

Article History

Article # 24-663

Received: 14-Jun-24

Revised: 13-Sep-24

Accepted: 15-Sep-24 Online First: 20-Oct-24

RESEARCH ARTICLE

Assessing the Potential of Indigenous Kikuyu Grass (*Pennisetum clandestinum*) as High-Quality Equine Forage from Different Accessions in Tropical Regions

Mansyur ¹*, Nyimas Popi Indriani ¹, Windu Nagara ², Dimar Sari Wahyuni ², Satria Maulana², Setiawan Martono², Herdis ², Karen J. Harper³, Nabila Fara Dega Rifianda¹, Vincent Niderkon⁴ and Yulianri Rizki Yanza ¹

¹Department of Animal Nutrition and Feed Technology, Faculty of Animal Husbandry, Padjadjaran University, Jatinangor, Sumedang, 45363 Indonesia

²Research Center for Animal Husbandry, Research National Research and Innovation Agency, Bogor, Indonesia
 ³School of Health, Medical and Applied Sciences, Central Queensland University, Rockhampton, Qld, 4702, Australia
 ⁴INRAE, VetAgro Sup, UMR Herbivores, Université Clermont Auvergne. F-63122 Saint-Genès-Champanelle, France
 *Corresponding author: mansyur@unpad.ac.id

ABSTRACT

Tropical small farmers face forage shortages during the dry season, impacting livestock productivity. C4 grasses in tropical regions, including Indonesia, offer high biomass production and drought resistance, though they pose nutritional challenges. Kikuyu grass (Pennisetum clandestinum), native to Africa, shows potential as a high-protein forage feed despite its high oxalic acid content, which can hinder mineral absorption. This study evaluated the biomass and nutritive value of three indigenous Kikuyu accessions in tropical conditions, aiming to promote its use among smallholder farmers. Three Kikuyu accessions (KBB, KTP, KBT) were planted at 1300 m elevation. The experimental design involved daily irrigation, fertilizer application, and sampling for biomass production and nutrient analysis. Dry matter, organic matter, crude protein, and fiber fractions were measured, along with mineral content and energy values. Results showed significant differences in leaf and stem proportions, with KBT having the highest leaf percentage. KTP had the highest dry matter yield. Nutrient analysis revealed higher crude protein and energy values in leaves, while stems had higher fiber content. Digestibility rates varied, with KBT showing the highest digestible energy. The study highlights the potential of Kikuyu grass, especially KBT and KBB accessions, as high-quality forage for equines in tropical regions. Factors such as defoliation schedule, fertilizer treatment, and soil conditions are crucial for optimizing forage quality. Promoting Kikuyu grass can improve livestock productivity for smallholder farmers in the tropics.

Keywords: Indigenous Kikuyu, Forage, Production, Nutrient content, Equine.

INTRODUCTION

Tropical small farmers often experience a shortage of forage during the dry season, which results in malnutrition and reduced livestock productivity due to a lack of adequate pasture land. This issue was highlighting the challenge of forage scarcity for tropical farmers due to long dry seasons, which affects production of livestock animals (Ngongo et al., 2021). Meanwhile grasses in tropical country like Indonesia, they are commonly classified as C4 grasses and widely recognized for their exceptional biomass production capabilities. These grasses possess a unique leaf anatomy that enables more efficient carbon fixation and generates higher concentrations of carbon compounds, such as oxaloacetic acid (Niyogi et al., 2015). In addition, C4 grasses exhibit superior water and drought resistance compared to C3 grasses (Marais, 2001). Their advantageous characteristics regarding plant productivity and adaptability may be more significant than the drawbacks related to their nutrient quality (Serrapica et al., 2019) and antinutritive compounds (Marais, 2001).

Cite this Article as: Mansyur, Indriani NP, Nagara W, Wahyuni DS, Maulana S, Martono S, Herdis, Harper KJ, Rifianda NFD, Niderkon V and Yanza YR, 2024. Assessing the potential of indigenous kikuyu grass (*Pennisetum clandestinum*) as high-quality equine forage from different accessions in tropical regions. International Journal of Agriculture and Biosciences 13(4): 701-708. https://doi.org/10.47278/journal.ijab/2024.173



A Publication of Unique Scientific Publishers

eISSN: 2306-3599; pISSN: 2305-6622

Kikuyu grass (Pennisetum clandestinum) is a plant species belonging to the Pennisetum family, an exceptional and unique plant with distinct characteristics. It is native to Africa, and several research studies conducted by García et al. (2014) and Royani et al. (2021) have confirmed the potential of Kikuyu as forage feed. This grass is highly regarded and widely used in pastures, sports fields, public spaces, and golf courses (Shahrivar et al., 2019). Kikuyu grass boasts a substantial percentage of CP (228g/kg DM) (García et al., 2014), surpassing the protein content of other tropical grasses. However, caution is warranted regarding mineral deficiencies when feeding Kikuyu grass to livestock because of its high oxalic acid content. This antinutritional substance can bind calcium, magnesium, and iron in both soluble and insoluble forms (Rahman et al., 2013). Importantly, oxalate salts can form an insoluble chelate complex with dietary calcium, potentially reducing its availability and absorption in the gut (Marais, 2001).

Compared to common tropical grasses like elephant and dwarf elephant grasses, The Kikuyu is underutilized as forage feed, especially for equine animals. The lack of information on its possible nutritional content, the scarcity of superior genetics, and its emerging biomass production needs to be examined. Despite the known nutritional challenges of C4 grasses in tropical regions, Kikuyu grass, demonstrates significant potential as a high-protein, digestible energy forage for equines and potentially other livestock (García et al., 2014; Charry et al., 2020; Royani et al., 2021). Therefore, the present study was conducted with the aim of evaluating the biomass production and nutritive value of three indigenous Kikuvu accessions in tropical conditions. The objective of this study was to investigate the potential of Kikuyu grass in different accessions as high-quality forage for equines, particularly in tropical countries like Indonesia. The study presents an innovation in promoting this African-native grass for regions like Indonesia, showing its adaptability and resilience, especially under drought-prone condition. This study was conducted in order to helps address forage shortages faced by small farmers, especially at tropical environment in optimizing forage quality by adjusting factors such as defoliation schedule, fertilizer application, and soil conditions to enhancing overall livestock productivity for smallholder farmers in the tropics.

MATERIALS & METHODS

The investigated Kikuyu Grass (*Pennisetum clandestinum*), comprising three accessions sourced from three distinct locations, was planted in an area at an elevation of 1300m above sea level. The average daily temperature and humidity were 23.85°C and 75.44%, respectively. The average daily precipitation during the experimental period fluctuated between 3.37 and 17.95mm.

Treatments and Variables

Three separate accession samples of *Penissetum* clandestinum (KBB, Burangrang accession; KTP, Tangkuban Perahu accession; and KBT, Bukit Tunggul accession) were evaluated over three distinct harvest periods. The grasses

were planted in an area measuring 3000m², with each plot measuring 50m² arranged in a randomized complete block design (accession and plant fraction). The grass was irrigated daily with a water sprinkler. Fertilizer application was carried out post-harvest using 11tons of sheep manure, 300kg of urea (46.6% N; PT. Petrokimia Gresik, Indonesia), and 200kg of NPK (16% N, 16% P, and 16% K; Yaramila®, PT. Yara Indonesia) per hectare.

We collected samples from five locations (0.5 square meters each) in each plot, four from the corners and one from the center, using a quadrant frame, and then weighed them. Annual biomass production was calculated from the yield data. Yield data were converted into dry matter (DM) production in kg/m² and tons/ha/year. Samples were separated into leaf and stem fractions (L/S) following the methodology described by Acero-Camelo et al. (2020). Fractionated samples from each collection spot were combined to create composite samples for further laboratory analysis.

Sample Measurements and Analysis

The dried samples were ground to a size of 1mm following their drying process in an oven at 55°C for three days. Analysis of dried matter (DM) was performed according to protocol SNI no 01-2891-1992. The sample was dried in an oven (Memmert UFE500, Memmert Unk +Co. KG, Schwabach, Germany) at 105°C for three hours. The organic matter (OM) of the samples was measured by tanning at 600°C for two hours in a Thermolyne FB 1400 furnace (Thermo Fischer Scientific, Massachusetts, USA). The disappeared matter from the tanning process is referred to as OM (Latimer, 2012). The crude protein (CP) content of each dried sample was analyzed in accordance with AOAC method no 2011.11 (Latimer, 2012) using a FoodALYT D3000 destructor and FoodALYT TS10 titration station (Omnilab Laborzentrum GmbH+Co. KG, Bremen, Germany). The fiber fraction such as neutral detergent fiber (NDF), acid detergent fiber (ADF), crude fiber (CF), and ether extract (EE) were analyzed using the ANKOM A200 fiber analyzer and ANKOM XT10 Extractor, following the manufacturer's protocols provided by ANKOM Technology, Macedon, NY, USA.

For the Hemicellulose content of samples, they were calculated by subtracting NDFa from ADF, while cellulose was calculated from ADF minus lignin. The nutrient quality of the whole plant was determined based on the L/S ratio for each accession. Mineral content analysis was conducted at IPB University laboratory using a Shimadzu AA-6880 atomic absorption spectrometer (Shimadzu Corp, Kyoto, Japan) in conjunction with the wet ashing method for sample preparation, as per Marshall (2010). The assessment included Ca, P, Mg, S, Fe, Zn, and Co. Meanwhile for the non-fibrous carbohydrate (NFC) was calculated based on the findings of Chavez et al. (2014) as follows:

NFC= 100% - (CP (%) + NDF (%) + EE (%) + ash (%) Gross energy (GE) in the samples was determined using the bomb calorimeter model CC01/M2, following the manufacturer's instructions provided by Toshniwal Technologies Pvt. Limited, New Delhi, India. Moreover, metabolic energy of each sample was calculated using the equations outlined by Kienzle and Zeyner (2010). For the digestible energy (DE) of each sample, it was calculated using the formula based on the guidelines provided by the National Research Council (NRC, 2007), as follows:

DE= 2,118 + 12.18 × CP (%) – 9.37 × ADF (%) – 3.83 × hemicellulose (%) + 47.18 × EE (%) + 20.35 × NFC (%) – 26.3 × ash (%)

Meanwhile for the percentages of nitrogen free extract (NFE) and total digestible nutrient (TDN) were calculated using equations as described by Detmann & Valadares Filho (2010), as follows:

NFE=100-((100-OM) + CP + EE + CF); TDN= 5.31 + 0.412 × CP (%) + 0.249 × CF (%) + 1.444 × EE (%) + 0.937 × NFE (%)

In vitro Evaluation through DAISY Experiment using Equine Faecal

The present DAISY *in vitro* digestibility evaluation was performed following Earing et al. (2010) protocol, performed using the Daisy II ANKOM (ANKOM Technology, New York, USA) method, where equine feces as the inoculum media. For instance, Two ANKOM buffer solutions (buffers A and B) were pre-warmed at 39°C and mixed in a 1:5 ratio. The fecal samples, as inoculants, were freshly collected in the afternoon from Sandalwood Equines and then transferred into a warmed insulated thermos jar under anaerobic conditions (CO₂ purged). The jars were transported to the laboratory at 39°C in a warm-insulated container.

Fecal samples were subsequently combined and mixed with two parts (w/v) of ANKOM buffer using a blender. The blended inoculant and buffer were then transferred into the digestion vessel, flushed with CO_2 for 15s, and placed in a Daisy II incubator at a temperature of 39°C. Ground Kikuyu samples were accurately weighed (approximately 0.5g) and sealed inside an F57 filter bag (ANKOM Technology, New York, USA). These bags were then incubated anaerobically in the Daisy II incubator for 48h in order to obtain the *in vitro* digestibility estimation may closely be similar to the *in vivo* value (Tassone et al., 2019). Furthermore, the filter bags were washed with fresh water until they were clear and then dried after the incubation process was completed, before being analyzed for DM, OM, and NDF, as described in the chemical analysis.

Statistical Analysis

Collected data were statistically analyzed using the randomized complete block design (RCBD) analysis whereas accession and plant fractions serving as main factors. The data structure for biomass production, mineral content, and in vitro digestibility only had accession as a single factor in the RCBD analysis. Statistical analyses were conducted using SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Between accessions were considered significantly different at P<0.001 (**), and most significantly different at P<0.001 (***). Furthermore, the analysis was continued with using Tukey-Kramer's test in interpreting the different superscript letter.

RESULTS

In the present study, the leaf's proportion of KBT was significantly higher than the KTP (P<0.05), whereas the KBB and KTP were considered similar (Fig. 1A). However, the proportion of stems was statistically similar between accessions. Moreover, the descriptive results (Fig. 1B and 1C) of Kikuyu grass overall dry matter (DM) production from KBB accession was 0.32kg/m² or 28.94 tons/ha/year. However, the highest average DM production was found in the KTP accession at 0.38kg/m² or 34.22ton/ha per year.

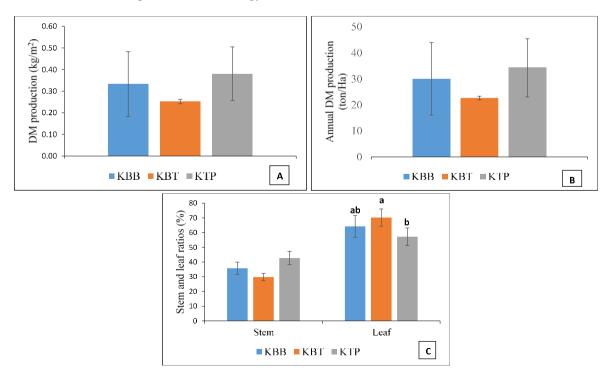


Fig. 1: Dry matter production per harvesting (A) and per year (B), and stem:leaf ratios (C) of three accession of Kikuyu in Indonesia.

Proximate analysis of the plant accessions revealed that the OM proportion was significantly higher in KBT than in KBB accessions (P<0.05; Table 1). No significant differences were observed in the other nutrient parameters among the accessions. However, the leaves fraction of Kikuyu grass was significantly higher in CP, EE, and TDN proportion than the stem fraction (P<0.0001). In contrary, the CF and NFC proportion in stem of Kikuyu grass from all accessions was significantly higher in than the leaves (P<0.0001). Furthermore, gross energy (GE), digestible energy (DE), and metabolizable energy (ME) content in leaves were higher than the stem (P<0.001). However, only the DE content showed a significant difference among accessions whereas the KBT considered as the highest (8.61MJ/kg DM).

The results of the fiber fraction analysis revealed distinct differences in the levels of ADF, cellulose, and lignin between the plant accessions (P<0.05), with no interaction effect between plant fraction and accessions (Table 2). Specifically, the KBB accession exhibited significantly lower levels of ADF and cellulose, whereas the lowest lignin level

was in the KBT accession. Moreover, variations in the concentrations of ADF, cellulose, within the stem in each accession was also higher than the leaves (P<0.0001), whereas silica content in leaves was higher than the stem in each accession (P<0.0001). Moreover, the KBB possess lower cellulose and ADF content than the other accessions. Additionally, there were no discernible variations between the three Kikuyu grass accessions in terms of their DM, OM, and NDF invitro digestibility values (Table 2).

DISCUSSION

Biomass Production and Nutrient Content of Kikuyu Grass

Biomass production is a pivotal determinant in the quest for high-quality forage and represents the culmination of intricate biochemical processes within plants. Through photosynthesis, plants adeptly transmute humble substrates, such as carbon dioxide, water, and various inorganic elements, into a rich array of organic compounds, predominantly comprising carbohydrates,

Table 1: The mean value of chemical composition and energy in three Kikuyu accessions and plant fractions

Chemical composition		KBT		KTP		KBB		Accessio	n	SEM		P-Val	ues
	Leaf	Stem	Leaf	Stem	Leaf	Stem	KBT	KTP	KBB		А	F	AxF
DM (%)	27.02	25.12	27.79	24.55	29.69	25.35	26.12	26.02	27.72	0.83	NS	NS	NS
				Nutrient co	omposition	(%DM)							
OM	90.44	90.04	89.62	87.97	89.8	89.65	90.62 ^B	88.93 ^{AB}	89.67 ^A	0.22	*	NS	NS
CP	22.38 ^b	15.8ª	21.9 ^b	16.5ª	23.45 ^b	17.97ª	20.28	19.56	21.58	0.56	NS	***	NS
CF	22.61ª	27.76 ^b	23.76 ^a	27.50 ^b	21.53ª	29.97 ^b	24.38	25.48	24.23	0.78	NS	***	NS
EE	2.82 ^b	1.56ª	2.96 ^b	1.2ª	3.02 ^b	1.45ª	2.41	2.20	2.49	0.15	NS	***	NS
NFE	42.63	44.92	41	42.76	41.81	40.26	43.54	41.68	41.37	0.64	NS	NS	NS
NFC	8.52ª	12.62 ^b	7.77ª	11.18 ^b	8.56 ^a	11 ^b	9.96	9.09	9.68	0.52	NS	*	NS
TDN	64.18 ^b	63.07 ^a	62.93 ^b	60.76 ^a	63.86 ^b	60 ^a	64.03	61.95	62.59	0.51	NS	*	NS
			Ene	rgy conten	t (Mega Jo	ule/kg DM))						
GE	16.49 ^b	14.3ª	16.53 ^b	14.06 ^a	15.46 ^b	14.31ª	15.95	15.59	15.18	0.20	NS	***	NS
DE	8.72 ^b	8.27 ^a	8.53 ^b	7.87 ^a	8.78 ^b	8.2ª	8.61 ^B	8.23 ^A	8.61 ^B	0.08	*	**	NS
ME	7.99 ^b	6.94ª	7.66 ^b	6.48 ^a	8.08 ^b	6.27ª	7.68	7.14	7.48	0.17	NS	***	NS

KBT=Bukit Tunggul; KTP=Tangkuban Perahu; KBB=Burangrang; DM=Dry matter; OM=Organic matter; CP=Crude protein; CF=Crude fibre; EE=Extract ether. NFE=N-free extract. NFC=Non-Fibrous Carbohydrate. TDN=Total Digestible Nutrient; GE=Gross Energy DE=Digestible Energy. ME=Metabolism Energy. Different superscript letters in the same column are showing significant differences. *=P<0.05, **=P<0.001, ***=P<0.0001; NS=not significant; A=Accession, F=Plant Fraction, AxF=Interaction between Accession and Plant Fraction.

Table 2: The mean proportion of fibre fractions in different accessions and plant fractions of Kikuyu grass

Fibre Fraction content (%)	KB		вт ктр		KBB			Accessions		SEM	P-Values		
	Leaf	Stem	Leaf	Stem	Leaf	Stem	KBT	KTP	KBB		А	F	AxF
NDFa	56.83	60.05	56.99	59.09	54.78	59.23	57.96	58.07	55.91	0.56	NS	NS	NS
NDFom	56.72	59.99	56.88	59.01	54.66	59.14	57.88	57.97	55.81	0.57	NS	NS	NS
ADF	28.50ª	33.70 ^b	29.78ª	34.43 ^b	28.30 ^a	32.93 ^b	30.34 ^{AB}	31.93 ^B	29.86 ^A	0.75	*	***	NS
Hemicellulose	28.22	26.36	27.20	24.66	26.47	26.29	27.62	26.14	26.06	0.34	NS	NS	NS
Cellulose	23.94ª	29.12 ^b	24.29 ^a	29.01 ^b	23.57ª	27.62 ^b	25.76 ^{AB}	26.43 ^B	25.01 ^A	0.70	*	***	NS
Lignin	4.57	4.58	5.49	5.42	4.73	5.31	4.58 ^A	5.50 ^B	4.85 ^{AB}	0.14	*	NS	NS
Silica	0.18 ^b	0.11ª	0.18 ^b	0.11ª	0.17 ^b	0.11ª	0.16	0.15	0.15	0.01	NS	***	NS

KBT= Bukit Tunggul; KTP= Tangkuban Perahu; KBB= Burangrang. NDFa= Neutral Detergent Fibre (amylase). NDFom= Free ash NDF. ADF= Acid Detergent Fibre. Different superscript letters in the same row are showing significant differences. ***=P<0.0001; NS=not significant; A=Accession, F=Plant Fraction, AxF=interaction between Accession and Plant Fraction. Same remarks as above.

Table 3: Macro and micro minerals content of three indigenous accession Kikuyu in Indon	iesia

Minerals		Accession	าร	Mean	SEM	P values	
	KBT	КТР	KBB				
			Macro minerals (g/	kg DM)			
Ca	3.3	3.5	3.6	3.5	0.11	NS	
Р	1.6	1.7	1.7	1.7	0.05	NS	
Mg	1.3	1.3	1.2	1.3	0.09	NS	
S	8.3 ^b	8.7 ^b	9.5ª	8.8	0.20	*	
			Micro minerals mg,	/kg DM			
Fe	121.50	134.52	140.78	132.27	23.14	NS	
Zn	30.00	34.36	32.41	32.26	4.99	NS	
Со	0.13 ^b	0.12 ^b	0.19 ^a	0.15	0.01	***	

KBT=Bukit Tunggul; KTP=Tangkuban Perahu; KBB=Burangrang. Different superscript letters in the same row are showing significant differences. *=P<0.05; ***=P<0.0001; NS=not significant.

proteins, and lipids (Baslam et al., 2020). These organic reserves, meticulously stored within plant structures, play a crucial role in sustaining the vital functions of equines and facilitating their growth, reproduction, and overall sustenance. The annual dry matter (DM) production of three distinct Kikuyu accessions exhibited noteworthy variability, ranging from 22.65 to 34.22tons/ha. Notably, figures surpassed previous reports, these which documented DM yields ranging from 7.03 to 16.24tons/ha (Feedipedia, 2021). It is clear that the complex relationship between factors such as nitrogen application rates and prevailing climatic conditions has a significant impact on DM yield. Indeed, as elucidated by Shahrivar et al. (2019), optimal DM yields of Kikuyu grass were attained under conditions characterized by elevated N application levels during the summer season, resulting in a commendable vield of up to 16.24tons/ha.

Moreover, an analysis of the stems among the three Kikuyu grass accessions showed a remarkable consistency, except for the KBT accession, which displayed a noticeably greater leaf proportion, accounting for 70.23% of its composition. However, this proportion falls short of the 80% dry matter (DM) yield attributed to two, four, and six leaves per tiller interval, as reported by Fulkerson et al. (1999). The reciprocal relationship between leaves and stems in determining the leaf-to-stem ratio is intricately tied to plant maturity. As forage matures, there is a discernible decrease in the proportion of leaves, as evidenced by the findings of Grev et al. (2020). Notably, the research was conducted by Fulkerson et al. (1999) delineated that the most favorable leaf yield was attained by defoliating Kikuyu grass at 2 leaves per tiller to a height of 3 cm, yielding a remarkable leaf proportion of 90.2%. Consequently, identification of an optimal defoliation timeframe is imperative to achieve the desired leaf-tostem ratio in aggregation with the plant's maturation trajectory.

The present study also explores the nourishing properties of Kikuyu grass as a potent nutritional forage choice for horses in tropical regions. Notably, the crude protein content of Kikuyu grass in this study reached 21.00% of the dry matter, surpassing levels found in other commonly utilized C4 grasses in Indonesia, such as Pennisetum purpureum variants and native grasses (Dahlanuddin et al., 2014; Zailan et al., 2016; Tuturoong et al., 2019). Such elevated protein content has significant implications for equine nutrition, particularly for meeting the dietary requirements of mature horses engaged in moderate exercise. Drawing upon the recommendations of the NRC (2007), a mature equine weighing 500kg and with heavy exercise needs daily intake about 2 to 2.5kg DM/100 kg body weight (BW) of equine, consist of 375g of CP. Remarkably, feeding Kikuyu grass alone to 500kg BW equine could fulfill 781g of crude protein, doubling the minimum requirement. However, despite its high protein content, Kikuyu grass exhibited a relatively low energy content, corroborating previous findings (García et al., 2014), as per the daily DE requirement of 38.91MJ/kg (NRC, 2007), equines consuming Kikuyu grass fall short of meeting their energy demands by 9.79MJ/kg DE. Hence,

addressing this energy deficit requires dietary supplementation to ensure optimal energy intake and sustain equine performance (Collas et al., 2015).

Further analysis of the Kikuyu grass components revealed significant variations in nutrient composition between the leaves and stems. The leaf fraction had significantly higher concentrations of CP, EE, TDN, GE, DE, and ME than the stems, as reported by Buchanan et al. (2015). These findings align with the predominant cellular and plasma membrane structures in leaf tissues, and the accession with the highest leaf proportion, KBT and KBB was poised to offer better digestibility compared to other accessions examined in this study. Conversely, the stem fraction displayed higher concentrations of crude fiber (CF) and neutral detergent fiber (NFC) than the leaves, consistent with the supportive functions of cell walls in plant structures (Carpita and McCann, 2015). These findings collectively enrich our understanding of the nutritive dynamics of Kikuyu grass and underscore the importance of strategic dietary management in equine nutrition.

A comparison of the mineral content in sampled Kikuyu grass with the findings reported by García et al. (2014) indicated lower concentrations of phosphorus (P) (0.17% vs. 0.35%), magnesium (Mg) (0.13% DM vs. 0.28% DM), and iron (Fe) (132.27mg/kg DM vs 210mg/kg DM). Meanwhile, (S) levels were higher (0.88mg/kg DM vs 0.25mg/kg DM) than the recommended levels (Garcia et al., 2014). However, the concentrations of S, Mg and Fe in dried Kikuyu surpassed the daily those minerals quotation for equines by 429, 62.5, and 133%, respectively (NRC, 2007). The essential minerals in animal feed are crucial for health, production, growth, and reproduction. Given the mineral requirements for light breed equines (Martin-Rosset, 2015), a diet consisting solely of Kikuyu grass lacks sufficient calcium (Ca), phosphorus (P), cobalt (Co), and zinc (Zn), necessitating supplementation. If the equine fed alone with Kikuyu grass with lower content (Ca), phosphorus (P), cobalt (Co), and zinc (Zn), might influence microbial diversity and fermentation rates in equine's cecal environment, whereas further may affects the digestive metabolism and health performance of equine (Martin-Rosset, 2015; Onvinvechukwu, 2017; Fehlberg et al., 2019; Paßlack et al., 2021). Mineral supplementation can be administered through commercial products, such as free-choice salt and mineral blocks, or by incorporating legumes into the diet as a mineral source (Morones et al., 2017).

Sulfur content in Kikuyu grass, particularly at the KBB accession, may reflect interactions between soil conditions and plant metabolism, influencing the synthesis of sulfurcontaining amino acids essential for plant growth (Havlin et al., 2013). The improved nitrogen fertilization may increase Kikuyu grass growth, suggesting that nitrogen uptake plays a significant role in biomass production (Kaur et al., 2016). Furthermore, the high levels of cobalt (Co) in Kikuyu grass of the KBB accession may be also influenced by soil conditions. However, attributing these levels entirely to well-drained soils and soil conditions requires further empirical evidence. Recent studies on Kikuyu grass have shown a synergistic and/or antagonistic interaction between trace elements that might affects the amino acid synthesis and bioavailability (Mejía-Taborda et al., 2014). Nonetheless, increased levels of minerals such as cobalt, copper, and selenium in Kikuyu pastures may improve health, productivity, and increase trace element uptake in livestock animals (Cloete et al., 1994).

Fiber Fractions and In Vitro Degradability Rates of Kikuyu Grass

Tropical grasses primarily comprise C4 grasses, which are superior in converting solar energy into biomass through a photorespiration-suppressing mechanism in their photosynthetic systems (Weijde et al., 2013). Despite their superiority in biomass production, C4 grasses are characterized by high lignification in the secondary cell walls, limiting their digestibility (Cesarino et al., 2016). In accordance with the KTP accession, the environmental factors, soil profile, and fertilizer treatments influence nutrient uptake for Kikuyu growth, underscoring the complex interplay of these factors in determining the carbohydrate structure of the plant. However, the ADF, cellulose, and lignin contents in Kikuyu grass under the KTP accession were notably higher than the others, suggesting that soil composition plays a crucial role in determining the fiber content of grasses. Different soil types result in varying levels of fiber fractions, such as ADF and neutral detergent fiber (NDF), further highlighting the complexity of this research.

Stopa et al. (2023) found that the application of soil conditioners, such as biochar, in conjunction with nitrogen-phosphorus-potassium (NPK) fertilizers leads to enhanced soil quality and increased grass biomass while maintaining consistent fiber content. This promising finding suggests that with the right soil conditioners and nitrogen fertilization, it is possible to improve soil quality and increase grass biomass, offering a potential solution for enhancing productivity in tropical grasses. Nitrogen fertilization is another important factor that influences the structural carbohydrate composition of grasses. Increasing nitrogen rates up to 270kg/ha, as reported by Leite et al. (2021), led to a reduction in ADF, NDF, and lignin content of Marandu palisade grass, while the crude protein levels increased. Moreover, the use of poultry manure alongside nitrogen fertilizers improves dry matter digestibility in tropical grasses, balancing nutrient ratios to enhance productivity (Ewetola et al., 2020). This suggests that the soil condition, Kikuyu's ability to absorb minerals, and treatment with N fertilizer in the present study modulated the structural carbohydrate accumulation of Kikuyu grass, offering hope for improved grass quality and digestibility.

In the present study, equine feces were used as an inoculum source to evaluate the nutrient digestibility of feed using the Daisy II in vitro method. This method is considered a reliable indicator that may reflect the true digestibility of evaluated forage in vivo trials (Earing et al., 2010). Although Kikuyu grass from different accessions displayed similar digestibility rates after 72h of in vitro incubation, the long-term utilization of Kikuyu grass from the KTP might yield unsatisfactory results because of its high fibrous fraction and lower DE content compared to Kikuyu grass planted in KBT and KBB. This relationship between digestibility rates and nutrient content in the feed was also confirmed by Hansen and Lawrence (2017).

The digestibility rates of forage can be influenced by its neutral detergent fiber (NDF) content, particularly the indigestible part of NDF, which can be predicted from the acid detergent lignin (ADL) content (Harper & McNeill, 2015). The observed evidence suggests that the digestibility rates of Kikuyu from the KTP accessions were relatively low because of the high ADL content in the plant. Indeed, the KTP accession in this study had significantly higher acid detergent fiber (ADF), ADL, and cellulose contents than the other accessions. High levels of ADF and other fiber components negatively affect the digestibility of dry matter (DM) and organic matter (OM) (Harris et al., 2017). Furthermore, Kikuyu grass planted in the KBT accession is a more promising source of high-quality forage for equines based on its nutrient content, fibrous fraction analysis, and in vitro digestibility rates.

Conclusion

In this study, the KBT accession of Kikuyu grass demonstrated a significantly higher leaf proportion than KTP, whereas KBB and KTP exhibited similar stem proportions. However, the stem proportions remained consistent across all accessions. The KTP accession produced the highest overall dry matter (DM) yield at 0.38 kg/m² or 34.22tons/ha annually. Proximate analysis revealed that accession KBT had the highest organic matter (OM) proportion. Leaf fractions exhibited significantly higher crude protein (CP), ether extract (EE), and total digestible nutrients (TDN) contents, whereas stem fractions contained higher crude fiber (CF) and nonfiber carbohydrate (NFC) contents. Additionally, Kikuyu leaves from all accessions displayed higher gross energy (GE), digestible energy (DE), and metabolizable energy (ME), with KBT leaves showing the highest DE content. Fiber analysis showed that KBB had significantly lower acid detergent fiber (ADF) and cellulose contents, whereas KBT had the lowest lignin levels. Although differences in fiber fractions were observed across accessions and plant parts, the digestibility values of DM, OM, and neutral detergent fiber (NDF) were similar. Differences in nutrient composition and energy content between accessions emphasize the importance of selecting appropriate Kikuyu grass varieties for optimal forage quality. In conclusion, essential factors such as the optimum defoliation schedule, fertilizer treatment, soil condition, and environment can enhance Kikuyu grass as a high-quality forage source for equines, particularly when focusing on accessions such as KBT and KBB.

Conflict of Interest: All authors declared there is no conflict of interest.

Ethical Approval Statement: No ethical approval was required as this research did not involve experimenting with rare plant species or conducting research with living animals.

Acknowledgement: The present study's completion was made possible by the support of Padjadjaran University, Indonesia, which the authors would like to express their gratitude towards. Additionally, the authors extend their appreciation to the Research Centre for Animal Husbandry, National Research and Innovation Agency, Bogor Indonesia, and Bio Farma Co. Ltd. for their support and facilitation as the major partner.

Source of Funding: This research was supported by statutory funding for research from Padjadjaran University, Indonesia, under the project entitled "The enhancement of feed quality for equine".

Author's Contribution: Mansyur: Designed research methodology, experimentation, data analysis, write & edited, original draft of the article, supervised the study. Nyimas Popi Indriani, Nabila Fara Dega Rifianda: Designed research methodology, performed experimental study and statistical analysis. Windu Nagara, Dimar Sari Wahyuni, and Satria Maulana: formal analysis data curation, writing original draft. Setiawan Martono, Herdis, and Karen Harper: data curation and reviewed the article. Vincent Niderkon, Yulianri Rizki Yanza: data curation, reviewed and edited the final article.

REFERENCES

- Acero-Camelo, A., Pabón, M.L., Fischer, G., & Carulla-Fornaguera, J.E. (2020). Optimum harvest time for Kikuyu grass (*Cenchrus clandestinus*) according to the number of leaves per tiller and nitrogen fertilization. *Revista Facultad Nacional de Agronomia Medellin*, 73(3), 9243-9253.
- Baslam, M., Mitsui, T., Sueyoshi, K., & Ohyama, T. (2020). Recent Advances in Carbon and Nitrogen Metabolism in C3 Plants. *International Journal of Molecular Sciences*, 22(1), 318. <u>https://doi.org/10.3390/ijms22010318</u>
- Buchanan, B.B., Gruissem, W., Jones, R.L., & Vickers, K. (2015). Biochemistry and Molecular Biology of Plants. John Wiley & Sons, Incorporated, Hoboken, UNITED KINGDOM.
- Carpita, N., & McCann, M. (2015). The cell wall. In B. B. Buchanan, W. Gruissem, R. L. Jones and K. Vickers (Eds.), Biochemistry and Molecular Biology of Plants (pp. 52-108). John Wiley & Sons, Incorporated, Hoboken, United States.
- Cesarino, I., Simões, M.S., dos Santos Brito, M., Fanelli, A., da Franca Silva, T., & Romanel, E. (2016). Building the wall: recent advances in understanding lignin metabolism in grasses. *Acta Physiologiae Plantarum*, 38(11), 269. https://doi.org/10.1007/s11738-016-2293-5
- Chavez, S.J., Siciliano, P.D., & Huntington, G.B. (2014). Intake estimation of horses grazing tall fescue (*Lolium arundinaceum*) or fed tall fescue hay. *Animal Science Journal*, 92(5), 2304-2308. <u>https://doi.org/10.2527 /jas.2013-7119</u>
- Charry, M., Rocha, E., & Fornaguera, J. (2020). Effect of altitude and defoliation frequency in the quality and growth of Kikuyu grass (Cenchrus clandestinus). Revista Facultad Nacional de Agronomía Medellín. <u>https://doi.org/10.15446/rfnam.v73n1.77330</u>
- Cloete, S.W., van Niekerk, F.E., Kritzinger, N.M., van der Merwe, G.D., Heine, E.W., & Scholtz, A.J. (1994). Production responses of sheep supplemented with copper, cobalt and selenium on Kikuyu ryegrass pastures. *Journal of the South African Veterinary Association*, 65(2), 52– 58.
- Collas, C., Dumont, B., Delagarde, R., Martin-Rosset, W., & Fleurance, G. (2015). Energy supplementation and herbage allowance effects on daily intake in lactating mares. *Animal Science Journal*, 93(5), 2520-2529. <u>https://doi.org/10.2527/jas.2014-8447</u>
- Dahlanuddin, Yanuarianto, O., Poppi, D.P., McLennan, S.R., & Quigley, S.P. (2014). Liveweight gain and feed intake of weaned Bali cattle fed grass and tree legumes in West Nusa Tenggara, Indonesia. *Animal Production Science*, 54(7), 915-921. <u>https://doi.org/10.1071/AN13276</u>
- Detmann, E., & Valadares Filho, S.C. (2010). On the estimation of nonfibrous carbohydrates in feeds and diets. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, 62, 980-984

- Earing, J., Cassill, B., Hayes, S., Vanzant, E., & Lawrence, L. (2010). Comparison of in vitro digestibility estimates using the Daisyll incubator with in vivo digestibility estimates in horses. *Animal Science Journal*, 88(12), 3954-3963
- Ewetola, I.A., Amisu, A., Jimoh, S.O., Muraina, T.O., Olanite, J., & Arigbede, O. (2020). Impact of fertilizer types on the nutritive quality of two tropical grass species harvested at different stages of growth during wet season. *Nigerian Journal of Animal Production*, 47(3). <u>https://doi.org/ 10.51791/NJAP.V47I3.169</u>
- Feedipedia, (2021). Kikuyu (*Pennisetum clandestinum*). Retrieved from https://www.feedipedia.org/node/398
- Fehlberg, L.K., Lattimer, J.M., Drouillard, J.S., & Douthit, T.L. (2019). Effect of Cobalt Chloride on Fermentation of Alfalfa and Smooth Bromegrass Hays by Horse Cecal Microorganisms. *Journal of Equine Veterinary Science*, 77, 75-79. <u>https://doi.org/10.1016/j.jevs.2019.02.020</u>
- Fulkerson, W., Slack, K., & Havilah, E. (1999). The effect of defoliation interval and height on growth and herbage quality of Kikuyu grass (*Pennisetum clandestinum*). *Tropical Grasslands-Forrajes Tropicales*, 33(3), 138-145.
- García, S., Islam, M., Clark, C., & Martin, P. (2014). Kikuyu-based pasture for dairy production: a review. *Crop and Pasture Science*, 65(8), 787-797.
- Grev, A.M., Wells, M.S., Catalano, D.N., Martinson, K.L., Jungers, J.M., & Sheaffer, C.C. (2020). Stem and leaf forage nutritive value and morphology of reduced lignin alfalfa. *Agronomy Journal*, 112, 406-417.
- Hansen, T.L., & Lawrence, L.M. (2017). Composition Factors Predicting Forage Digestibility by Horses. *Journal of Equine Veterinary Science*, 58, 97-102. doi: <u>https://doi.org/10.1016/j.jevs.2017.08.015</u>
- Harper, K.J., & McNeill, D.M. (2015). The Role iNDF in the Regulation of Feed Intake and the Importance of Its Assessment in Subtropical Ruminant Systems (the Role of iNDF in the Regulation of Forage Intake). Agriculture, 5(3):778-790. <u>https://doi.org/10.3390/ agriculture5030778</u>.
- Harris, P.A., Ellis, A.D., Fradinho, M.J., Jansson, A., Julliand, V., Luthersson, N., & Vervuert, I. (2017). Review: Feeding conserved forage to horses: recent advances and recommendations. *Animal*, 11(6), 958-967. <u>https://doi.org/10.1017/S1751731116002469</u>.
- Havlin, J.L., Tisdale, S.L., Nelson, W.L., & Beaton, J.D. (2015). Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 8th ed. Pearson Education. North Carolina State Univ. United States.
- Kaur, N., Phillips, I., & Fey, M. (2016). Amelioration of bauxite residue sand by intermittent additions of nitrogen fertiliser and leaching fractions: The effect on growth of Kikuyu grass and fate of applied nutrients. *Science of the Total Environment*, 550, 362-371. <u>https://doi.org/10. 1016/j.scitotenv.2016.01.012</u>
- Kienzle, E., & Zeyner, A. (2010). The development of a metabolizable energy system for horses. *Journal of Animal Physiology and Animal Nutrition*, 94(6). 231-240. <u>https://doi.org/10.1111/j.1439-0396.2010.01015.x</u>
- Latimer Jr., G., Ed. (2012) Official Methods of Analysis of the AOAC International. 19th Edition, AOAC.
- Leite, R.G., Cardoso, A.D., & Fonseca, N.V.B. (2021). Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass. *Scientific Reports*, 11, 14786. <u>https://doi.org/10.1038/</u> s41598-021-94098-4
- Marais, J. (2001). Factors affecting the nutritive value of Kikuyu grass (Pennisetum clandestinum)-a review. *Tropical Grasslands-Forrajes Tropicales*, 35(2), 65-84.
- Marshall, M.R. (2010). Ash Analysis. In S. S. Nielsen (Ed.), Food Analysis (pp. 105-115). Boston, MA: Springer US.
- Martin-Rosset, W. (2015). Equine nutrition: INRA nutrient requirements, recommended allowances and feed tables: Wageningen Academic Publishers, Wageningen, Netherlands.
- Mejía-Taborda, A., Ochoa-Ochoa, R., & Medina-Sierra, M. (2014). Effect of different doses of compound fertilizer on the quality of Kikuyu grass (Pennisetum clandestinum Hochst. Ex Chiov.). Pastos y Forrajes, 37, 31-37.
- Morones, E., Mendoza, G.D., Martínez, J.A., Plata, F.X., Palancares, C., & Hernández, P.A. (2017). Effect of Mineral Block Supplementation on In Vivo Digestibility and In Vitro Gas Production with Equine Fecal Bacteria. Journal of Equine Veterinary Science, 53, 81-85. <u>https://doi.org/10.1016/j.jevs.2017.01.009</u>
- Ngongo, Y., Hau, D., Kotta, N., & Nulik, J. (2021). Forage tree legumes and sustainable upland farming in Timor Island – Indonesia. *IOP Conference Series: Earth and Environmental Science*, 807. <u>https://doi.org/10.1088/1755-1315/807/3/032035</u>
- Niyogi, K.K., Wolosiuk, R.A., & Malkin, R. (2015). Photosynthesis. In B. B. Buchanan, W. Gruissem, R. L Jones and K. Vickers (Eds.), Biochemistry and Molecular Biology of Plants (pp. 508-565). John Wiley & Sons, Incorporated, Hoboken, United States.

- NRC, (2007). Nutrient Requirements of Horses: Sixth Revised Edition. The National Academies Press.Washington, DC, United States.
- Onyinyechukwu, A.A. (2017). Haematology and Clinical Biochemistry Findings Associated with Equine Diseases - a Review. *Notulae Scientia Biologicae*, 9(1). <u>https://doi.org/10.15835/nsb919939</u>.
- Paßlack, N., van Bömmel-Wegmann, S., Vahjen, W., & Zentek, J. (2021). Impact of dietary zinc chloride hydroxide and zinc methionine on the faecal microbiota of healthy adult horses and ponies. *Journal of Equine Veterinary Science*, 103804. <u>https://doi.org/10.1016/j.jevs.2021</u>. 103804
- Rahman, M.M., Abdullah, R.B. & Wan Khadijah, W.E. (2013). A review of oxalate poisoning in domestic animals: tolerance and performance aspects. *Journal of Animal Physiology and Animal Nutrition*, 97(4), 605-614. <u>https://doi.org/10.1111/j.1439-0396.2012.01309.x</u>.
- Royani, J.I., Utami, R.N., Maulana, S., Agustina, H., Herdis, Herry, R., Sarmedi, & Mansyur, (2021). Biodiversity of Kikuyu Grass (*Penisetum clandestinum Hochst.* ex. Chiov) in Indonesia as high protein forage based on morphology and nutrition compared. *IOP Conference Series: Earth and Environmental Science*, 902 012006. <u>https://doi.org/10.1088/</u> <u>1755-1315/902/1/012006</u>
- Serrapica, F., Masucci, F., Raffrenato, E., Sannino, M., Vastolo, A., Barone, C.M.A., & Di Francia, A. (2019). High Fiber Cakes from Mediterranean Multipurpose Oilseeds as Protein Sources for Ruminants. *Animals*, 9(11), 918. <u>https://doi.org/10.3390/ani9110918</u>.

- Shahrivar, A.A., Rahman, M.M., Hagare, D., & Maheshwari, B. (2019). Variation in Kikuyu grass yield in response to irrigation with secondary and advanced treated wastewaters. *Agricultural Water Management*, 222, 375-385.
- Stopa, W., Wróbel, B., Paszkiewicz-Jasińska, A., Strzelczyk, M. (2023). Effect of biochar application and mineral fertilization on biomass production and structural carbohydrate content in forage plant mixture. *Sustainability*, 15(19). <u>https://doi.org/10.3390/su151914333</u>
- Tassone, S., Renna, M., Barbera, S., Valle, E., & Fortina, R. (2019). In Vitro Digestibility Measurement of Feedstuffs in Donkeys Using the Daisyll Incubator. *Journal of Equine Veterinary Science*, 75, 122-126. <u>https://doi.org/10.1016/j.jevs.2019.02.002</u>
- Tuturoong, R.A.V., Malalantang, S.S., & Moningkey, S.A.E. (2019). Assessment of the nutritive value of corn stover and king grass in complete feed on Ongole steer calves productivity. *Veterinary World*, 13(4), 801-806. https://doi.org/10.14202/vetworld.2020.801-806
- Weijde, T., Alvim Kamei, C., Torres, A., Vermerris, W., Dolstra, O., Visser, R. & Trindade, L. (2013). The potential of C4 grasses for cellulosic biofuel production. 4(107). *Frontiers in Plant Science*, 4, 107. <u>https://doi.org/10 .3389/fpls.2013.00107</u>
- Zailan, M., Yaakub, H., & Jusoh, S. (2016). Yield and nutritive value of four Napier (*Pennisetum purpureum*) cultivars at different harvesting ages. *Agriculture Biology Journal of North America*, 7, 213-219