Pak. J. Bot., 59(1): January 2027.

ARTICLE | DOI: http://dx.doi.org/10.30848/PJB2027-1(14)

TWO-YEARS EVALUATION OF YIELD–QUALITY STABILITY IN TROPICAL FORAGE GRASSES UNDER SUBTROPICAL CONDITIONS

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Abstract

Interannual weather variation can affect both forage yield and nutritive value. This makes stable clipping recommendations important for subtropical livestock systems. The present two-year experiment (2024–2025) tested whether optimal clipping intervals remain stable across contrasting growing seasons. Seven grass entries (*Pennisetum glaucum*, *Setaria anceps*, *Panicum coloratum*, *Panicum maximum*, *Pennisetum orientale*, *Pennisetum purpureum*, and *Sorghum almum*) were clipped at 45, 60, 75, and 90-day intervals in a randomized complete block design (RCBD) with three blocks over two consecutive years. Mixed-effects models, with year and block nested within year as random effects, were applied to dry matter (DM) yield, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), *In vitro* dry matter digestibility (IVDMD), and relative feed value (RFV). Year-to-year variation was more evident in early-clipping yields (45–60 days) than in late-clipping yields (75–90 days) for most species. Across the two study years, *Pennisetum purpureum* maintained relatively stable nutritive value and tolerated delayed clipping up to 90 days, whereas *Setaria anceps* showed greater year-to-year sensitivity in IVDMD. The 60–75-day window remained a robust compromise across both years, but species-specific adjustments are recommended. *Sorghum almum* and *Panicum maximum* maintained quality at 60 days, while *Pennisetum purpureum* retained comparatively higher nutritive value when clipping was delayed to 90 days. The second year of data confirmed the general 60–75-day clipping recommendation. However, species differed in interannual stability, which should guide grass selection in areas with variable rainfall.

Key words: Interannual variation; Forage stability; Tropical grasses; Mixed-effects model; Subtropical climate

Introduction

In cut-and-carry ruminant systems, cultivated forages provide most of the dietary energy, protein, and fiber required for animal production. High-quality forage encourages voluntary intake and animal performance, while low-quality forage requires costly concentrates (McCarthy and Mulliniks, 2021). In subtropical conditions, C4 grasses grow rapidly, but their nutritive value declines sharply with maturity because structural carbohydrates and lignin increase (Junges *et al*., 2024; Tahmasebi *et al*., 2020).

A single-year field evaluation in Peshawar, Pakistan, compared seven tropical grasses over four clipping intervals (45, 60, 75, and 90 days), and the results showed that 60–75 days produced the highest DM yield and good nutritive value for most grasses (Jan *et al*., 2026). The seven grasses were selected because they are regionally relevant forage resources in Pakistan and had shown contrasting yield and nutritive responses in the previous Peshawar clipping-interval trial. That study, like most clipping-interval trials, was conducted over one growing season and could not determine whether the same intervals would perform similarly in other years. Subtropical Pakistan is characterized by high interannual variability in rainfall and temperature, making clipping recommendations based on a single year potentially unreliable. Although livestock contributes approximately 14% to GDP and 63% to agricultural value addition in Pakistan, feed deficits persist, with estimated national shortages of 19% in DM, 37% in CP, and 38% in ME (Habib *et al*., 2016). Therefore, evidence-based and climate-resilient forage management is needed to reduce dry matter, crude protein, and metabolizable energy deficits while maintaining forage yield and nutritive value across variable years.

Clipping interval recommendations for tropical forages in Pakistan are still mainly based on single-year trials. Such studies, while valuable for identifying general yield-quality trade-offs, cannot distinguish between genuine species responses and year-specific weather effects. Therefore, multiyear testing is needed before clipping interval recommendations can be considered reliable under variable subtropical conditions. Interannual stability, defined here as the consistency of a forage species' yield and nutritive value across climatically contrasting growing seasons, remains unquantified for most tropical grasses in subtropical production systems, yet recent two-year stability analyses of

Received: 19-05-2026 | Revised: 10-08-2026 | Accepted: 15-08-2026 | Online: 30-08-2026

tropical grass mixtures have demonstrated that annual forage yield can vary by nearly 4,000 kg DM ha^{-1} between years, even under consistent management (Garcia-Carvalho *et al*., 2025). Farmers need to know which species maintain quality across different years. This information can support climate-resilient forage selection. The present study addresses this gap by using mixed-effects modeling, with year as a random effect, to quantify interannual stability in seven tropical grasses commonly used in Pakistan's smallholder livestock systems. This modeling approach is supported by multiyear forage research (Winter *et al*., 2025).

The present two-year experiment (2024–2025) was designed to extend the preliminary single-year experiment by: (i) quantifying changes in yield and nutritive traits between years under the same management conditions; (ii) testing whether the 60–75 day compromise identified in the preliminary single-year experiment is maintained in a climatically second year; (iii) determining whether species differed in the interannual stability of their nutritive value; and (iv) developing species-specific clipping recommendations that account for the yield-quality trade-off and interannual reliability.

This study is a two-year extended follow-up to our previously published single-year investigation (Jan *et al*., 2026). The earlier paper reported only single-year responses. The present study evaluates two-year patterns and interannual stability, which were not assessed in the earlier publication. The previously published single-year study is appropriately cited and used as the basis for comparison. The two papers are complementary, with the first reporting short-term responses and the present study providing long-term stability insights.

Materials and Methods

Study site: The region has a humid subtropical climate, characterized by hot summers and mild winters, with monsoonal rainfall. Mean annual precipitation ranges from 450 to 550 mm, and mean summer temperature ranges from 30 to 39°C. The soil is sandy loam with a pH of about 7.5, low organic matter (≈ 0.8%), and moderate drainage. The present two-year experiment continued the single-year field evaluation reported by Jan *et al*., (2026), using the same study site, tropical forage grass entries, and clipping-interval structure.

The trials were initiated in August each year. The 75- and 90-day clipping intervals extended into the following calendar year, usually until February, but all observations were assigned to the trial year of initiation. Daily rainfall, maximum temperature, and minimum temperature were obtained from a nearby weather station. These data were summarized to describe growing conditions and interannual variation. During the active growing period from August to February, monthly mean temperature in Peshawar ranged from 11.5 to 31.2°C, while monthly rainfall ranged from 9.7 to 68.3 mm.

Experimental design and plant materials: Seven tropical forage grasses were assessed. These grasses were selected because of their local adaptation, availability in Pakistani forage germplasm resources, prior performance under Peshawar conditions, and use in smallholder ruminant feeding systems: *Pennisetum glaucum*, *Pennisetum purpureum*, *Pennisetum orientale*, *Panicum coloratum*, *Panicum maximum*, *Setaria anceps*, and *Sorghum almum*. Seed and vegetative planting materials were obtained from the Forage Research Institute, Sargodha, and the PFI germplasm collection.

The experiment was conducted in a randomized complete block design (RCBD) using three replications. Each experimental plot measured 4 m × 3 m (12 m²). Biomass was clipped from the central rows of each plot to minimize border effects. All possible combinations of the seven grass entries and four clipping intervals (45, 60, 75, and 90 days) were randomly assigned to each block. In both years, the same plots were kept under cultivation, perennial stands were allowed to regrow after each clipping, and no replanting was done in the second year. Establishment was completed in the season before the first experimental year, and one full season was allowed for stand establishment and uniformity before treatment application.

Clipping procedures: Clipping was done by hand, and the plants were clipped 8 cm above ground level. The clipping interval (45, 60, 75, and 90 days) was determined from the last clipping date. After each clipping, plots were allowed to regrow for the assigned clipping interval before the next clipping. All clippings were conducted between 07:00 and 09:00 local time to reduce diurnal moisture fluctuations.

The fresh weight of clipped biomass from each plot was recorded in the field using an electronic scale. A representative subsample was collected from the central rows of each plot, avoiding border plants to minimize border effects, placed in labelled polyethylene bags, and transported immediately to the laboratory for processing.

Laboratory analyses

Dry matter: Subsamples were chopped to 2–3 cm, placed in paper bags, and oven-dried at 65°C for 72 hours to constant weight. Dried samples were ground through a Wiley mill fitted with a 1-mm screen. Total dry matter was confirmed by further drying at 103°C overnight (McCleary, 2023).

$$\text{Dry matter content (\%)} = \frac{\text{Oven-dried sample weight}}{\text{Fresh sample weight}} \times 100$$

Fiber fractions: Fiber fractions (neutral detergent fiber and acid detergent fiber) were analyzed using the detergent system described by McCleary (2023) using an ANKOM200 Fiber Analyzer. For NDF, an amylase-assisted procedure without sodium sulfite was used. Fiber values were ash-corrected and expressed as a percentage of DM.

Ash: The ash content was measured by incineration at 550°C for 4 hours according to ISO 5984 (Quirino *et al*., 2023). All analyses were conducted in duplicate and expressed on a DM basis.

Crude protein: For crude protein determination, total nitrogen was determined by the Kjeldahl digestion-distillation-titration method (McCleary, 2023).

Crude protein concentration was calculated as total nitrogen × 6.25 and expressed as g kg^{-1} DM.

***In vitro* dry matter digestibility**: IVDMD was determined using the two-stage technique of Tilley & Terry (1963) adapted to an ANKOM DaisyII incubator. Ground forage samples (0.5 g) were sealed in nylon bags and incubated for 48 hours at 39 ± 0.5°C in buffered rumen fluid.

IVDMD (%) was calculated as [(initial dry matter − residual dry matter after incubation) / initial dry matter] × 100.

Relative feed value: Relative feed value (RFV) was calculated from digestible dry matter (DDM) and dry matter intake (DMI). ADF and NDF values were first converted from g kg^{-1} DM to percentage, as described by Guamán Rivera *et al*., (2023). DDM (%) = 88.9 – (0.779 × ADF%); DMI (% body weight) = 120 / NDF% and RFV = (DDM × DMI) / 1.29.

Statistical analysis

All statistical procedures were performed using R software version 4.0.2 (R Core Team, 2020) with the lme4, lmerTest, and emmeans packages (Bates *et al*., 2015; Kuznetsova *et al*., 2017; Lenth, 2023). Data from 2024 and 2025 were first analyzed separately using a linear mixed model with block as a random effect, and entry, interval, and their interaction as fixed effects. We then fitted a pooled two-year linear mixed-effects model for each response variable (DM yield, DM content, CP, NDF, ADF, ash, IVDMD, and RFV), treating entry (E), interval (I), and E×I as fixed effects (Table 1). Year (Y) and block nested within year (Y:block) were treated as random effects. Estimated marginal means for E×I combinations were obtained via the emmeans package, and pairwise comparisons were adjusted using Tukey's method (α = 0.05). Model assumptions (normality and homoscedasticity of residuals) were checked by visual inspection of Q–Q plots and residuals-versus-fitted plots.

Table 1. Pooled mixed-effects models – fixed-effect significance.

Response variable	Entry	Interval	Entry x interval
Forage yield (kg DM ha^{-1})	P<0.001	P<0.001	P<0.001
IVDMD %	P<0.001	P<0.001	P<0.001
Dry matter content %	P<0.001	P<0.001	P=1.000
Crude protein g kg^{-1} DM	P<0.001	P<0.001	P=0.727
NDF g kg^{-1} DM	P<0.001	P<0.001	P=0.299
ADF g kg^{-1} DM	P<0.001	P<0.001	P=0.878
Ash g kg^{-1} DM	P=0.202	P<0.001	P=0.981
RFV	P=0.529	P<0.001	P=0.893

IVDMD = *In vitro* dry matter digestibility; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; RFV = Relative feed value

Results

Forage yield: A longer clipping interval generally resulted in higher pooled two-year DM yields across all entries, though the increase varied by species (Table 2). At 90 days, *Pennisetum purpureum* had the highest yield (17,686 kg DM ha^{-1}), followed by *Setaria anceps* (15,483 kg DM ha^{-1}), while *Sorghum almum* reached 13,009 kg DM ha^{-1} at 75 days. The lowest DM yield across all entry × interval combinations was recorded for *Pennisetum glaucum* at 45 days (5,632 kg DM ha^{-1}). Yields differed significantly among species at 90 days. However, *Panicum maximum* reached its highest yield at 75 days (12,181 kg DM ha^{-1}), followed by a lower yield at 90 days (8,447 kg DM ha^{-1}). *Sorghum almum* also reached its highest yield at 75 days (13,009 kg DM ha^{-1}), with no significant gain at 90 days. The entry x interval interaction was significant (p<0.001), indicating that the effect of clipping interval on DM yield differed among grass entries.

***In vitro* dry matter digestibility (IVDMD):** The effect of the clipping interval was significant (p<0.001) for all entries, and IVDMD decreased with increasing interval (Table 3). *Sorghum almum* (66.31%) and *Pennisetum glaucum* (64.56%) had the highest digestibility at 45 days. In several species, IVDMD decreased to 29–33% by 90 days. However, *Pennisetum purpureum* showed a comparatively smaller decline in IVDMD across clipping intervals, decreasing from 59.81% at 45 days to 47.12% at 90 days. This indicates comparatively greater digestibility stability under delayed clipping than in most other grasses. The entry × interval interaction was significant (p<0.001), indicating that the effect of clipping interval on IVDMD differed among grass entries.

Dry matter content (% FW): Dry matter content was significantly affected by clipping interval (p<0.001; Table 4), with generally higher values at the longer clipping intervals. *Pennisetum orientale* had comparatively high DM content, whereas *Sorghum almum* had comparatively low DM content, with values ranging from 35.81 to 43.07% and 18.05 to 23.69%, respectively. The entry × interval interaction was not significant (p=1.000), indicating that the response of DM content to clipping interval did not differ significantly among grass entries.

Crude protein (CP): Crude protein concentration generally declined with longer clipping interval (p<0.001 for interval; Table 5), but the reduction was less dramatic than for IVDMD. Across all treatments, CP ranged from 46.8 to 65.6 g kg^{-1} DM. *Setaria anceps* and *Panicum coloratum* had relatively higher CP concentrations at 45 days (65.6 and 63.6 g kg^{-1} DM, respectively), declining to approximately 50–51 g kg^{-1} by 90 days. The entry × interval interaction was not significant (p = 0.727), indicating that the effect of clipping interval on CP did not differ significantly among grass entries.

Neutral detergent fiber (NDF): NDF concentration generally increased with increasing clipping interval (p<0.001 for interval; Table 6). *Pennisetum purpureum* had the lowest NDF concentration at 45 days (508.2 g kg^{-1} DM), while *Panicum maximum* had the highest NDF concentration at 90 days (788.9 g kg^{-1} DM). The entry × interval interaction was not significant (p=0.299), indicating that the effect of clipping interval on NDF did not differ significantly among grass entries.

Acid detergent fiber (ADF): ADF concentration generally increased with increasing clipping interval (p<0.001 for interval; Table 7). Across all treatments, ADF ranged from 357.4 to 527.0 g kg^{-1} DM. *Sorghum almum* had the lowest ADF concentration at 45 days (357.4 g kg^{-1} DM), while *Panicum coloratum* and *Pennisetum purpureum* exceeded 500 g kg^{-1} DM at 90 days. The entry × interval interaction was not significant (p = 0.878), indicating that the effect of clipping interval on ADF did not differ significantly among grass entries.

Table 2. Effect of clipping interval on forage yield (kg DM ha^{-1}) of forage grasses.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	5631.83^{a}	7474.33^{b}	7052.20^{b}	8329.00^{b}	323.215
Panicum coloratum	6431.50^{a}	5795.17^{a}	8192.00^{a}	7552.67^{a}	698.957
Panicum maximum	9547.40^{a}	6737.00^{a}	12180.67^{b}	8447.33^{a}	738.584
Pennisetum orientale	7280.67^{a}	6344.17^{a}	7334.83^{a}	7884.67^{a}	503.995
Pennisetum purpureum	9103.00^{a}	12152.50^{a}	17457.00^{b}	17686.00^{b}	1403.997
Setaria anceps	8502.60^{a}	8516.00^{a}	10522.33^{a}	15483.33^{b}	531.683
Sorghum almum	8001.83^{a}	8167.33^{a}	13008.83^{b}	11666.00^{b}	877.821

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 3. *In vitro* dry matter digestibility (IVDMD, %) of forage grasses at different clipping intervals.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	64.56^{a}	46.41^{b}	36.99^{c}	29.27^{d}	1.564
Panicum coloratum	56.27^{a}	38.88^{b}	35.23^{b}	28.57^{c}	1.102
Panicum maximum	49.40^{a}	47.01^{a}	46.12^{a}	41.82^{b}	0.912
Pennisetum orientale	55.82^{a}	42.65^{b}	38.74^{c}	31.19^{d}	0.953
Pennisetum purpureum	59.81^{a}	52.63^{b}	52.15^{b}	47.12^{c}	1.033
Setaria anceps	44.52^{a}	37.27^{b}	32.14^{c}	29.17^{c}	0.832
Sorghum almum	66.31^{a}	43.85^{b}	36.91^{c}	32.90^{c}	1.539

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 4. Effect of clipping interval on dry matter content (% FW) of forage grasses.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	17.53^{a}	20.27^{a}	19.57^{a}	23.57^{a}	1.642
Panicum coloratum	24.60^{a}	27.82^{a}	26.15^{a}	30.24^{a}	1.895
Panicum maximum	21.98^{a}	25.22^{a}	22.23^{a}	27.56^{a}	2.162
Pennisetum orientale	37.07^{a}	41.04^{a}	35.81^{a}	43.07^{a}	3.511
Pennisetum purpureum	30.23^{a}	32.21^{a}	27.86^{a}	35.75^{a}	3.566
Setaria anceps	21.13^{a}	23.42^{a}	22.96^{a}	27.47^{a}	2.078
Sorghum almum	18.05^{a}	20.70^{a}	19.71^{a}	23.69^{a}	1.855

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 5. Crude protein (CP, g kg^{-1} DM) of forage grasses as affected by clipping interval.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	57.2^{a}	50.1^{a}	52.2^{a}	46.9^{b}	2.33
Panicum coloratum	63.6^{a}	57.0^{a}	49.4^{b}	51.5^{b}	2.56
Panicum maximum	55.4^{a}	52.4^{a}	54.1^{a}	49.5^{a}	2.35
Pennisetum orientale	56.1^{a}	52.9^{a}	48.9^{a}	48.8^{a}	1.98
Pennisetum purpureum	61.1^{a}	53.9^{a}	49.4^{a}	46.8^{b}	3.15
Setaria anceps	65.6^{a}	57.1^{a}	54.5^{a}	50.5^{b}	3.42
Sorghum almum	60.2^{a}	58.5^{a}	52.6^{a}	50.9^{b}	2.24

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 6. Neutral detergent fiber (NDF, g kg^{-1} DM) contents of forage grasses as affected by clipping interval.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	580.2^{a}	625.0^{a}	672.8^{b}	707.4^{b}	11.39
Panicum coloratum	611.9^{a}	648.5^{a}	674.0^{b}	726.6^{c}	11.10
Panicum maximum	630.7^{a}	681.8^{b}	749.1^{c}	788.9^{d}	7.27
Pennisetum orientale	642.0^{a}	676.0^{a}	701.0^{b}	763.5^{c}	11.58
Pennisetum purpureum	508.2^{a}	573.3^{b}	584.6^{b}	629.8^{c}	13.33
Setaria anceps	616.9^{a}	656.7^{b}	706.2^{c}	747.6^{d}	8.52
Sorghum almum	632.5^{a}	683.4^{b}	697.8^{b}	758.7^{c}	10.04

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 7. Acid detergent fiber (ADF, g kg⁻¹ DM) of forage grasses as influenced by clipping interval.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	380.6[a]	426.6[a]	436.9[a]	483.7[b]	15.37
Panicum coloratum	444.1[a]	450.7[a]	482.9[a]	527.0[b]	17.81
Panicum maximum	378.0[a]	406.5[a]	454.9[b]	491.3[b]	12.53
Pennisetum orientale	376.4[a]	416.2[a]	461.5[b]	492.2[b]	10.71
Pennisetum purpureum	380.5[a]	427.0[b]	468.2[c]	508.0[d]	7.95
Setaria anceps	376.0[a]	433.2[b]	459.7[b]	500.4[c]	9.92
Sorghum almum	357.4[a]	381.3[a]	418.1[b]	460.9[b]	12.09

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 8. Ash content (g kg⁻¹ DM) of forage grasses with respect to clipping interval.

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	124.3[a]	127.9[a]	150.1[a]	151.9[a]	9.18
Panicum coloratum	97.8[a]	93.9[a]	106.5[a]	117.5[a]	9.53
Panicum maximum	103.4[a]	111.0[a]	120.5[a]	137.2[b]	7.86
Pennisetum orientale	120.1[a]	123.2[a]	137.0[a]	155.1[b]	8.14
Pennisetum purpureum	106.3[a]	126.1[a]	131.7[b]	148.3[b]	6.13
Setaria anceps	74.4[a]	105.8[b]	120.4[b]	134.5[c]	3.77
Sorghum almum	106.0[a]	120.9[a]	138.4[a]	135.9[a]	9.03

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Table 9. Relative feed value (RFV) of forage grasses at different clipping intervals (means with Tukey letters).

Species	45 days	60 days	75 days	90 days	SEM
Pennisetum glaucum	103.29[a]	98.10[a]	88.29[a]	78.59[a]	8.075
Panicum coloratum	92.08[a]	82.43[a]	73.37[a]	63.08[b]	4.659
Panicum maximum	91.56[a]	79.92[a]	68.80[b]	60.22[b]	3.584
Pennisetum orientale	98.67[a]	85.52[b]	74.84[c]	64.58[d]	3.479
Pennisetum purpureum	134.27[a]	115.16[b]	112.28[b]	93.78[c]	4.372
Setaria anceps	99.04[a]	83.53[b]	72.77[c]	62.28[d]	3.206
Sorghum almum	97.77[a]	85.37[b]	80.00[b]	68.01[c]	3.440

Note: Means within a row followed by different letters are significantly different at p≤0.05 by Tukey's test
SEM = pooled standard error of the mean

Ash content: Ash content ranged from 74.4 to 155.1 g kg⁻¹ DM (Table 8). The interval main effect was significant ($p<0.001$), whereas the entry effect was not significant ($p=0.202$), and the entry × interval interaction was also non-significant. Ash concentration generally increased with longer clipping intervals in several species. The non-significant entry × interval interaction indicates that the effect of clipping interval on ash content did not differ significantly among grass entries.

Relative feed value (RFV): RFV generally decreased with increasing clipping interval ($p<0.001$ for interval; Table 9). The highest RFV (134.27) was recorded for *Pennisetum purpureum* at 45 days, while the lowest RFV (60.22) was recorded for *Panicum maximum* at 90 days. Although RFV of *Pennisetum purpureum* declined from 134.27 at 45 days to 93.78 at 90 days, it remained higher than that of all other species at 90 days, supporting its comparatively greater tolerance to delayed clipping. The entry × interval interaction was not significant ($p=0.893$), indicating that the effect of clipping interval on RFV did not differ significantly among grass entries.

Discussion

This two-year study confirmed the inverse relationship between forage yield and nutritive value across seven tropical grasses, consistent with previous reports from tropical and subtropical regions (Junges *et al*., 2024; Tahmasebi *et al*., 2020). Extending the clipping interval generally increased DM yield, reduced IVDMD and RFV, and increased NDF and ADF. This two-year dataset quantified the stability of yield-quality trade-off across contrasting growing seasons. The present findings extend the preliminary single-year report from the same location by evaluating responses across two years (Jan *et al*., 2026).

The extent of the digestibility loss observed is similar to that reported for other tropical grasses. For example, comparable CP values of 78.5 g kg⁻¹ were reported for early-clipping *Pennisetum purpureum*, and *Tripsacum laxum* clipping at 45- and 70-day intervals in the Peruvian Amazon (Rupay Taboada *et al*., 2023). The present finding that a 60–75-day clipping window provided a useful compromise between yield and nutritive value is broadly consistent with findings for *Megathyrsus maximus* in Ethiopia, where later clipping increased yield while affecting forage quality

(Wolele *et al*., 2025). Likewise, Taiwan grass (*Pennisetum purpureum*) clipping at 60-day intervals also showed satisfactory IVDMD (66.5% for leaves) until a sudden drop later on (Mendoza-Pedroza *et al*., 2022).

The species × interval interaction (p=0.727) was not significant, and CP only decreased 15–20% from 45 to 90 days after clipping. IVDMD, however, decreased in all cases by 35–55% with high variation between entries (p<0.001). This suggests that while CP dilution is an important determinant of quality loss when mature, NDF accumulation is a more important factor, as indicated by the meta-analyses that demonstrated that energy supply and intake limitation (mediated by NDF) are important constraints in mature forages (Orzuna *et al*., 2021; Yanza *et al*., 2021). In practice, the choice of grass species with respect to digestibility at delay is more important than CP retention, because CP reduction is comparable among species.

The entry × interval interaction for CP was not significant (p=0.727), and CP showed a comparatively moderate decline across clipping intervals. IVDMD declined more strongly and showed a significant entry × interval interaction (p<0.001), indicating greater species-specific variation in digestibility response to delayed clipping. This suggests that while CP dilution contributes to quality loss with maturity, increasing fiber concentration, particularly NDF, may impose a stronger limitation on forage intake and energy availability, consistent with previous evidence (Orzuna *et al*., 2021; Yanza *et al*., 2021). In practical terms, species differences in digestibility response to delayed clipping may be more informative than CP retention alone.

A major conclusion is that the nutritive value of some species showed greater interannual stability than others. *Pennisetum purpureum* showed comparatively stable nutritive value across the two study years and retained relatively higher IVDMD and RFV under delayed clipping. In contrast, *Setaria anceps* showed greater year-to-year sensitivity in IVDMD, with a stronger decline under delayed clipping in one of the study years. This pattern suggests that *Setaria anceps* may be more sensitive to interannual variation than *Pennisetum purpureum*. Farmers using *Setaria anceps* should therefore avoid extending clipping intervals beyond 60 days when seasonal conditions are variable. These observations provide additional evidence on interannual differences in forage quality beyond the earlier single-year recommendation.

These interannual differences are consistent with findings from multiyear forage trials conducted elsewhere. Garcia-Carvalho *et al*., (2025) reported a 3,974 kg DM ha^{-1} year^{-1} difference in annual forage yield between years for tropical grass mixtures in Brazil (p=0.0017). Winter *et al*., (2025) demonstrated that multi-year stability assessments can quantify inter-annual variation with CVs below 30% under consistent management. These studies reinforce that single-year trials are insufficient for robust clipping recommendations and that species-specific multi-year assessments can improve climate-resilient forage management.

Consistent with the preliminary single-year study (Jan *et al*., 2026), pooled two-year means confirm that the 60–75-day interval provides the most consistent compromise across most entries. However, the two-year data refine this recommendation in three ways. First, *Pennisetum purpureum* showed greater tolerance to extended clipping intervals: yields increased significantly from 75 to 90 days (17,457 to 17,686 kg DM ha^{-1}), but values of IVDMD declined slightly (52.15% to 47.12%), and RFV remained above 90. This shows that species can be clipping at 90 days without significant quality penalties and is an option for farmers interested in bulk forage production or who cannot manage clipping regimes.

The relative tolerance of *Pennisetum purpureum* for delayed clipping is consistent with findings from Bangladesh, where *Pakchong Napier* retained comparatively better nutritive value at later clipping stages. Clipping at the 6-leaf stage (approximately 45-50 days) producing CP of 184 g kg^{-1} DM and supporting cattle growth rates of 610 g day^{-1}, whereas delaying to the 14-leaf stage (approximately 75-90 days) reduced CP to 118 g kg^{-1} DM and growth rates to 270 g day^{-1}, underscoring the substantial reduction in animal performance and the practical importance of our species-specific recommendations (Roy *et al*., 2025).

Second, *Setaria anceps* and *Panicum coloratum* showed severe quality losses beyond 60 days (IVDMD <30% at 90 days, RFV <65), so they should be clipping at 45–60 days when quality is prioritized. Third, *Sorghum almum* and *Panicum maximum* performed optimally at 60–75 days: yields at 75 days were significantly higher than at 60 days, but further delay to 90 days did not increase yield, while IVDMD and RFV continued to decline. For these species, the 75-day interval represents a true optimum rather than a compromise.

Our recommendation for *Panicum maximum* (syn. *Megathyrsus maximus*) aligns with those reported for Mexico and Ethiopia. In Tanzania grass (*Panicum maximum* cv. Tanzania), the optimal clipping point was 50 days, with maximum DM accumulation at 80 days, but better leaf proportion and quality at earlier clipping (Maldonado-Peralta *et al*., 2024). The Ethiopia guinea grass study further confirmed that 90-day clipping age offers a better balance of yield and nutritional quality (CP 11.48% at 90 days). Collectively, these studies support our finding that a 60-75-day clipping window provides the most consistent compromise for *Megathyrsus maximus* under subtropical conditions.

The progressive increase in NDF and ADF with advancing maturity mirrors findings from detailed clipping-interval studies on *Pennisetum purpureum*. In Taiwan grass, leaf NDF increased from 60.4% at 30 days to 72.5% at 150 days, while ADF increased from 36.7% to 46.8% over the same period (Mendoza-Pedroza *et al*., 2022). These comparisons should be interpreted in relation to differences in production environment and clipping intervals. Mendoza-Pedroza *et al*. (2022) evaluated Taiwan grass under different clipping intervals, including wider maturity stages than those used in the present study, while Roy *et al*., (2025) examined Pakchong Napier under a different tropical production environment. Although their climates and clipping intervals do not fully match the subtropical conditions of Peshawar, both studies support the same biological pattern observed here: delayed clipping increases maturity-related quality losses, but *Pennisetum purpureum*-type grasses can retain comparatively better nutritive value than several other tropical grasses. These patterns of fiber accumulation were also evident across the seven species. NDF concentrations above 600–650 g kg^{-1} DM can restrict voluntary intake through rumen fill, while high ADF concentrations are associated with reduced digestibility (Van Soest *et al*., 1991). In the present study, NDF concentrations at 90 days exceeded 700 g kg^{-1} DM in all entries except *Pennisetum purpureum* (629.8 g kg^{-1} DM), while ADF exceeded 500 g kg^{-1} DM in several entries. These high fiber concentrations corresponded with the lower IVDMD and RFV values observed at the longer clipping intervals.

Originally developed for temperate forages, RFV proved useful as a comparative ranking tool in this subtropical context. The observed RFV range (60 to 134) clearly separated high-quality early-clipping forages from low-quality late-clipping forages. The entry × interval interaction for RFV was not significant ($p = 0.981$), indicating that the response of RFV to clipping interval did not differ significantly among grass entries. However, absolute RFV values for late-clipping forages such as *Panicum maximum* at 90 days (RFV = 60.2) were low, indicating reduced feeding value at longer clipping intervals.

Dry matter content of fresh forage generally increased at longer clipping intervals. *Pennisetum orientale* had the highest DM content, approximately 35–43%, which may reduce the amount of water transported with fresh forage and may influence preservation characteristics. However, high DM content alone does not indicate high nutritive value, as shown by its moderate IVDMD and RFV. Conversely, *Pennisetum glaucum* and *Sorghum almum* had relatively low DM content, approximately 17–24%, while still showing comparatively high IVDMD at early clipping intervals. Therefore, DM content should be interpreted together with digestibility and fiber fractions when evaluating forage quality.

Ash content ranged from 74–155 g kg^{-1} DM and generally tended to increase with longer intervals in several species. Although Table 8 showed numerical differences in ash concentration among species, the entry effect was not statistically significant ($p=0.202$). This may reflect relatively homogeneous soil conditions across the experimental site, similar mineral availability among plots, or greater within-species sampling variability. In contrast, the interval effect was significant, suggesting that clipping maturity influenced ash concentration more strongly than species identity under the present field conditions.

If mineral supply to animals is a major consideration, future studies should focus directly on selected macro- and micro-minerals. In subtropical Pakistan, where smallholder farmers have limited access to commercial concentrates, early clipping of *Pennisetum purpureum*, *Sorghum almum*, and *Setaria anceps* may be preferable when higher digestibility and RFV are prioritized. Clipping at 75–90 days may be considered when greater DM yield is the main objective, particularly for *Pennisetum purpureum* and *Panicum maximum*, which retained comparatively better digestibility at longer clipping intervals. *Pennisetum purpureum* may also be preferred where greater interannual stability is required, whereas *Setaria anceps* should be used more cautiously when clipping is delayed beyond 60 days under highly variable seasonal conditions.

The main limitation of this study was that data were collected from a single site in Peshawar over two years. Multilocation trials are therefore needed to assess the generalizability of these findings across other subtropical regions of Pakistan, including Faisalabad, Multan, and irrigated areas of Sindh. Although weather data were collected, specific relationships between climatic variables and interannual variation in forage responses were not explicitly modeled. Future analyses should identify the weather variables most strongly associated with changes in forage yield and nutritive value under delayed clipping. Finally, animal performance was not measured directly, and the nutritive-value indices reported in this study should therefore be interpreted as proxies for feeding response. On-farm feeding trials would strengthen the practical relevance of these forage-quality findings.

Another limitation is that relative feed value (RFV) was originally developed for temperate forages. Although RFV was useful in this study as a comparative index for ranking grass entries and clipping intervals, it may not fully represent the feeding value of tropical forages under all production conditions. Future studies should consider using relative forage quality (RFQ) and direct animal performance measurements to provide a more comprehensive assessment of feeding value. Based on the pooled two-year responses in DM yield, digestibility, fiber concentration, and RFV, species-specific clipping recommendations are summarized in Table 10.

Table 10. Recommended clipping intervals for tropical forage grasses under subtropical conditions.

Species	Recommended clipping interval	Practical basis
Pennisetum glaucum	60 days	Better yield than early clipping with less quality loss than delayed clipping
Panicum coloratum	45–60 days	Quality declines markedly after 60 days
Panicum maximum	75 days	Highest yield at 75 days with no benefit from delaying to 90 days
Pennisetum orientale	60 days	Earlier clipping is preferred because digestibility declines with delay
Pennisetum purpureum	75–90 days	Highest yield and stronger nutritive stability under delayed clipping
Setaria anceps	45–60 days	Sensitive to delayed clipping and interannual variation
Sorghum almum	60–75 days	Good yield-quality compromise before quality declines at 90 days

Conclusion

This two-year field study conducted under irrigated subtropical conditions in Peshawar, Pakistan, evaluated seven tropical forage grasses by clipping at four intervals (45, 60, 75, and 90 days) and compared the results with a previously published single-year study from the same location. Three main findings emerged.

First, the general yield–quality relationship observed in the single-year study was also evident across the two study years. Longer clipping intervals generally increased DM yield, reduced IVDMD and RFV, and increased NDF and ADF. The response of CP to clipping interval was comparatively moderate, and the non-significant entry × interval interaction indicated that CP responses did not differ significantly among grass entries.

Second, the 60–75-day clipping window provided an appropriate compromise between DM yield and nutritive value for most species, although species-specific adjustments were evident. *Pennisetum purpureum* showed comparatively greater tolerance to delayed clipping and retained relatively better nutritive value at 75–90 days. *Setaria anceps* and *Panicum coloratum* should be clipped at 45–60 days when forage quality is prioritized. *Panicum maximum* and *Sorghum almum* performed favorably at 60–75 days.

Third, species differed in their apparent interannual stability. *Pennisetum purpureum* showed comparatively stable nutritive value across the two study years, whereas *Setaria anceps* showed greater year-to-year sensitivity in IVDMD. These findings suggest that *Pennisetum purpureum* may be a more reliable option under variable seasonal conditions, while delayed clipping of *Setaria anceps* beyond 60 days should be approached cautiously.

These results show that data from more than one year are needed to detect year × interval interactions. Future studies across more sites would further strengthen clipping recommendations to account for the yield-quality relationship and year-to-year consistency. For smallholder farmers in subtropical Pakistan, we recommend prioritizing species with demonstrated inter-annual stability, particularly *Pennisetum purpureum*, while exercising caution with year-sensitive species such as *Setaria anceps* when clipping is delayed beyond 60 days. Future research should expand this multi-year approach to additional locations and integrate direct measurement of climatic variables to model the weather factors driving inter-annual variation in forage quality and yield.

Acknowledgments

The authors are grateful to the Pakistan Forest Institute, the Centre of Animal Nutrition, the Directorate General of Livestock and Dairy Development (Research Wing), Khyber Pakhtunkhwa, and the College of Animal Science and Technology, Yangzhou University, China, for providing laboratory facilities, equipment, and technical support during the study.

Conflicts of Interest: The authors declare no conflicts of interest.

Author's Contributions: Conceptualization, visualization, writing–original draft, R.U.J.; writing–review and editing, M.I and M.T.K.; validation, software, data analysis, M.S and Z.Y.; table preparation, manuscript revision, drafting and revision, Y.Z.; conceptualization, study design, project administration, supervision, interpretation, funding acquisition, drafting, and revision. Y.J and M.W. All authors have read and agreed to the published version of the manuscript.

Funding Disclosure: This work was supported by the Key Project for Intergovernmental International Science and Technology Innovation Cooperation (2026YFE0106600), the High-end Foreign Experts program (H20250797), the Huaian Talent Enclave Expert Workstation Program of Ministry of Science and Technology of China, and the Priority Academic Program Development of Jiangsu Higher Education Institution (PAPD), P.R. China.

Data availability: The datasets used and/or analyzed in the current study are available from the corresponding author upon request.

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