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Nutrient Value and *In Vitro* Digestibility of *Pennisetum purpureum* cv. Mott under Varying Gamma Irradiation Doses in Acidic Soil

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ABSTRACT

Gamma irradiation is an emerging technique in agriculture with the potential to enhance the nutritional quality of forage crops and improve their adaptability to infertile environments. This study examined how different doses of gamma irradiation affected the nutrient content, calcium (Ca) and phosphorus (P) uptakes, and the *in vitro* digestibility of *Pennisetum purpureum* cv. Mott, a forage grass cultivated on acidic soil. The experiment involved the application of various gamma irradiation doses (0 Gy, 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy). Four replications were done using a randomized block design, with 25 *P. purpureum* cv. Mott seeds in each repetition, totaling 700 seeds. The plants were grown in acidic soil with a pH of approximately 4.5–5. After two months of growth, the plants were harvested, and various parameters were analyzed. The research results indicated that the treatment had a significant effect on increasing organic matter content (p<0.01), NDF (neutral detergent fiber) (p<0.01), ADF (acid detergent fiber) (p<0.01), cellulose (p<0.01), hemicellulose (p<0.01), crude protein (p<0.01), crude fat (p<0.05), non-nitrogen-free extract (p<0.05), fiber fractions (p<0.01), nutrient digestibility (p<0.01), and fiber fraction digestibility (p<0.01). Overall, it can be concluded from this study that a gamma irradiation dose of 15 gy can enhance the nutritional content of *P. purpureum* cv. Mott cultivated on acidic soil and improves its utilization efficiency for livestock due to the increased digestibility.

***Keywords:** digestibility; gamma irradiation; marginal land; Pennisetum purpureum cv. Mott*

INTRODUCTION

Acidic soils are characterized by their poor soil quality and limited agricultural productivity. These lands pose a significant challenge to sustainable agriculture (Csikós & Tóth, 2023). Despite their potential for cultivation, acidic soils are often neglected due to their inherent limitations, including soil degradation, low fertility, and vulnerability to environmental stressors (Sallustio *et al.*, 2022). Acidic soils are frequently characterized by nutrient deficiency, particularly in essential nutrients such as nitrogen, phosphorus, and potassium (Abay *et al.*, 2022). These nutrients are critical for plant growth and crop development (Yahaya *et al.*, 2023). Additionally, the low pH levels in many marginal soils further exacerbate nutrient availability issues, leading to stunted crop growth and reduced yields (Saleem *et al.*, 2023).

In acidic soil, there is a unique potential for developing forage crops (He *et al.*, 2022). Forage crops, such as *Pennisetum purpureum* cv. Mott can thrive in challenging environments and offer valuable biomass for livestock feed (Shehzadi *et al.*, 2021). Exploring the potential of forage crops in acidic soil can contribute to sustainable agriculture practices and address the issue of food production in resource-constrained areas (Haberzettl *et al.*, 2021). Plants grown in acidic soil often exhibit inefficient nutrient utilization due to unfavorable soil conditions (Csikós & Tóth, 2023). Nutrient uptake processes are hindered, limiting the amount of nutrients plants can absorb from the soil (Tan *et al.*, 2023). This inefficiency affects crop productivity and contributes to nutrient runoff, which can have adverse environmental impacts (Fageria & Nascente, 2014).

P. purpureum cv. Mott, also known as elephant grass or Napier grass, is a versatile forage crop with remarkable characteristics (Chouychai & Somtrakoon, 2022). It is well-suited for cultivation in marginal lands due to its adaptability to various environmental conditions (Putra *et al.*, 2022b). However, the cultivation of this forage crop in such lands is often hampered by nutrient deficiencies and suboptimal soil conditions (Putra *et al.*, 2022a).

Gamma irradiation, a form of ionizing irradiation, has been utilized in agriculture for several purposes, including pest control, mutation breeding, and crop enhancement (Udage, 2021). This technology has shown promise in inducing beneficial changes in plant characteristics, making them more adaptable and productive (Katiyar *et al.*, 2022). In marginal land agriculture, gamma irradiation offers a unique approach to address nutrient deficiencies and enhance crop performance (Chaudhary *et al.*, 2019).

Gamma irradiation has been employed in agriculture for decades, offering a range of applications (Kato *et al.*, 2020). It can stimulate plant genetic and physiological responses, resulting in altered growth patterns, increased resistance to pests and diseases, and enhanced nutrient uptake (Katiyar *et al.*, 2022). Researchers have investigated gamma irradiation to improve crop performance, particularly in suboptimal soil conditions (Yunita *et al.*, 2023). In plants propagated vegetatively, the exposure of rooted stem cuttings, detached leaves, and dormant plants to irradiation has led to the emergence of numerous mutants. According to the FAO/IAEA database, of the 465 mutants released for vegetatively propagated plants, the majority are found in ornamental plants, with a smaller representation in fruit trees (Maluszynski *et al.*, 1992). Examples of these mutants encompass chrysanthemum, Alstroemeria, dahlia, bougainvillea, rose, Achimenes, begonia, carnation, Streptocarpus, and azalea. The use of gamma irradiation on vegetative materials has played a pivotal role in broadening genetic diversity and enhancing desirable traits in diverse plant species (Ahloowalia & Maluszynski, 2001).

This study presents a new method for addressing agricultural difficulties caused by acidic soils, by utilizing gamma irradiation on *P. purpureum* cv. Mott. Gamma irradiation possesses a distinctive capacity to provoke advantageous alterations in plant physiology, which might potentially augment the efficiency of nutrient uptake, enhance tolerance to environmental stresses, and improve the overall quality of crops. The objective of this study is to determine the maximum nutrient content and digestibility of the *P. purpu*reum cv. Mott mutant under varying gamma irradiation doses in acidic soil.

MATERIALS AND METHODS

Study Area

The research was conducted in Kota Baru Santan, Tubei, Lebong Regency, Bengkulu, Indonesia, located at a latitude of -3.1667240 and a longitude of 102.1432690, with an elevation of 97 meters above sea level. Figure 1 shows the more detailed location.

Procedures

Irradiation process. In this study, an unconventional approach was adopted by utilizing cutting stems of *P. purpureum* cv. Mott as the primary research material for gamma irradiation. This deviation from the conventional practice of applying gamma irradiation to seeds or generative material was prompted by the limited availability of seeds in *P. purpureum* cv. Mott. The selection of cutting stems as the research material aims to explore alternative avenues for mutation induction, demonstrating the adaptability of gamma irradiation in enhancing the genetic traits of *P. purpureum* cv. Mott.

The irradiation treatment was done at BATAN (National Nuclear Energy Agency). *P. purpureum* cv. Mott seeds, obtained from stem cuttings, were irradiated with acute doses of gamma irradiation, including doses of 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy, in addition to a control group.

The gamma irradiation source was Cobalt 60, administered through a gamma chamber irradiator model 4000A at an approximately 0.0775 rad/second dose rate. Each irradiation dose was applied to 100 plant stems, resulting in an irradiated total of 1100 stems, with an additional 100 stems serving as the control group.

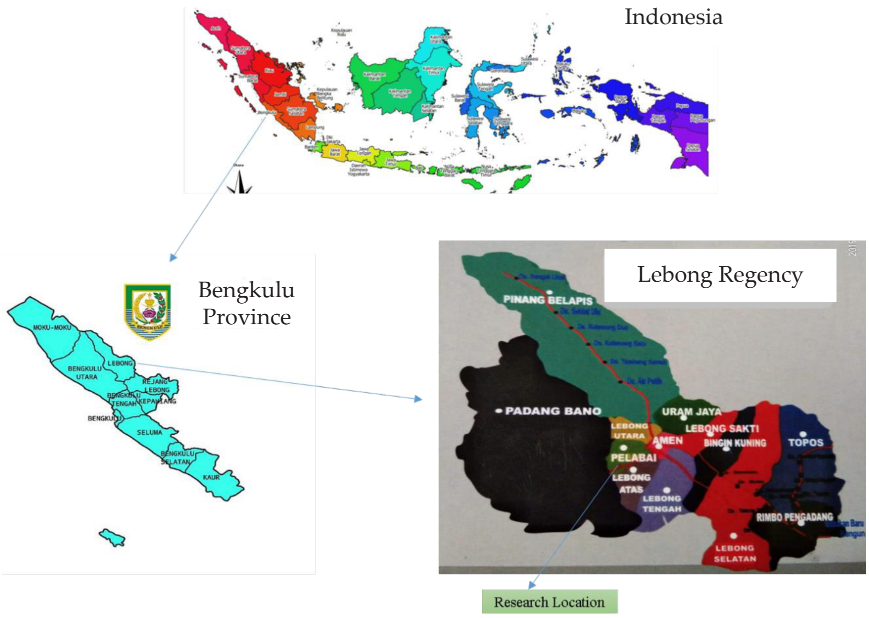

Figure 1. The research was conducted in acidic soil at Tikteleu Village, Pelabai District, Lebong Regency, Bengkulu Province, Indonesia

Land preparation. The field research was conducted on marginal land in Kota Baru Santan, Tubei, Lebong Regency, Bengkulu, Indonesia, with coordinates Lat -3.1667240 and Long 102.1432690. The research land has a pH range of 4.5-5.6 and an Aldd content of 2.26 ppm with 10.68% Al saturation. The experimental design used a completely randomized block design (CRBD) with four replications. The adoption of a randomized group design at the block level in this research study is justified by the need to account for the potential influence of varying sunlight exposure, unequal wind patterns, and the presence of westward-facing trees. This meticulous approach enhances the internal validity of the study, allowing for more accurate interpretations of the observed effects and contributing to the overall advancement of scientific knowledge in the field. Each experimental plot consisted of 4 rows (5 m in length and 3 m in width), and then, un-irradiated and irradiated seeds were sown, and the distance was 75 cm apart on the top of the row. The plants carefully controlled weeds and insects that could affect their growth throughout the period. After a growth period of two months, the plants were harvested, and the shoots were separated from the roots. Subsequently, observations were conducted based on the predetermined test parameters.

Chemical Composition Analysis

Samples of *P. purpureum* cv. Mott grown for two months in the experimental field were collected. The samples included the plants' upper parts, such as leaves and stems. Both will be analyzed to evaluate proximate compositions such as moisture, crude lipid, ash, crude fiber, and crude proteins, according to Aganduk *et al*. (2023). Meanwhile, the acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were evaluated according to the methodology described by Van Soest *et al*. (2020). This technique involves using a mild detergent to eliminate protein and hemicellulose from the sample. The outcome is a fiber that consists of cellulose, hemicellulose, and lignin. The cellulose content is determined by subtracting the ADF from the lignin content and is commonly referred to as crude cellulose (Navarro *et al*., 2018).

In Vitro Digestibility

In vitro digestibility analysis was done according to a modification of the Tilley & Terry (1963) procedure using the Daisy incubator (ANKOM Technology method) based on Leon *et al.* (2023). Rumen fluid was obtained from FH cows fitted with fistulas and located at the University of Andalas barn in Padang. The care provided for animals undergoing surgical modifications adhered to established guidelines (Bayne, 1998). The collection and preparation of rumen inoculum and buffer were described in Aguerre *et al*. (2023).

Data Analysis

The research data were analyzed for variance based on a randomized complete block design with seven treatments and four replications. In addition to employing ANOVA (analysis of variance), a multivariate analysis was also utilized for a more comprehensive examination of the data. Furthermore, Duncan's multiple range test was employed to determine the differences among the treatments, ensuring a thorough exploration of the experimental outcomes.

RESULTS

Chemical Composition

Table 1 provides information on the average values and variations for each parameter throughout a range of treatments, from IR 0 (no irradiation) to IR 30 (the greatest irradiation dose). The research results indicate that gamma irradiation significantly affected the organic matter, crude fiber, crude protein, ether extract, and nitrogen-free extract contents. However, gamma irradiation did not significantly affect dry matter or TDN (total digestible nutrients).

The highest organic matter content was found in the treatment with a gamma irradiation dose of 30 Gy, with a value of 85.37%, significantly different from the other treatments. Treatments with irradiation up to a dose of 20 Gy showed no significant differences. The sequential average contents of organic matter from treatments IR 0, IR 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy were 81.51%, 81.49%, 82.44%, 82.70%, 82.23%, 84.79%, and 85.37%, respectively.

Gamma irradiation treatment had a significant impact on crude fiber. The highest fiber content was observed in the treatment with a dose of 30 Gy. Statistical analysis showed that the treatment with a dose of 30 Gy had significantly different results for plant crude fiber. The lowest crude fiber content was found in the treatment with a dose of 15 Gy. The sequential results of crude fiber content after treatment, arranged from the highest to the lowest values, are as follows: IR 30 (30.65%), IR 25 (26.92%), IR 20 (23.57%), IR 0 (23.35%), IR 5 (22.805%), IR 10 (22.125%), and IR 15 (21.97%).

Gamma irradiation significantly impacted the crude protein in plant treatments. The highest crude protein content was obtained in the treatment with a dose of 15 Gy and was significantly different from the non-radiated treatment (IR 0), dose of 5 Gy (IR 5), dose of 25 (IR 25), and dose of 30 (IR 30). However, it was not significantly different from the treatment with a dose of 10 Gy (IR 10) and 20 Gy (IR 20).

As the irradiation dose increased to the 15 Gy level, crude protein content significantly increased but decreased at the 20 Gy to 30 Gy levels. The sequential results of crude protein content were arranged from the highest to the lowest values, is as follows: IR 15 (20.13%), IR 10 (20.04%), IR 20 (19.69%), IR 5 (17.08%), IR 0 (13.24%), IR 25 (13.23%), and IR 30 (11.53%).

Gamma irradiation treatment had a significant impact on ether extract. The lowest fat content was found in the treatment with a irradiation dose of 30 Gy (0.82%), significantly different from the other treatments. Statistically, irradiation treatments from IR 0 (no irradiation) to IR 25 (25 Gy) showed no significant difference. The sequential results of ether extract content after treatment, arranged from the highest to the lowest values, are as follows: IR 10 (2.81%), IR 0 (2.76%), IR 20 (2.67%), IR 5 (2.34%), IR 15 (1.92%), IR 25 (1.94%), and IR 30 (0.82%).

The effects of gamma irradiation treatment on nitrogen-free extract content were significant. The highest nitrogen-free extract content was found in treatment IR 25 (42.71%) and was significantly different from treatment IR 10 (37.48%) and treatment IR 20 (36.31%). However, it was not statistically different from treatments IR 0 (42.16%), IR 5 (39.28%), IR 15 (38.68%), and IR 30 (42.37%).

Fiber Fraction Content

Table 2 presents the results of the study of fiber fractions, including NDF, ADF, cellulose, hemicellulose, lignin, and silica contents in the plant tissue. The NDF content in *P. purpureum* cv. Mott exhibited a distinct response to gamma irradiation exposure, as depicted in Table 2. Commencing with the untreated sample (Un irradiated) possessing an NDF concentration of 74.50%, we noted a systematic decrease in NDF content as the gamma irradiation doses increased. At the maximum dosage of 30 Gy (IR 30), the NDF level was reduced to 68.83%. The ADF content exhibited a multifaceted response to gamma irradiation exposure, with diverse patterns at different dosage levels. At an intermediate dose of 10 Gy (IR 10), the ADF content increased slightly to 31.08%. Nevertheless, the ADF contents declined at elevated doses, such as IR 20 and IR 25, with values of 33.13% and 36.30%, respectively.

The cellulose content remained relatively stable at the lower irradiation doses, as indicated by values around 24% (24.36%, 25.045, 24.22%, and 23.84%) for doses ranging from 0 to 15 Gy (IR 0, IR 5, IR 10, and IR 15). However, a notable increase in cellulose content was observed at higher irradiation doses. At 20 Gy (IR 20), the cellulose content increased to 25.44±4.67, and this trend continued at 25 Gy (IR 25) with a cellulose content of 28.26%. The hemicellulose content showed varying responses to different levels of gamma irradiation exposure. At lower irradiation doses (IR 0 to IR 15), ranging from 0 to 15 Gy, the hemicellulose content exhibited only minor fluctuations. Values around 43.50%, 41.68%, 38.87%, and 37.13% were recorded. At 20 Gy (IR 20), the hemicellulose content dropped to 37.76%, and this trend continued at 25 Gy (IR 25) with a further reduction to 39.02%. The most significant decrease in hemicellulose content was observed at 30 Gy (IR 30), with 32.41% indicating a substantial reduction compared to the lower-dose treatments. The decrease in hemicellulose content with increasing gamma irradiation doses can be attributed to the susceptibility of hemicellulose to irradiation-induced depolymerization.

Table 1. Nutrient composition analysis of different treatments (IR 0 to IR 30) with mean values and standard deviations of *Pennisetum purpureum* cv. Mott

Treatments	Nutrient composition						
	DM%	OM (%DM)	CF (%DM)	CP (%DM)	EE (%DM)	NFE (%DM)	TDN (%DM)
Un irradiated	13.99±0.73	81.51±0.65^{A}	23.35±0.33^{A}	13.24±1.45^{A}	2.76±0.08^{b}	42.16±0.12^{a}	60.07±0.44
IR 5	14.08±0.57	81.49±0.70^{A}	22.80±0.77^{A}	17.08±0.42^{B}	2.34±0.83^{b}	39.28±0.89^{ab}	58.21±0.52
IR 10	13.86±0.21	82.44±0.36^{A}	22.12±0.72^{A}	20.03±0.79^{C}	2.81±0.69^{b}	37.48±1.02^{b}	58.25±0.89
IR 15	14.17±0.76	82.70±0.75^{A}	21.97±2.09^{A}	20.13±0.59^{C}	1.92±0.68^{b}	38.68±2.31^{b}	58.08±0.77
IR 20	14.46±0.26	82.23±2.26^{A}	23.57±1.31^{A}	19.69±0.62^{C}	2.67±0.62^{b}	36.31±2.59^{b}	57.17±1.29
IR 25	13.52±0.87	84.79±1.18^{B}	26.92±1.03^{B}	13.22±3.29^{A}	1.94±0.98^{b}	42.71±5.04^{a}	60.28±2.03
IR 30	14.03±0.81	85.37±0.27^{C}	30.65±0.69^{C}	11.53±0.31^{A}	0.82±0.31^{a}	42.37±0.28^{a}	58.58±0.06
p	>0.05	<0.01	<0.01	<0.01	<0.05	<0.05	>0.05

Note: Treatment IR 5 until IR 30 indicated the application of various gamma radiation doses (5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy). DM= dry matter, OM= organic matter, CF= crude fiber, CP= crude protein, EE= ether extract, NFE= nitrogen-free extract, and TDN= total digestible nutrients are nutritional parameters analyzed for each treatment. Means in the same column with different superscripts differ significantly at uppercase (p<0.01) and lowercase (p<0.05).

Table 2. Fiber fraction content of of *Pennisetum purpureum* cv. Mott under different gamma irradiation exposure

Treatments	Fiber fraction contents					
	NDF%	ADF%	Cellulose%	Hemicellulose%	Lignin%	Silica%
Un irradiated	74.50±1.95^{AB}	31.00±0.95^{AB}	24.36±3.44^{A}	43.50±4.21^{A}	4.19±1.47^{A}	2.45±0.28
IR 5	72.82±1.82^{ABC}	31.13±3.24^{AB}	25.04±3.21^{A}	41.68±4.29^{AB}	3.58±1.92^{B}	2.51±0.15
IR 10	69.95±2.27^{CD}	31.08±0.37^{AB}	24.22±1.26^{A}	38.87±0.75^{BC}	3.50±0.14^{BC}	3.37±1.03
IR 15	66.90±2.64^{D}	29.76±0.68^{A}	23.84±2.66^{A}	37.13±2.03^{C}	3.47±0.53^{CD}	2.46±0.43
IR 20	70.88±2.02^{BC}	33.13±0.88^{B}	25.44±4.67^{A}	37.76±1.96^{BC}	4.88±0.41^{D}	2.80±0.57
IR 25	75.32±3.29^{A}	36.30±1.44^{C}	28.26±2.62^{B}	39.02±0.92^{BC}	5.61±1.98^{D}	2.43±1.02
IR 30	68.83±2.18^{CD}	36.43±1.05^{C}	25.44±4.24^{A}	32.41±5.46^{D}	7.52±0.48^{D}	3.47±0.85
p	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05

Note: Treatment IR 5 until IR 30 indicated the application of various gamma radiation doses (5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy). NDF= neutral detergent fiber, ADF= acid detergent fiber. The data in the table are presented in the format of Mean±SD (Mean is the average value, and SD is the standard deviation). Means in the same column with different superscripts differ significantly (p<0.01).

At the lowest irradiation dose, 0 Gy (IR 0), the lignin content was recorded as 4.19. Table 2 presents the study results of fiber fractions, including NDF, ADF, cellulose, hemicellulose, lignin, and silica contents in the plant tissue. These findings yield insights into the effects of gamma irradiation on the makeup of crucial fiber constituents. These findings enhance comprehension of plant adaptation and potential use in various scenarios. As the irradiation dose increased to 5 Gy (IR 5), the lignin content slightly decreased to 3.58%. Subsequently, at 20 Gy (IR 20), there was a noticeable increase in lignin content to 4.88%. The lignin content increased at 25 Gy (IR 25), reaching 5.61%. The highest irradiation dose, 30 Gy (IR 30), resulted in the highest lignin content of 7.52%, while the lowest lignin value at IR 15 was 3.47%, providing a comprehensive overview of the lignin variations across different irradiation doses.

The statistical analysis indicated that the differences in silica content across different irradiation doses were not statistically significant (p>0.05). This means that the variations observed in silica content could likely be due to random fluctuations, and there is no strong evidence to suggest a direct impact of gamma irradiation on the silica content of *P. purpureum* cv. Mott in this study.

Digestibility of Nutrients

Table 3 presents the results of a study investigating the impact of gamma irradiation on the proximate digestibility of various feed components. The results demonstrate a significant improvement in DMD with increasing gamma irradiation dose, with "IR 15" exhibiting the highest DMD (69.19%). Organic matter digestibility (OMD) evaluates the digestibility of organic matter in the feed. It follows a similar trend to DMD, with the highest OMD observed in "IR 15" (64.78%), signifying improved organic matter digestibility due to gamma irradiation. Crude protein digestibility (CPD) assesses the digestibility of crude protein and reveals that "IR 15" (67.00%) exhibits the highest CPD, indicating significantly enhanced crude protein digestibility due to irradiation.

Crude fiber digestibility (CFD) measures the digestibility of crude fiber, an essential component in animal nutrition. The data indicates that "IR 15" (60.00%) displays the highest CFD, signifying improved crude fiber digestibility with gamma irradiation. Ether extract digestibility (EED) evaluates the digestibility of ether extract content, with relatively consistent values across treatments, suggesting no significant impact from irradiation. Nitrogen-free extract digestibility (NFE-D) assesses the digestibility of nitrogen-free extracts, including carbohydrates, with variations among treatments but no significant influence from gamma irradiation.

Digestibility of the Fiber Fraction

Table 4 shows the results of gamma irradiation's effect on the digestibility of fiber fractions in *P. purpureum* cv. Mott. The study indicates that the increased irradiation levels significantly reduce the digestibility of ADF, NDF, cellulose, and hemicellulose. Higher irradiation levels, such as in the IR 15 treatment, exhibited higher NDF digestibility values (60.01%) than the IR 0 treatment (57.51%).

These results indicate that gamma irradiation at certain levels can significantly reduce NDF digestibility. Furthermore, the IR 30 treatment also showed low NDF digestibility values (55.68%).

The ADF digestibility values varied among the treatments. The lowest ADF digestibility was observed in the IR 30 treatment (51.31%). On the other hand, the IR 15 treatment exhibited the highest ADF digestibility (56.29%). Lower digestibility values at higher irradiation levels, as shown in the IR 30 treatment, indicate that excessive irradiation may decrease ADF digestibility. Conversely, the IR 15 treatment demonstrates that moderate irradiation levels can enhance ADF digestibility.

Gamma irradiation could enhance cellulose digestibility. The most substantial impact was observed in the IR 15 treatment (62.70%). Conversely, the IR 30 treatment had lower cellulose digestibility (55.21%). This suggests that excessive irradiation can have a detrimental effect on cellulose digestibility. Gamma irradiation did not significantly impact hemicellulose digestibility. The hemicellulose digestibility values across different treatments showed no substantial variations.

Table 3. The digestibility of dry matter, organic matter, crude protein, crude fiber, ether extract, and nitrogen-free extract in *Pennisetum purpureum* cv. Mott treated with gamma irradiation

Treatments	Nutrient digestibility					
	DMD%	OMD%	CPD%	CFD (fiber)%	EED (fat)%	NFE-D%
Un irradiated	63.34±3.18^{AB}	60.72±1.10^{AB}	63.00±1.0^{A}	56.00±0.82^{A}	58.33±1.00^{A}	58.67±1.00^{A}
IR 5	65.77±1.19^{A}	61.10±1.56^{AB}	64.00±0.001^{AB}	57.00±1.00^{AB}	58.67±1.00^{A}	59.00±1.00^{A}
IR 10	65.11±0.79^{AB}	62.85±0.67^{AC}	63.67±1.00^{A}	58.00±0.82^{B}	60.33±1.00^{A}	59.33±0.58^{A}
IR 15	69.19±0.57^{C}	64.78±0.83^{C}	67.00±1.00^{C}	60.00±1.00^{C}	63.33±0.58^{B}	63.00±1.00^{B}
IR 20	63.26±2.02^{AB}	60.12±0.12^{B}	64.00±0.82^{AB}	58.67±2.00^{BC}	61.00±0.82^{B}	60.00±1.00^{A}
IR 25	62.62±1.38^{B}	60.33±2.34^{B}	60.33±0.82^{B}	57.00±0.001^{AB}	59.33±2.08^{A}	57.33±2.08^{A}
IR 30	57.41±1.42^{D}	55.09±1.10^{D}	56.00±1.00^{D}	54.00±1.00^{D}	55.33±3.79^{C}	55.33±1.00^{C}
p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Note: Treatment IR 5 until IR 30 indicated the application of various gamma radiation doses (5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy). This table presents data on the digestibility of DMD= dry matter digestibility, OMD= organic matter digestibility, CPD= crude protein digestibility, CFD= crude crude fiber digestibility, EED= ether extract digestibility, and NFE-D= nitrogen-free extract digestibility. The data in the table are presented in the format of Mean±SD (Mean is the average value, and SD is the standard deviation). Means in the same column with different superscripts differ significantly (p<0.01).

Multivariate Analysis of Chemical Composition and Digestibility

Figure 2 shows a hierarchical correlation heatmap that illustrates the relationships among these parameters. The correlation analysis results for nutrient data at different treatment levels offer valuable insights into the intricate interactions among many nutritional factors. Within this particular context, the heatmap provides a potent visual depiction of the correlation patterns among crucial elements in the stream.

A notable positive association is discovered between DM and OM, NFE, and TDN. These findings suggest that a rise in dry matter content is typically accompanied by an increase in the nutritional value of digestible nutrients. Furthermore, there exists a compelling correlation between the content of crude protein (CP) and fiber constituents such as detergent fiber (DF) and ADF. This indicates a correlation between the protein content and fiber content in the feed, which offers valuable information on the composition of the feed that affects the availability of nutrients.

The heatmap effectively illustrates the intricate nature of interactions between dietary components. The correlations among crude protein digestibility (CPD), crude fiber digestibility (CFD), nitrogen-free extract digestibility (NFE-D), and neutral detergent fiber digestibility (NDFD) suggest that alterations in one parameter may be linked to alterations in the other parameters, demonstrating a wide-ranging interconnectedness.

Table 4. The digestibility of fiber fraction in *Pennisetum purpureum* cv. Mott treated with gamma irradiation

Treatments	Fiber fraction digestibility			
	NDFD%	ADFD%	CeD%	HmD%
Un irradiated	57.51±0.73[A]	52.85±1.09[A]	58.59±0.79[A]	60.85±0.51
IR 5	57.87±0.43[A]	53.90±1.15[A]	59.50±0.95[A]	60.97±0.61
IR 10	58.31±0.11[A]	54.16±0.31[A]	61.77±0.32[B]	61.64±0.55
IR 15	60.01±0.40[B]	56.29±0.27[B]	62.70±2.08[C]	63.01±1.00
IR 20	57.67±1.03[A]	54.29±0.48[A]	59.71±1.52[A]	60.65±1.45
IR 25	57.18±0.43[A]	53.94±1.37[A]	58.61±0.68[A]	60.21±3.52
IR 30	55.68±0.67[A]	51.31±0.45[A]	55.21±1.46[A]	59.64±0.73
p	<0.01	<0.01	<0.01	>0.05

Note: Treatment IR 5 until IR 30 indicated the application of various gamma radiation doses (5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, and 30 Gy). NDFD= neutral detergent fiber digestibility, ADFD= acid detergent fiber digestibility, CeD= cellulose digestibility, and HmD= hemicellulose digestibility. The table displays each measured parameter's mean values and their corresponding SD= Standard Deviations. Means in the same column with different superscripts differ significantly (p<0.01).

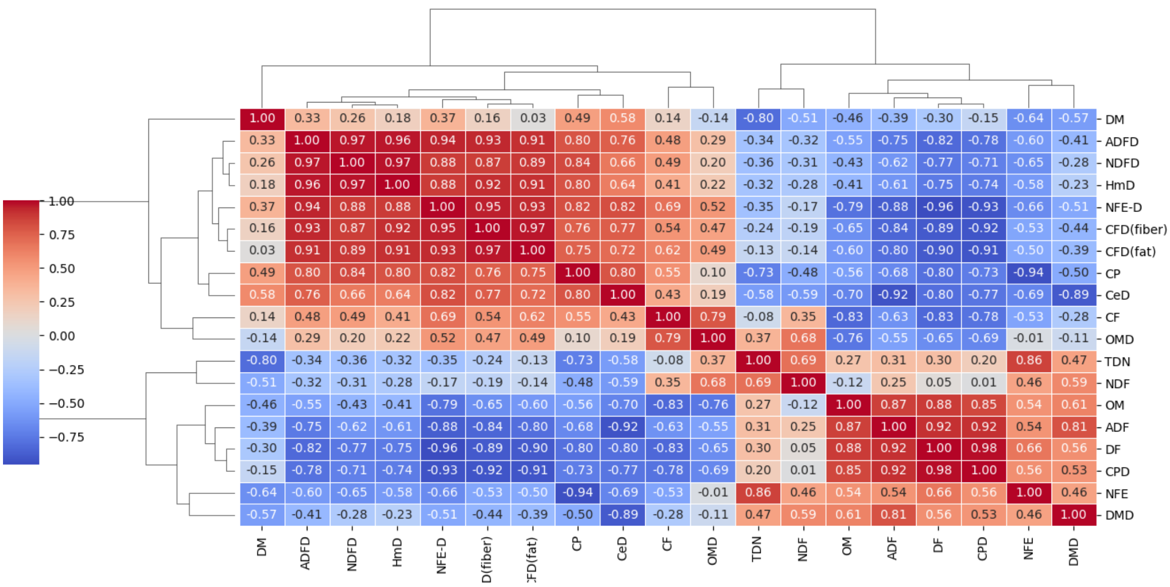

Figure 2. Hierarchical correlation analysis of gamma radiation effects on multiple parameters in *Pennisetum purpureum* cv. Mott. Note: The correlations between these parameters are visually represented by the colors and intensities of cells in the heatmap. Red colors indicate positive correlations, while blue colors represent negative correlations. The relationships among these parameters, including DM= dry matter, OM= organic matter, DF= dietary fiber, CP= crude protein, CF= crude fat, NFE= nitrogen-free extract, TDN= total digestible nutrients, NDF= neutral detergent fiber, ADF= acid detergent fiber, DMD= dry matter digestibility, OMD= organic matter digestibility, CPD= crude protein digestibility, CFD= crude fiber digestibility, CFD= crude fat digestibility, NFE-D= nitrogen-free extract digestibility, NDFD= neutral detergent fiber digestibility, ADFD= acid detergent fiber digestibility, CeD= cellulose digestibility, and HmD= hemicellulose digestibility.

DISCUSSION

The application of gamma irradiation led to the development of a mutant (putative) fodder plant. This mutant is known as *P. purpureum* cv. Mott exhibited improved nutritional content when cultivated in acidic soil. This improvement is attributed to several factors, including the ability of gamma irradiation to break chemical bonds within organic matter, crude fiber, and ether extract molecules, rendering them smaller and more digestible by livestock (Mapato & Wanapat, 2018). During growth, cell development may be influenced by irradiation-induced cell disruption, leading to cellular mutations that potentially alter metabolic processes such as amino acid synthesis and the accumulation of structural carbohydrates in response to photosynthetic rates within the plant organism. Furthermore, agronomic characteristics may provide insights into the effects of irradiation on nutrient levels in forage. Gamma irradiation, for instance, can instigate structural modifications in the cellular composition of Pennisetum purpureum cv Mott, thereby enhancing the accessibility of digestive enzymes to organic matter, crude fiber, and ether extract. Furthermore, gamma irradiation can enhance the activity of digestive enzymes, thus aiding livestock in more efficient digestion of organic matter, crude fiber, and ether extract (Taghinejad *et al.*, 2009).

Gamma irradiation-induced enhancement of organic matter content in *P. purpureum* cv. Mott can significantly improve its nutritional value. Organic matter is a source of energy, protein, and vitamins for livestock. Elevated organic matter can also enhance other nutritional components, such as crude fiber, fat, and protein (Owens *et al.*, 2010). Crude fiber is a vital component in livestock feed. It aids digestion, maintains gastrointestinal health, and prevents obesity (Mapato & Wanapat, 2018).

Gamma irradiation treatment has been shown to significantly impact the composition of *P. purpureum* cv. Mott, leading to the increased levels of ADF (acid detergent fiber), cellulose, and lignin while reducing the presence of hemicellulose. These research findings differ from those of Cheng *et al*. (2022), which indicated that a high dose of gamma irradiation can reduce the fiber fraction value. These changes are a result of several factors. Firstly, gamma irradiation can break chemical bonds within the plant cell walls, including those of lignin, cellulose, and hemicellulose, rendering these molecules smaller and more easily digestible by livestock (Aslaniyan *et al.*, 2023). Additionally, gamma irradiation-induced alterations in cell structure within *Pennisetum purpureum* cv. Mott facilitates the improved access for digestive enzymes to lignin, cellulose, and hemicellulose. Moreover, the heightened activity of digestive enzymes, prompted by gamma irradiation, contributes to the more efficient digestion of these components. Consequently, the increased ADF and cellulose contents hold implications for the enhanced crude fiber value and improved digestibility (Chand *et al.*, 2022). In contrast, the elevated lignin content may reduce nutritional value and enhance fiber digestibility (Raffrenato *et al.*, 2017). The decrease in hemicellulose content is associated with lower crude fiber value and improved fiber digestibility (Dilaga *et al.*, 2022). Notably, at a irradiation dose of 30 gray, the increased lignin content can reduce hemicellulose content due to the competitive relationship between these two components in the plant cell wall synthesis process (Mnich *et al.*, 2020).

The study emphasizes the importance of IR 15 treatment, emphasizing its unique features. Remarkably, IR 15 distinguishes itself by having the lowest lignin value, indicating its potential influence on feed quality (Firsoni *et al.*, 2019). Furthermore, the study shows that IR 15 exhibits improved nutritional digestibility, as indicated by higher values for parameters such as dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD), and nitrogen-free extract (NFE). The data indicate that IR 15 may have a crucial role in enhancing feed efficiency and nutrient utilization. IR 15 shows promise as a treatment for optimizing nutritional results in different agricultural contexts due to its reduced lignin content and increased digestibility measures. As we explore these results further, the potential uses and consequences of IR 15 in improving cattle nutrition and overall agricultural output become more evident.

The correlation analysis performed on nutrient data across various treatment levels provides significant insights into the intricate relationships among crucial nutritional elements within the stream environment. A direct association exists between dry matter and key nutritional components such as organic matter, NFE, and TDN (Darma *et al.*, 2023). Consequently, a rise in the amount of solid material present is linked to an improvement in the nutritional quality of nutrients that can be broken down and absorbed. Furthermore, a notable association is shown between the content of CP and fiber components such as DF and ADF. This association elucidates the connection between the protein and fiber content in the feed, offering useful information about the composition of the feed and its influence on the availability of nutrients (Adams *et al.*, 2018).

The heatmap serves as an effective visual representation, elucidating the intricate interactions among dietary components. The correlations among CPD, CFD, NFE-D, and NDFD further emphasize the interconnectedness within the system. These correlations suggest that alterations in one parameter may be associated with changes in the other parameters, highlighting the widespread interdependence among various nutritional aspects. The hierarchical correlation heatmap proves to be a powerful tool in unraveling the complexity of these relationships and contributes valuable information for understanding the dynamics of nutrient availability and digestibility in the studied context.

CONCLUSION

Overall, it can be concluded from this study that a gamma irradiation dose of 15 Gy resulted in a mutant (putative) forage plant, which donated better forage nutritional content of *Pennisetum purpureum* cv. Mott cultivated on acidic soil and improved its utilization efficiency for livestock due to the increased digestibility. This research provides insights into the potential and limitations of gamma irradiation in improving this plant's nutritional value and adaptability in marginal lands, potentially enhancing forage grass production in challenging agricultural landscapes.

CONFLICT OF INTEREST

The authors declare no apparent conflicts of interest or personal relationships that could potentially influence the presented findings in this research article.

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