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Evaluation of Super Napier (*Pennisetum purpureum* × *P. glaucum*) as Ruminant Fodder by *In vitro* and *In situ* Methods

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ABSTRACT

In India, the ruminant animal production faces challenges due to insufficient availability of feedstuffs and low-quality of forage crops. To address these issues, cultivating high-yielding, nutritious varieties like Super Napier (*Pennisetum purpureum* × *P. glaucum*) and conserving them is vital to sustain ruminant farming. Hence, an attempt was made to assess Super Napier (SN) hay as ruminant feedstuff by*in vitro* and *in situ* methods. Initially, SN hay was subjected to rumen *in vitro* gas production(RIVGP) study, with cumulative gas production (GP) measured at various time intervals up to 96 h post incubation. Subsequently, *in* vitro true dry matter digestibility (IVTDMD) and neutral detergent fiber digestibility (NDFD) were determined using a modified two-stage *in vitro* technique. Further, SN hay was subjected to a 96 h ruminal incubation to evaluate dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF) degradability using *in situ* method. The chemical analysis of SN hay revealed that it contains organic matter (OM)-86.9%, CP - 9.96%, NDF - 72.5% and acid detergent fibre (ADF)- 47.0% on DM basis. In the rumen kinetics study, the potential gas production (*D*) was 57.0 mL, with the rate of gas production (*c*) 0.035 h^{-1}. The IVTDMD and NDFD of SN hay were 68.7% and 40.5%, respectively. The potential *in situ* degradability and degradation rate for DM, CP, and NDF of SN hay were 68.5 % at 0.049 h^{-1}, 73.0% at 0.048 h^{-1} and 62.0% at 0.044 h^{-1}, respectively. Further, the estimated metabolizable energy (ME) of SN hay was 7.22 MJ/kg DM. Thus, SN hay with good amount of GP, *in vitro* DM and NDF digestibility and *in situ* DM, CP and NDF degradability can be considered as a good source of basal fodder feedstuff for ruminant production.

KEYWORDS: Chemical composition, *In vitro* digestibility, *In situ* degradability, Super Napier

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INTRODUCTION

India is currently grappling with a severe fodder crisis, exacerbated by challenges in producing sufficient feed and fodder for livestock amidst diminishing land resources, erratic monsoons, and drought-related shortages. With an 11.2% deficit in green fodder, a 23.4% shortage in dry fodder, and a 29% shortfall in concentrates (Roy et al., 2019), the Nation faces decreased livestock productivity compared to more developed countries. To address this, exploring high-biomass perennial fodder varieties is imperative. Napier Pakchong 1 (NP) grass, a hybrid napier (*Pennisetum purpureumand* × *P. americanum*) developed by Thailand's Nakhonratchasima Animal Nutrition Research and Development Center(Sarian, 2013), presents a promising solution, with 7.9% CP, 73.1% NDF, and 45.7% ADF on a DM basis (Cherdthong et al., 2015). Commonly known as Super Napier(SN), this fodder holds potential for mitigating feed scarcity. Limited data on the nutritional value of SN hay under Indian conditions necessitates this study, which aims to bridge the gap through *in vitro*and *in situ* evaluations of SN hay for ruminant feeding.

MATERIALS AND METHODS

Sample collection and preparation

The SN fodder samples were harvested at different intervals from the fodder plots maintained

at the Department of Livestock Farm complex, Veterinary College, Bangalore, Karnataka (13°01' 27.42" N, 77°35' 3.59" E; elevation 920 m). The mean annual rainfall of 830.1 mm, with maximum rainfall during the months of July and August, respectively. Mean minimum and maximum temperatures are 16.1 and 33.8°C. For hay preparation, SN fodder samples from 4 different plots were harvested at approximately 45th day of previous harvest, pooled and chaffed and sun dried until moisture of the forage reduced to 10-12%. In addition, samples of SN fodder at different growth stages viz., stage I (45 to 50 days), stage II (60 to 65 days) and stage III (75 to 80 days) were harvested, collected separately and processed. The samples were oven-dried at 60°C for 48 hours to determine their dry matter (DM), then milled to pass through a 1 mm sieve and preserved for subsequent analysis.

Chemical analysis

Forage samples were analyzed for their proximate composition as per AOAC (2005) and the fibre fractions were determined according to Van Soest et al. (1991).

***In vitro* Hohenheim gas test**

Air-equilibrated SN hay sample (200±10 mg) was incubated at 39°C for 96 hours with 30 mL mixed rumen inoculum (rumen buffer and rumen liquor in a 2:1 ratio) (Menke and Steingass, 1988), using 100 mL calibrated syringes in triplicate. Rumen liquor was collected from donor cow fed on roughage (Finger millet straw) and CFM based diet to meet nutrient (DM, CP and TDN) requirements for maintenance (ICAR, 2013). Cumulative GP was recorded at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 h of incubation. Data on GP was fitted to the following exponential equation

$$Y= a+b\ (1-e^{-ct})$$

Where, Y (mL),GPat time t; a (mL) is the initialGP; b (mL) =GP during incubation; a+b or D (mL) = potential GP; c (mL/h) is rate of GP.

The ME, *in vitro* organic matter digestibility (IVOMD) and short chain fatty acids (SCFA) of SN hay sample were determined according to Menke and Steingass (1988). Further, partitioning factor (PF) and microbial biomass production (MBP) were estimated according to Blummel et al. (1997) and Blummel (2000), respectively.

***In vitro* true dry matter digestibility (IVTDMD)**

and neutral detergent fibre digestibility (NDFD)

The IVTDMD and NDFD analysis was conducted according to Goering and Van Soest (1970) modification of Tilley and Terry (1963) in *in vitro* batch fermentor. Approximately 400 mg of dried SN hay sample was weighed into F57 Ankom Filter bags and incubated for 48 h in sealed Erlenmeyer flasks with a mixture of Mold's buffer and rumen fluid (4:1 ratio) in triplicate. After incubation, leftover residues in bags were treated with neutral detergent solution in an Ankom200 fiber analyzer, and the remaining dry residues were weighed. *Further,* the flask contents were transferred to centrifuge tubes, and centrifuged at 5000 rpm for 20 minutes at 4°C. The supernatant (800 ìl) was mixed with 25% metaphosphoric acid (200 ìl) and stored at -20°C for subsequent VFA analysis. The concentration of VFA were determined using a gas chromatograph (Filipek and Dvorak, 2009).

Protein fractions

The determination of various nitrogen (N) fractions including total N, buffer-insoluble N (BIN; Licitra et al., 1996), protease insoluble N (PIN; Krishnamoorthy et al., 1995; Licitra et al., 1998) and acid-detergent insoluble N (ADIN; Licitra et al., 1996) were done by Kjeldahl method according to AOAC (2005). The PIN which form the rumen undegraded N, was determined using commercial broad-spectrum protease of *Streptomyces griseus* (type XIV, Sigma P-5147, St Louis, MO, USA). About 0.5 g of SN hay sample was incubated in 40 mL of borate-phosphate buffer (pH 7.8 to 8.0) for 1 h in 125 mL of Erlenmeyer flask. Further, the content in flasks were incubated with 10 mL of *Streptomyces griseus*protease solution containing 330×10^{-3} units/ mL for 48 h with intermittent shaking. Afterwards,

the contents were filtered through Whatman No. 54 filter paper and the filter paper with residue was transferred to Kjeldahl digestion tube for N estimation, which was assumed to be rumen undegradable protein (Licitra et al., 1998).

***In situ* evaluation**

In the *in situ* procedure (Singh et al., 1995), 5 g of SN hay sample (1 mm size) was placed in triplicate bags, sealed with plastic ties, and subjected to ruminal incubation at intervals of 0, 1, 3, 6, 9, 12, 24, 48, 72, and 96 h. Each incubation time had three bags anchored to a weight, suspended in the rumen in reverse order, and removed simultaneously. Following removal, the bags underwent washing and were subsequently dried at 60°C for 48 h. Residual DM, CP and NDF were quantified (AOAC, 2005). The exponential model of nonlinear regression (Orskov and McDonald, 1979) was employed to determine soluble or rapidly degradable fraction (a), insoluble but potentially degradable fraction (b), the rate of degradation(k_d), potential degradability [$Y=a+b(1-e^{-kd\times t})$], effective degradability [$P=a+b(K_d/K_d+K_p)$] with Kp as the rate of passage (0.056/h for DM, CP, and NDF).

All analyses in the study were conducted at least three times in triplicate, and the reported values are presented as mean ± standard error of the mean. RIVIGP data were analyzed for gas kinetics using GraphPad Prism software (Version 8.0.2, 2019). *In situ* nutrient degradation was assessed using the exponential model of nonlinear regression in the same software, providing insights into both degradation rate and extent.

RESULTS AND DISCUSSION

The chemical composition of SN forage varies depending on the stage of cutting(Table 1). The SN grass harvested at an early stage (Stage I) contained lower dry matter (DM) and subsequently harvest contained higher DM. As the SN plant matures, the moisture content decreases leading to an increase in DM content, as evident from the higher DM content in the later stages of cutting (Stage II and III). Concurrently, the crude protein (CP) content decreases as the grass matures. This trend is supported by the findings of Wadi et al. (2004) and Molla et al. (2018), who observed that the CP content decreases with an increased age at harvest. The reduction in total ash content with advancing stages of cutting is also in line with findings of Diriba and Vaars (2000) and Molla et al. (2018). This reduction can be attributed to natural processes, such as mineral translocation to the roots and dilution within the plant associated with maturation. Moreover, the mineral content of the plant is influenced by several factors, including the type of soil and its nutrient content, as well as environmental conditions (Molla et al., 2018). However, with increasing maturity, the fibrous carbohydrate content including NDF and ADF tends to increase. This is because, as the grass matures, the prominence of cell walls increases leading to a higher fiber content. The observed differences in chemical composition at various stages of cutting underscore the importance of selecting the optimal harvest time to achieve the desired nutritional properties. As the plant ages, there is a notable increase in cell wall components while CP content decreases, ultimately impacting the digestibility of the forage. Therefore, choosing the right stage of harvest is crucial to obtain forage with optimal nutritive value and digestibility.

In this context, high-quality SN hay was obtained by harvesting at the optimum age, around 45 to 50 days. On dry matter basis, SN hay contained approximately 9.96% CP, 1.92% EE, 13.1% TA, 72.5% NDF, 47.0% ADF and 6.99% acid detergent lignin (ADL). The CP, NDF and ADF concentration in SN hay obtained in the present study was higher than the reported values (Ravindra et al., 2021). The CP, NDF and ADF concentration in SN hay obtained in the present study were lower than the reported values (12 % CP, 80.12 % NDFand 49.50 % ADF) (Sravani et al., 2021).The chemical constituents such as CP, NDF and ADF of SN hay prepared in our study were consistent with the research findings of Liangco et al. (2019). The NDF and ADF values for SN hay obtained in our study were in line with the values reported by Cherdthong et al. (2015)

(73.1% NDF and 45.7% ADF) while the CP content (7.9%) reported was lower. The CP content at 9.96%, slightly exceeds the minimum CP requirement of 60 to 80 g/kg for sustaining rumen microbial growth and normal rumen functioning, as suggested by Weiss et al. (1992) and Van Soest (1994). The variation in chemical composition observed in different studies can be attributed to several factors such as types of soil, climatic condition, growth conditions, agronomical practices and stage of harvest.

Table 1. Chemical composition of Super Napier at different stages of harvest and its hay

Nutrient	Stage I (45-50 days)	Stage II (60 -65)	Stage III (70-85 days)	Super napier hay[1]
Dry matter	15.0 ± 0.02	16.7 ± 0.04	19.2 ± 0.09	88.0 ± 0.01
Organic matter	85.2 ± 0.04	85.6 ± 0.04	86.5 ± 0.07	86.9 ± 0.08
Crude protein	8.98 ± 0.02	6.73 ± 0.04	4.27 ± 0.03	9.96 ± 0.06
Ether extract	2.05 ± 0.02	1.98 ± 0.03	2.01 ± 0.02	1.92 ± 0.02
Total ash	14.8 ± 0.04	14.4 ± 0.03	13.5 ± 0.02	13.1 ± 0.03
Acid insoluble ash	6.01 ± 0.02	5.52 ± 0.02	5.45 ± 0.03	5.10 ± 0.10
Neutral detergent fibre	73.1 ± 0.01	75.6 ± 0.02	81.9 ± 0.05	72.5 ± 0.05
Acid detergent fibre	49.2 ± 0.01	51.5 ± 0.07	55.1 ± 0.07	47.0 ± 0.08
Acid detergent lignin	6.81 ± 0.01	7.15 ± 0.01	7.78 ± 0.07	6.99 ± 0.06
Cellulose	42.4 ± 0.05	44.3 ± 0.03	47.3 ± 0.03	40.0 ± 0.01
Hemicellulose[2]	23.9 ± 0.05	24.2 ± 0.01	26.8 ± 0.06	25.5 ± 0.06
TCHO[3]	74.2 ± 0.02	76.9 ± 0.04	80.2 ± 0.03	76.0 ± 0.05

1SN hay was prepared with fodder harvested at 45 -50 days
2Hemicellulose = NDF – ADF; 3TCHO (Total carbohydrates) = 100 – (CP+EE+Ash)

The total N available to animals from SN hay, both in the rumen and the intestine, was found to be 85.3% (Table 2). Notably, SN hay contains 14.7% of its total N in the form of ADIN, which is lower compared to the reported value for Napier grass by Krishnamoorthy et al. (1995).This percentage is, however, higher than what's typically found in protein sources. The elevated ADIN levels in roughages compared to grains and protein supplements are likely attributed to the presence of lignin and tannin protein complexes (Van Soest, 1982). It's important to note that tropical forages such as SN hay tend to be more fibrous than temperate forages. Consequently, a larger portion of their N content is not readily available to ruminants due to its binding within indigestible vascular bundles (Van Soest, 1982).

Table 2. Protein fractions of Super Napier hay

CP, %	N fraction, % Total N				Available N, %				
					Total	Rumen, % RDN		Intestine	
	ADIN	BIN	PIN	RDN		Rapid	Slow	% total N	% of PIN
9.96 ± 0.06	14.7 ± 0.93	63.8 ± 0.05	42.6 ± 0.05	57.5 ± 0.03	85.3 ± 0.04	29.7 ± 0.06	37.0 ± 0.05	27.9 ± 0.06	65.5 ± 0.07

ADIN, Acid detergent insoluble nitrogen; BIN, Buffer insoluble nitrogen; PIN, Protease insoluble nitrogen; RDN, Rumen degradable nitrogen

Regarding the composition of N in SN hay, BIN makes up 63.8% of the total N. Within BIN, variable proportions are distributed between protease soluble and protease insoluble fractions. The protease soluble N (BIN-PIN) accounts for 21.2% and represents the slowly degradable N. Notably, this value is lower compared to what was reported for hybrid Napier grass (Krishnamoorthy et al., 1995).A significant portion of N in SN hay is protease insoluble (PIN), comprising 42.6% of the total N. When considering the total available N (85.3% of the total N), the rumen degraded N (RDN) makes up approximately 57.5%. Within RDN, the slow-degraded fraction constitutes about 37.0%, while the rapidly degraded fraction is about 29.7%. In the context of indigestible N, ADIN serves as an estimate. By subtracting this fraction from PIN, we can estimate the rumen undegradable nitrogen (UDN) that is digestible in the intestine. In the case of SN hay, the UDN digestible at the intestine varies and is estimated to be approximately 27.9% of the total N or 65.5% of the PIN.

GP techniques provide vital insights into feed degradation kinetics in the rumen, which are crucial for evaluating nutritional value. This technique allows for the continuous monitoring of GP at various incubation intervals, making it a valuable tool for studying fermentation kinetics. In this context, data pertaining to GP kinetics of SN hay is presented in Table 3. The *D* for SN hay was 57.0 mL and $t_{1/2}$was 19.9 h. Furthermore, *c* was 0.035 h^{-1}. GP from the soluble fraction (*a*) and the insoluble fraction (*b*) was measured at 2.92 mL and 54.1 mL, respectively. The value of *a* observed in the present study was consistent with the findings of Okoruwa and Igene (2014), where *a* was reported as 3 mL. However, the observed *b* and $t_{1/2}$ were higher as compared to those reported by Okoruwa and Igene (2014), where *b* was 27 mL, and $t_{1/2}$ was 18 h. This discrepancy in $t_{1/2}$ can be attributed to the lower rate of degradation (c), which was measured at 0.035 h^{-1} in the present study.

Relying solely on chemical analysis is insufficient to fully comprehend the nutritional value of animal feedstuff. To gain a more comprehensive understanding of its degradability and utilization, the RIVGP technique proves to be a potent tool for evaluating a feed's true worth, surpassing its chemical composition. The data on the *in vitro* gas profile of SN is summarized in Table 3. It was observed that the GP at 24 h for SN hay was 32.0 mL/0.2 g DM. Additionally, IVOMD, SCFA, MBP and PF for SN hay were measured at 56.1%, 0.71 mmol/ 0.2 g DM, 209.2 mg and 3.51, respectively. The PF values obtained in this study fell within the theoretical range of 2.75 to 4.41 (Blummel et al., 1997). The observed IVOMD for SN hay was comparable to the IVOMD reported for hybrid napier at 6 weeks of age (Weerathunga et al., 2023). The observed IVOMD for SN hay was comparable to the IVOMD reported for hybrid napier at 6 weeks of age (Weerathunga et al., 2023). The estimated ME value for SN hay, at 7.22 MJ/kg DM, was within the range of ME values reported by Garg et al. (2012) for various hybrid napier strain (PBN233) under tropical conditions (6.99 to 7.66 MJ/kg DM), although it was slightly lower than the ME reported for hybrid napier at 6 weeks of age (Weerathunga et al., 2023). These variations in energy value seem to be associated with differences in GP volume and chemical composition (CP, EE, Ash) of the feedstuff since the formula for estimating ME takes these individual chemical constituents into account.

The IVTDMD and NDFD of SN hay were 68.7% and 40.5%, respectively (Table 4). The IVTDMD of SN hay was found to be comparable to values reported in certain studies (Bhatta et al., 2016; Turano et al., 2016). Additionally, the NDFD was in line with findings for the hybrid napier variety PMN2 (38%) as reported by Turano et al. (2016). The IVTDMD observed in the present study was similar to observation for *in vitro* dry matter digestibility of other grass hays, as reported by Nelson et al. (1972). Moreover, the observed TVFA at 48 h of incubation was 16.9 mM (Table 4). The ratio of acetate, propionate, and butyrate was within the range (75:15:10 to 40:40:20) typically produced in the rumen, as suggested by Bergman (1990).

Table 3. *In vitro* rumen fermentation profile and ME value of Super Napier hay

Parameters	Mean ± SE
a (mL)	2.92± 0.70
b (mL)	54.1 ± 0.98
D (mL)	57.0 ± 1.58
c (h^{-1})	0.035± 0.01
$t_{1/2}$	19.9 ± 0.71
GP 24 h (mL/0.2 g DM)	32.0 ± 0.13
IVOMD (%)	56.1 ± 0.11
MBP (mg)	209.2± 0.27
PF	3.51± 0.01
SCFA (mmol/0.2g DM)	0.71 ± 0.01
ME (MJ/kg DM)	7.22± 0.02

a, Gas production from soluble fractions;b, Gas Production from insoluble fractions; *D*, Potential Gas production;c, rate constant; $t_{1/2,}$ half life GP_{24h}, GP for 24 h; MBP, Microbial biomass production; PF, Partitioning factor; SCFA, Short chain fatty acids; IVOMD – calculated *in vitro* organic matter digestibility; ME – Metabolisable energy

It was observed that the DM degradability of SN hay reached 68.7% after 96 h of incubation. The *Y* for DM was 68.5%, with a *k* of 0.049 h^{-1}(Table 5). The *P* for DM was 41.9% at a passage rate of 0.056/ h. The rumen undegradable DM (100- *P*) in SN hay was 58.1%. The *a* for DM fraction accounted for approximately 18.7%, while the *b* constituted roughly 49.8%. The *Y* for DM observed in this study was lower than the values reported by Jagadeesh et al. (2017), who found a *Y* for DM of 75.3 % for hybrid napier at 45 days and Ningal (2020), who reported a *Y* for DM of 69.9% for napier in goats.

Table 4. *In vitro* rumen fermentation parameters, true dry matter digestibility and neutral detergent fibre digestibility of Super Napier hay

Parameters	Mean ± SE
IVTDMD (%)	68.7 ± 0.54
NDFD (%)	40.5 ±0.81
TVFA (mM)	16.9 ± 1.17
Acetate (%)	58.4 ± 0.57
Propionate (%)	24.9 ± 0.29
Butyrate (%)	13.0 ± 0.08
Isobutyrate (%)	0.58± 0.004
Valerate (%)	2.05 ± 0.02
Isovalerate (%)	1.13± 0.01

IVTDMD – *In vitro* true dry matter digestibility measured at 48 h incubation; NDFD – Neutral detergent fibre digestibility measured at 48 h incubation; TVFA –Total volatile fatty acids measured at 48 h incubation

Table 5. *In situ* rumen degradability of DM, CP and NDF of Super Napier hay

Variables	Degradability (%)		
	DM	CP	NDF
a	18.7 ± 0.58	22.8 ± 0.50	0.64± 0.15
b	49.8 ± 1.08	50.2 ± 0.71	61.4 ±1.01
K_d (h^{-1})	0.049± 0.001	0.048± 0.005	0.044± 0.001
Y	68.5 ± 0.55	73.0 ± 0.71	62.0 ± 0.92
P	41.9 ± 1.03	45.9 ± 1.10	27.6 ± 1.21

a= soluble or rapidly degradable fraction; *b*= insoluble but potentially degradable fraction; k_d=0.693/$t_{1/2}$, degradation rate (h^{-1}); [*a*, *b* and k_d are based on nonlinear regression using the exponential model]; *Y*, potential degradability at time *t*, *Y*=a+b ($l-e^{-kd*t}$); Effective degradability, $P= a+b(K_d/K_d+K_p)$ [K_p is the rate of passage, taken as0.056/h]

In terms of CP degradability, assessed over the same incubation periods, it was noted that CP degradability at 96 h in SN hay reached 74.8% out of 9.96% CP content, indicating an 8.24% breakdown of protein components after 48 h. The *a* of CP fraction in SN hay accounted for around 22.8%, while *b* was 50.2%(Table 5). Rumen undegradable CP (100- *P*) in SN hay amounted to 54.1%, higher than what was observed in the *in vitro* protein fractionation system of SN hay. The *Y* for CP stood at 73.0% with a *k* of 0.048 h^{-1}, and *P* was 45.9% at a passage rate of 0.056/h. These values were higher compared to the results of Chiou et al. (1995), who reported a 40% degradability in napier grass, and Jagadeesh et al. (2017), who found a 36.7% degradability in hybrid napier.

In the case of NDF degradability, assessed across the same incubation periods, the NDF degradability in SN hay reached 61.7% after 96 h of incubation. The*a* of NDF fraction in SN hay accounted for around 0.64%, while the *b* constituted approximately 61.4%. The *Y* for NDF was 62.0 % with a k of 0.044 h^{-1}, and the *P* was 27.6% at a passage rate of 0.056/ h(Table 5). These values were higher compared to the results of Ningal (2020), who reported a 24.8% and 12.7% NDF degradability in napier grass at passage rates of 0.02 h^{-1} and 0.05 h^{-1}, respectively.

CONCLUSION

The results of chemical composition analyses and *in vitro* and *in situ* degradability studies indicated that Super Napier hay could be a promising feed resource for ruminants during lean season. Additionally, its outstanding yield capacity, positions it as an ideal choice for farmers seeking to elevate their livestock nutrition and productivity.

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