coloratum* recorded the highest dry matter content (32.87%). These increases may be explained by moisture loss and greater structural tissue development as plants mature. This increase may support forage preservation, but it can also reduce the nutritive value because of higher fiber accumulation.

Crude protein (CP) content: The crude protein content was also decreased as the harvesting interval increased (Table 4). In the factorial response-combination block design (RCBD), there were significant main effects of harvesting interval on crude protein ($p<0.001$), but not for species × interval interaction ($p = 0.988$), suggesting that the declines were relatively consistent across species. The highest crude protein contents were found at 45 days in *Setaria anceps* (8.78%) and *Panicum coloratum* (8.25%), decreasing to 5.93% and 6.67%, respectively, at 90 days. For example, *Pennisetum purpureum* decreased from 8.23% (45 days) to 5.32% (90 days).

Crude fiber (CF) content: Crude fiber content increased with harvesting duration, and there was a statistically significant difference among all species ($p<0.001$). *Setaria anceps* and *Panicum maximum* also had high crude fiber content at 90 days (46.92% and 45.74%, respectively), suggesting higher lignification of tissues. In contrast, *Pennisetum glaucum* recorded the lowest fiber content at 45 days (26.58%). This tendency of having a higher indigestible fiber in older forages could reduce the amount of energy available for ruminants (Table 5).

Ash content: The ash content of forage tended to decrease as the intervals between cuts increased for most species, thereby reducing metabolic activity and nutrient absorption into differentiated tissues. *Pennisetum glaucum* showed the greatest decrease, with an ash content of 13.76% after 90 days versus 18.77% for 45 days.

However, the patterns were not clear for all species. Notably, the pattern in *Sorghum almum* and *Panicum maximum* was unusual in that the greatest ash content occurred at 90 days (18.39% and 17.34%, respectively), indicating that species-specific differences may exist in mineral accumulation and retention.

The effect of harvesting interval on ash content was significant for *Pennisetum glaucum* ($p = 0.0366$) and *Pennisetum orientale* ($p = 0.0236$), suggesting that the response in terms of minerals to the time at which it was harvested differed by species (Table 6).

Table 1. Dry matter yield (kg ha^{-1}) of various forage grasses (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum, Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) under various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	5,540	8,690	4,340	14,390	9,647	6,370	4,310
60	6,977	9,982	6,810	16,665	8,910	6,560	5,540
75	6,530	10,497	6,945	17,267	10,940	7,042	6,205
90	7,820	14,990	7,512	18,350	11,272	8,455	7,500
P-value	0.7846	0.4023	0.6589	0.8510	0.9356	0.8814	0.6249

Table 2. *In vitro* dry matter digestibility (%) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, and *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	64.24^{a}	44.79^{a}	55.82^{a}	58.25^{a}	66.75^{a}	49.25^{a}	55.82^{a}
60	46.15^{b}	38.18^{b}	38.91^{b}	52.73^{ab}	44.25^{b}	46.53^{ab}	42.72^{b}
75	37.65^{c}	32.82^{c}	35.66^{c}	51.91^{b}	36.36^{c}	45.42^{b}	38.47^{c}
90	28.53^{d}	28.33^{c}	29.72^{c}	46.72^{c}	33.35^{c}	41.19^{c}	30.69^{d}
P-value	0.0001	0.0001	0.0001	0.0007	0.0003	0.0001	0.0001

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test ($p\leq0.05$). Means sharing the same letter are not significantly different

Table 3. Fresh dry matter (%) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum, Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	16.79^{d}	20.19^{d}	27.29^{c}	19.57^{d}	15.43^{c}	19.50^{c}	35.13^{c}
60	19.29^{c}	23.15^{c}	30.25^{b}	21.61^{c}	17.56^{b}	22.46^{b}	38.40^{b}
75	22.05^{b}	25.00^{b}	31.87^{a}	23.65^{b}	20.10^{a}	24.50^{a}	40.40^{a}
90	24.10^{a}	26.57^{a}	32.87^{a}	25.01^{a}	21.53^{a}	25.60^{a}	41.63^{a}
P-value	0.0000	0.0001	0.0001	0.0000	0.0003	0.0002	0.0000

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test ($p\leq0.05$). Means sharing the same letter are not significantly different

Table 4. Crude protein content (%DM) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at varying harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	7.29[a]	8.78[a]	8.25[a]	8.23[a]	8.20[a]	8.15[a]	7.23[a]
60	6.78[b]	7.44[b]	7.51[b]	6.94[b]	7.72[b]	6.91[b]	6.88[b]
75	6.13[c]	6.52[c]	6.41[c]	6.07[c]	6.18[c]	6.33[c]	5.84[c]
90	5.45[d]	5.93[c]	6.67[c]	5.32[d]	6.56[c]	5.81[c]	5.86[c]
P-value	0.0034	0.0849	0.0017	0.0001	0.3304	0.0918	0.3242

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Table 5. Crude Fiber (%DM) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	26.59[d]	32.72[d]	34.95[d]	34.30[d]	28.91[d]	35.83[d]	33.39[d]
60	29.74[c]	38.01[c]	37.82[c]	36.34[c]	33.42[c]	37.27[c]	36.74[c]
75	35.13[b]	43.95[b]	40.06[b]	38.02[b]	38.13[b]	41.98[b]	39.99[b]
90	37.32[a]	46.92[a]	41.56[a]	39.66[a]	40.26[a]	45.74[a]	42.78[a]
P-value	0.0001	0.0001	0.0003	0.0002	0.0001	0.0001	0.0001

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Table 6. Ash (%DM) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	18.77[a]	12.73[b]	16.28[b]	17.35[b]	14.18[b]	13.00[b]	18.77[a]
60	18.45[a]	13.15[b]	18.45[a]	12.97[b]	17.23[b]	19.13[b]	19.41[a]
75	15.48[b]	18.21[b]	16.69[b]	16.82[b]	12.33[b]	15.69[b]	13.68[b]
90	13.76[c]	15.23[b]	16.60[b]	13.45[b]	18.39[b]	17.34[b]	10.36[b]
P-value	0.0366	0.2763	0.9363	0.3887	0.0900	0.4730	0.0236

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Table 7. NDF (%) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, and *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	72.36[a]	71.66[a]	78.61[a]	71.07[a]	72.56[a]	67.38[ab]	71.10[a]
60	74.55[b]	75.63[b]	79.30[b]	77.51[b]	75.89[b]	75.10[b]	73.97[b]
75	78.61[b]	77.85[b]	78.33[b]	75.78[b]	76.28[b]	79.13[b]	74.12[b]
90	78.61[b]	78.61[b]	82.31[b]	78.04[b]	82.57[b]	82.44[b]	78.40[b]
P-value	0.0214	0.4475	0.7288	0.3153	0.2012	0.0854	0.3750

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Table 8. ADF (%DM) of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	46.00[a]	46.49[b]	55.36[c]	44.40[b]	45.70[b]	45.84[b]	45.31[b]
60	47.79[b]	48.17[b]	48.41[b]	47.42[b]	44.21[b]	46.11[b]	46.40[b]
75	45.95[b]	47.96[b]	52.26[c]	50.58[c]	47.56[b]	50.81[c]	48.65[b]
90	49.07[b]	51.19[b]	55.36[c]	54.30[c]	49.54[b]	54.44[c]	50.68[b]
P-value	0.7608	0.3881	0.0127	0.0714	0.0544	0.0530	0.2870

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Table 9. RFV of forages (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Pennisetum purpureum*, *Sorghum almum*, *Panicum maximum*, *Pennisetum orientale*) at various harvesting periods.

Harvesting interval (days)	*Pennisetum glaucum* (PG)	*Setaria anceps* (SA)	*Panicum coloratum* (PC)	*Pennisetum purpureum* (PP)	*Sorghum almum* (SOA)	*Panicum maximum* (PM)	*Pennisetum orientale* (PO)
45	68.33[a]	69.09[b]	64.76[c]	71.68[b]	68.47[b]	74.47[b]	70.55[b]
60	64.51[b]	63.17[b]	60.04[b]	62.46[b]	66.75[b]	66.05[b]	66.34[b]
75	62.92[b]	61.63[b]	57.51[b]	60.82[b]	63.61[b]	58.07[b]	64.06[b]
90	57.20[b]	58.25[b]	52.10[b]	55.47[b]	56.80[b]	52.60[b]	58.75[b]
P-value	0.1176	0.2289	0.1206	0.0441	0.0301	0.0396	0.0949

Note: Different lowercase letters within the same column indicate significant differences among harvesting intervals within the same species according to Tukey's HSD test (p≤0.05). Means sharing the same letter are not significantly different

Neutral detergent fiber (NDF): Neutral detergent fiber (NDF) increased with extended harvesting intervals, reflecting greater cell wall development associated with forage maturity. The largest increase was observed in Panicum maximum (67.38% at 45 days to 82.44% at 90 days). *Sorghum almum* and *Setaria anceps* also exceeded 80% NDF at the 90-day interval.

Nevertheless, statistically significant differences in neutral detergent fiber (NDF) content were observed only in *Pennisetum glaucum* ($p=0.0214$). Other species exhibited numerically greater NDF values; however, these differences were not statistically significant. These patterns suggest that NDF levels at plant age may vary depending on the species and subsequently influence voluntary feed consumption due to increased rumen fill and reduced digestibility (Table 7).

Acid detergent fiber (ADF): Extended harvesting periods increased ADF in most forage species, indicating reduced digestibility with plant maturity. At 90 days, relatively high ADF values were recorded in *Panicum coloratum* (55.36%), *Panicum maximum* (54.44%), and *Pennisetum purpureum* (54.30%), reflecting greater cell wall development at later maturity. Although some species did not show statistically significant changes, *Panicum coloratum* showed a significant interval effect ($p = 0.0127$). Overall, increasing ADF values indicate lower digestible energy due to greater fiber accumulation and lignification (Table 8).

Relative feed value (RFV): RFV declined with increasing harvesting intervals for all species (Table 9). Across treatments, RFV values ranged from approximately 52 to 74, with the highest value recorded in *Panicum maximum* at 45 days (74.47) and the lowest value recorded in *Panicum coloratum* at 90 days (52.10).

Discussion

Although a general inverse relationship exists between forage yield and quality, this research contributes by presenting the first comparison of seven studied grasses under standardized field tests in a subtropical environment, with particular emphasis on RFV as an integrated forage quality index. The results are applicable and species-specific, providing low-input advice suitable for small landholders in a South Asia-specific context.

The factorial structure of the experiment, all variables were reanalyzed using a factorial RCBD model, including the species × harvesting interval interaction. This interaction was significant for dry matter (DM), ether extract, crude fiber, and IVDMD ($p<0.05$); consequently, treatment comparisons were interpreted as simple effects (harvesting intervals within each species), where appropriate. However, the interaction was not significant for dry matter yield ($p = 0.739$).

This experiment indicates that harvesting interval influences both the yield and nutritive value of tropical forage grasses under subtropical environments. Longer intervals tended to produce a greater dry matter yield (interval main effect, $p<0.001$); however, within-species differences were often not significant. This trade-off between biomass accumulation and declining quality with maturity is widely reported for tropical forages and supports the cautious interpretation of yield patterns alongside quality outcomes.

The increased biomass allowed by this growth is associated with lower nutritional value, reflected in a reduction in crude protein (CP), *In vitro* dry matter digestibility (IVDMD), and mineral content. The decline in CP content between 45 and 90 days in all species aligns with known physiological changes: as grasses mature, the exchange of nitrogen among leaves and reproductive tissues decreases; hence, a lower protein content (Zetterlind *et al.,* 2025).

Nitrogen reallocation is characterized by the recycling of nutrients from leaves to stem and foliage tissues as hemicellulose and cellulose, resulting in a reduction in the protein content of mature tissues (Qin *et al.,* 2025). *Setaria anceps* and *Panicum coloratum* exhibited physiological responses of greater magnitude, consisting of a greater increase in crude protein. This consistent pattern is frequently observed in pasture management systems.

The indices of total mineral concentration and ash concentration of most of the species studied declined as the harvesting periods increased. This process can be explained by the dilution effect, which is caused by high levels of biomass production and a decrease in metabolic activity with maturity. Tissues are more metabolically active in young than in old age; young tissues have more minerals than aged tissues, which are more largely composed of indigestible structural compounds diluting the proportion of minerals per unit of dry matter. This has significant consequences for ruminant nutrition, especially in ruminant systems with limited mineral supplementation, such as extensive grazing or small landholder zero-grazing systems.

The fiber concentration, specifically acid detergent fiber (ADF) and neutral detergent fiber (NDF), was significantly higher with late harvesting. This finding is in line with previous studies that have linked forage maturity to high lignification and cell wall thickening. Such slow growth in crude protein loss in *Seteria anceps* might be attributed to the fact that the nutrient tends to distribute nitrogen in the structural components in the early growth stages. This characteristic would add to its value in the use of the forage in tropical systems, where delayed maturity normally lowers feed value (Sales-Silva *et al.,* 2023; Diniz *et al.,* 2023).

Increased NDF may restrict voluntary consumption by rumen fill, and higher ADF is mostly associated with low digestible energy. *Setaria anceps* and *Sorghum almum* had maximum NDF and ADF values at 90 days, which was consistent with the lower IVDMD and RFV at subsequent harvests (Facco *et al.,* 2024).

Across species, IVDMD fell by 45 to 90 days, most notably in *Pennisetum glaucum* and *Sorghum almum,* as expected for a negative relationship between cell wall buildup and digestibility. Plant maturity also leads to lignification and more supporting tissues, which lowers the accessibility of digestible portions to microbes (De Zutter *et al.,* 2023; Lelis *et al.,* 2025).

Relative feed value (RFV), which integrates acid detergent fiber (ADF) and neutral detergent fiber (NDF) through predicted digestibility (DDM) and dry matter intake (DMI), declined with advancing regrowth age,

consistent with increased fiber fractions and reduced IVDMD. In this study, RFV values generally ranged from 52 to 74. Considering that, RFV was developed for temperate forages; these values should be interpreted cautiously and primarily used for comparative ranking among tropical grasses and harvesting intervals, rather than as absolute market grades. The observed pattern supports earlier harvests (45–60 days) for higher-quality forage, while longer intervals favour biomass production.

These results align with findings from previous single-species studies. For example, in a study in India it was reported that longer harvesting intervals in hybrid grasses increased green and dry matter yields but reduced crude protein and increased crude fiber (Meel *et al.,* 2025). Similarly, Tadele & Ayinshte (2026) found a decline in the nutritional quality of *Pennisetum purpureum* with delayed harvesting in Ethiopia, highlighting the importance of earlier harvesting for preserving protein levels.

In contrast to previous studies conducted on a single species, the present study offers a comparative, multi-species assessment, which indicates considerable differences in the way various tropical grasses react to the frequency of harvesting. For example, *Pennisetum purpureum* and *Setaria anceps* exhibited a large biomass increase and decreased forage quality, whereas *Sorghum almum* and *Panicum maximum* continued to demonstrate a better ratio between yield and nutritional value.

These interspecific differences highlight the need for species-specific harvesting strategies, which is a key contribution to forage management in subtropical mixed-species systems, where such comparative data are limited.

These findings align with previous forage research, which demonstrated that advancing regrowth age increases fiber fractions and reduces digestibility in tropical grasses (Facco *et al.,* 2024; Tulu *et al.,* 2023). A similar pattern was observed across species in the present study. Although RFV was developed primarily for temperate forages, its use here provides a simple comparative index to rank grasses and harvesting intervals on expected intake and digestibility, rather than as an absolute market grade.

Differences in the response of various grass species to harvesting intervals varied among species. *Pennisetum purpureum* and *Setaria anceps* were the most useful but were affected by long harvesting periods to the extent that they began to suffer in quality. In contrast, *Panicum maximum* and *Sorghum almum* performed better when harvesting was at the intermediate stages (60–75 days), resulting in a trade-off between quality and yield. These differences could probably be explained by intrinsic differences in the leaf-to-stem ratio, photosynthetic efficacy, and assimilate partitioning. For example, C4 grasses, including Pennisetum, are more productive but lignify quickly, thus restricting their maximum harvest (Risso Barbosa *et al.,* 2024).

The strength of the methodology lies in the use of advanced laboratory procedures, notably the Daisy II incubator system, which closely simulates rumen fermentation. This enhances the reliability of IVDMD estimates and inferences regarding forage quality. Consistent trends observed across indicators of CP, IVDMD, NDF, ADF, and RFV further support the validity of the results and their relevance for practical management decisions.

The difference in the yield and quality might have arisen due to other factors than the harvesting period, like the levels of nitrogen in the soil, the level of water in the soil, and seasonal temperatures, which were not carefully regulated in this research. These conditions are in line with on-farm reality, which enhances the generalizability of the findings to smallholder farms; however, these parameters must be measured systematically in future studies, providing more special recommendations.

Although these are strong points, there are several weaknesses that should be mentioned. Relative feed value (RFV) was created and used in temperate forages thus, its values are mainly used for relative ranking of tropical grasses and different harvesting seasons. Despite having laboratory analyses done on duplicate subsamples, variability in the analysis (e.g., CV) was not reported. Also, no time-intervention was ensured in soil fertility, and the small size of the plots could have created an edge effect, which could have had an impact on growth and quality. To optimize the harvesting intervals based on the changes in the soil nutrient status, agronomy, and patterns of the temperature of seasons, species-specific recommendations should be refined in future studies (De Zutter *et al.,* 2023).

Comprehensively, the yield-quality trade-off in tropical forage grasses is controlled by harvesting intervals. Long intervals were more conducive to the accumulation of biomass, and shorter intervals were more favourable to the crude protein and digit ratio as well as RFV as a result of less deposition of fibers and lignin. Since the difference in yield among species was often not statistically significant, the same should be recommended alongside production objectives (quantity versus quality) and species performance. The current work, in comparison to other works in the region, which have used only one species, presents comparative evidence of seven grasses in the same subtropical field conditions that enhance future species-specific decisions regarding low-input livestock systems.

Conclusions

This study validates that the harvesting interval is a primary management factor impacting the quality of production and nutritive value of tropical forage grasses in subtropical areas. Although extended periods may positively affect biomass production, there is a decrease in crude protein, digestibility, and relative feed value due to fiber accumulation and plant maturity.

Notably, some species exhibited a more favourable yield-quality balance at intermediate harvesting intervals (60–75 days; for example, *Sorghum almum* and *Panicum maximum*), suggesting that species-specific harvest schedules are necessary.

This study provides useful recommendations for maximizing harvest time in low-input and mixed-species forage systems by assessing the digestibility and RFV parameters of several grass species.

Further research should be conducted on the interactions between harvesting ranges and environmental factors, such as soil fertility, temperature, and rainfall, to establish climate-resilient forage management practices.

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