

Prediction of the nutritional values by INRA (2018) feed evaluation system of *Megathyrus maximus* subjected to different grazing strategies

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Abstract: Grazing management is a key element to optimize growth cycle of forages, which are enhanced in their chemical composition leading to the reception of greater nutritive values for feeding ruminants. Several studies have showed that the accuracy and precision of the feeding values are critical to achieve this goal, unfortunately, in Ecuador there are not referential data, despite of *Megathyrus maximus* is the most forage used by livestock farmers. The predominant aim of the current study was to approach and estimate the feeding values by INRA feed evaluation system of Tanzania grass (*Megathyrus maximus*) subjected at different grazing strategies. The statistical design was a randomized complete block, with a 3×2 factorial arrangement. Where the treatments were combinations of three grazing frequencies (GF) (30 d; termed GF30, 45 d, GF45 and 60 d, GF60) and two cutting heights (CHs; 30 and 45 cm). The agronomic data did not vary by season effect ($P = 0.24$ to 0.82), but GF60 had higher plant height and dry matter (DM) contents ($P < 0.001$) than other GF, although with less tiller populations (241 vs. 304 tiller/m²). By CHs effect, Tanzania grass at 30 cm had a greater DM content than 45 cm of CHs (5565 vs. 4221 ± 603 kg/ha⁻¹; $P = 0.073$). All chemical determinations were affected by GF, CHs and their interaction ($P < 0.001$ to 0.004) with the exception on ADF between CHs ($P = 0.50$). Whereas only the CP and ADF values were conditioned by season effect ($P = 0.001$ to 0.011). Subsequently, Tanzania grass subjected at moderate rest periods and low defoliation intensities showed greater energy and proteins values than traditionally used GF60. Based in these findings, the defoliation frequency had influence on agronomic and chemical characteristics, whilst the feeding values was mainly affected by grazing intensities and weather conditions. So, the best combination regarding nutritive values should be at GF30 and GF45 combined with 45 cm of CHs.

Keywords: Feeding values; Forage quality; Methane emission; Small-livestock farmers.

Introduction

Livestock plays an important role in the livelihoods of rural households and the economies of developing countries (McDermott, Staal, Freeman, Herrero, & Van de Steeg, 2010; Herrero et al., 2013; Thornton & Gerber, 2010; Do-Lam, Trung-Thanh, & Grote, 2019; Torres, Heredia-r, Toulkeridis, & Estupiñ, 2022). Nevertheless, meat and dairy production in arid, equatorial and tropical regions is often lower than productive in temperate regions due to the lower nutritional quality of forage grasses, infertile soils and adverse climatic conditions (Lee, Davis, Chagunda, & Manning, 2017; B, Heredia-r, Valencia, & Torres, 2021; Meister et al., 2021). Sustainable intensification as a form of production wherein yield increase without adverse environmental impact and without the cultivation of more land, are being largely considered (Barreto-álvarez, Heredia-rengifo, Padilla-almeida, & Toulkeridis, 2020; Nicolás et al., 2018). In tropical regions, forages are the main food source for ruminants, however, the environmental conditions and the management of grasslands directly affect the yield and quality of the pastures, impacting its growth, animal performance, and the functioning of the pastoral ecosystem (Schons et al., 2021; Szymczak et al., 2021). In this sense, higher cut intervals increase total forage mass, but tend to reduce the nutritional value (Macdonald, Penno, Lancaster, & Roche, 2008; De Sousa, Alexandrino, Dos Santos, & Freitas, 2019). Since, maximum herbage accumulation is set as the optimal to start grazing, while intense grazing (i.e., low residual sward height) is usually imposed as the limit of sward depletion (Szymczak et al., 2021). Therefore, grazing management strategies that optimize herbage utilization and digestible dry matter intake (DMI) by grazing cows might improve land use and mitigate key environmental issues of pasture-based livestock systems (Lascano & Cárdenas, 2010; Souza et al., 2019).

Megathyrsus maximus is the main grass used in tropical livestock systems as it contains high forage production potential, nutritional value and adaptation to different climatic conditions and in poor soils (Carnevalli et al., 2006; de Lima Veras et al., 2020). This leads to the assumption that the grasses of Poaceae are ecologically dominant and by far the most economically important family in the world (Stromberg, 2011; Benabderrahim & Elfalleh, 2021). The Orellana Province, with a total population estimate 157,520 habitants, form part the Amazonian Region (RA) and represent 18.6% of a total 45.47%. Besides, this zone has showed a social composition many complexes, experimenting deep changes since the decade of 1960 as effect of several laws of agrarian reform and colonization and the begin the hydrocarbon production. In this sense, GADPO (2015) has mentioned that main activities for generate income producers in the RA are concentrated in the agriculture (56.5%), livestock (10%), while 30% are under mix production system (agriculture-livestock) however, all these actions employ extensive pasture-based systems with very low productivity and rentability level. In fact, although *Megathyrsus maximus* is the most relevant grass adapted for raising cattle in the RA, the use of continuous stocking combined with minimum rest periods, and high defoliation intensities are management practices that contribute to low availability and quality of forage, with negative effects on animal performance (Cedeño-Aristega, Luna Murillo, Espinoza Coronel, & Romero Garaicoa, 2021; Gómez Villalva, Pérez, Vásconez Galarza, & Moran Salazar, 2021). Subsequently, for a better forage utilization, it is fundamental to understand the adequate timing of

harvest from the point of view of the yield, quality and persistence of the plants (Fulkerson & Donaghy, 2001; Parsons et al., 2011; Ben & Toulkeridis, 2022). Since, maintaining high rates of forages intake, and high animal performance for tropical forages, require pastures with adequate canopy structures (Rao, 2001; Euclides et al., 2018). Therefore, the recent research with tropical forage grasses has been focused on identifying grazing management strategies that harmonize with and optimize the natural growth cycle of plants, favouring their growth and production (S. Da Silva, Sbrissia, & Pereira, 2015). Furthermore, considering that tropical grasses are of lower nutritional value compared to those from temperate areas, it is essential to identify tropical grass varieties with the highest nutritive value (Batistoti et al., 2012).

Forage quality is a central consideration in the design and implementation of grazing systems, as it is influenced by the length of both the grazing and the rest periods between subsequent grazing events (Briske et al., 2008). Whereas, nutritional values of forages is crucial in livestock nutrition, because effective livestock production is related to the amount of nutrients in the forages (Amiri, Rashid, & Shariff, 2012). Numerous feed evaluation systems among them those of the National Research Council, (NRC, 2001), the Cornell Net Carbohydrate and Protein System (Fox et al., 2004), the DVE/OEB system NorFor (Volden, 2011), and the Institut National de la Recherche Agronomique (INRA, 2018) have been developed and are regularly being updated in order to offer the possibility to formulate rations by matching nutrient supply with animal requirements (Daniel, Van Laar, Dijkstra, & Sauvant, 2020).

In Ecuador, there is very little research information available on the forage quality and feeding values of Tanzania grass (*Megathyrsus maximus*). Considering this, through the recently updated INRA (2018) feed evaluation system, our study aimed to do an accurate estimation of nutrient partitioning of Tanzania grass (*Megathyrsus maximus*) subjected at different grazing strategies.

Materials and Methods

Study area

This study was conducted in the Orellana Province, which is located in the northern area of the Ecuadorian Amazon (GADPO, 2015), as shown in Figure 1.

Agricultural land use in Orellana Province, according to the estimates by INEC-ESPAC (2019), covers a total area of 606,307 hectares (ha), distributed as mountains and forests, 485,039 ha (80%), permanent crops of some 43,582 ha (7.2%), other uses with 28,049 ha (4.6%), cultivated pastures with 25.162 ha (4.2%); natural pastures with 19,034 ha (3.1%) as well as transitory crops and fallow with 4959 ha (0.82%). The climate in the region is characterized by humid tropical rainforests (Inzunza, 2007; Holdridge, 1967; INAMHI, 2021). The average rainfall is 2942 mm annually, with an annual average temperature of 29.7 °C.

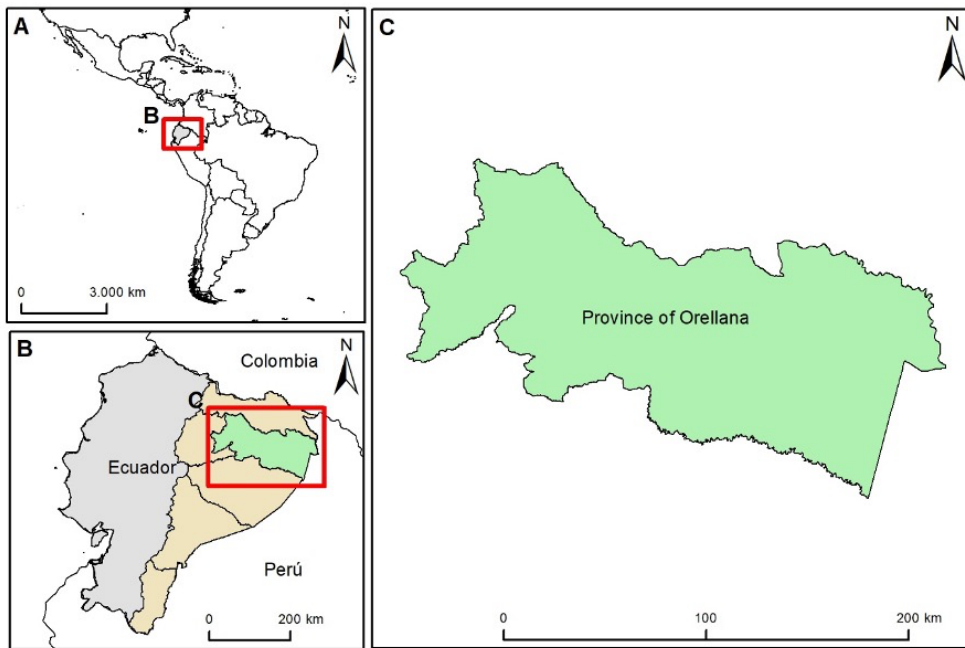


Figure 1 - Study area. A) Geographical location of Ecuador in America. B) Ecuadorian Amazonia. C) Province of Orellana.

The Tanzania grass (*Megathyrsus maximus*) was established in 2016 at 3kg/ha seeding rate. The physical-chemical properties of the soil were 7% of organic matter, 15.1 ppm of phosphorus (P), 0.26 meq of potassium (K) and 57% base saturation. The experimental area was fertilized with 195 kg N/ha applied at seeding. The rainfall and ambient temperature data during the study, are shown in Figure 2.

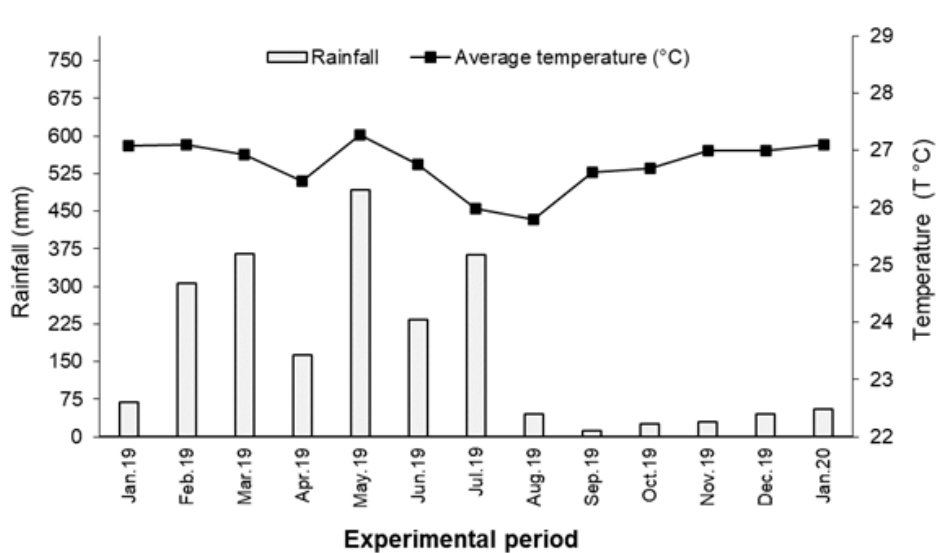


Figure 2 - Monthly mean temperatures and rainfall from January 2019 to January 2020 in the experimental area in Orellana Province.

Experimental design and treatments

Treatments were combinations of three grazing frequencies (30 d; termed GF30, 45 d, GF45 and 60 d, GF60) and two cutting heights (CHs) (30 and 45 cm, respectively). Then, the treatments were allocated to experimental units (24 plots; 5m × 5m with a 0.5m path between plots) according to a randomized complete block design, with a 3 × 2 factorial arrangement. In September 2018, grazing began using a frequency 35-day rest period and 30 cm of cutting height. Thereafter, in December 2018, the plots were assigned to different treatments and managed with 30 or 45 cm CHs. The cuts of the forages, according to each grazing frequency and cutting height, were performed using a handheld prop sickle. The experimental period was from January 2019 to January 2020.

Sampling and data determinations

Pasture height

Throughout the study, canopy height was measured using a 1-m ruler centimeter-graduate at several random points regarding each treatment prior each harvesting (Euclides-Batista, Junior, Carneiro da Silva, Difante, & Amorim Barbosa, 2016).

Tiller population density

Tiller population density (TPD; tiller/m²) was obtained by direct counting the number of tillers in an 1 × 0.25 m² sampling frame, allocated at the average height of the plot at each treatment and harvesting (Grant, Barthram, King, & Smith, 1983; Sbrissia, Carneiro, Augusto, Carvalho, & Pedreira, 2001; De Sousa et al., 2019).

Plant cover score

Plant scoring of the plots was realized in order to determine the extent of cover by the forage. To this end, scoring was yielded using five-point grading score as suggested by Onyeonagu & Asiegbu (2013) for subjective evaluation, where degree of cover was assessed as, 1 (< 20%) labeled as very low; 2 (20 to 39%) labeled as low; 3 (40 to 59%) labeled as medium; 4 (60 to 79%) labeled as high and 5 (80 to 100%) labeled as very high.

Forage mass

Forage mass (FM) was assessed through the double sampling technique described by NRC (1962). Therefore, for direct measurements, we used one 0.25 m² square to collect random samples in each treatment prior every grazing throughout the experimental period. The FM was estimated by cutting and weight the forage. A visual scale was used as an indirect measurement method, where the scale ranged from 1 to 3, meaning that 1 is the lowest herbage mass and 3 is the highest (Haydock & Shaw, 1975). In addition, during the direct determination of forage, two samples of approximately 0.5 kg for treatment were collected. The first sample was dried in the forced air circulation oven at 60 °C for 72 h in order to determine dry matter (DM kg ha⁻¹), while one second sample of fresh grass was frozen at -20 °C for the determination of its chemical composition.

Chemical composition

Prior to the analysis, the frozen grass samples were thawed at ambient temperature. Thereafter, they were conditioned at 60°C for 24 h, and then milled and homogenized through a cyclone mill (Retsch SM2000, Retsch, Haan, GE) with a 1 mm mesh. The chemical analysis was conducted in duplicate according to AOAC (2000) and expressed on a dry matter basis. Thus, dry matter (DM) was determined at 103°C for 24 h and ashes burnt at 550°C for 5 h. Crude protein was calculated as a percentage of N \times 6.25 by the Kjeldahl method (Bradstreet, 1954). Crude fiber (CF) was analyzed according to Weende method by acid hydrolysis with 1.25% H₂SO₄, followed by alkaline hydrolysis with 1.25% NaOH. By contrast, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were sequentially determined on an ash-free basis according to Van Soest et al. (1991) adding sodium sulphite and thermostable α -amylase (Ankom Technology, Fairport, NY, USA).

Forage Quality

The relative feed value (RFV) and relative forage quality (RFQ) were estimated using the nutrient values determined according to reference chemical analysis previously described (D Undersander & Moore, 2002). The RFV used as a forage quality estimator, is an index which combines important nutritional factors (potential intake and digestibility) into one number for a quick, easy and effective method of evaluating quality (Jeranyama & Garcia, 2004; Linn & Martin, 1989), as indicated in the quality assessment of roughages (Atalay & Kahriman, 2020), consequently, it was estimated according to equation (1).

$$RFV = DDM \times DMI \div 1.29 \quad (1)$$

Where:

$$DDM \text{ (Digestible dry matter)} = 88.9 - (0.779 \times \% \text{ ADF in base on DM}), \text{ and}$$

$$DMI \text{ (Dry matter intake)} = 120 / NDF \% \text{ of DM}$$

In contrast, RFQ index is more developed than the RFV index, as it reflects the expected performances of cattle consuming roughage more effectively. Therefore, the following equation was used (2).

$$RFQ = (DMI_{grass}) \times (TDN_{grass}) \div 1.23 \quad (2)$$

Where:

$$TDN_{grass} = (NFC \times 0.98) + (CP \times 0.87) + (FA \times 0.97 \times 2.25) + (NDF_n \times NDFD_p / 100) / 10$$

$$DMI_{grass} = -2.318 + 0.442 \times CP - 0.0100 \times CP^2 - 0.0638 \times TDN + 0.000922 \times TDN^2$$

$$+ 0.180 \times ADF - 0.00196 \times ADF^2 - 0.00529 \times CP \times ADF$$

DMI is expressed as % of BW, and CP, ADF, and TDN are expressed as % of DM (D Undersander & Moore, 2002).

Nutrient values

The chemical composition previously determined at each treatment, served to estimate the feeding values of Tanzania grass (*Megathyrsus maximus*) using equations proposed by INRA (2018). Subsequently, energy values expressed on kcal/kg DM were calculated for gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy for meat production (ENV). Hereby, a conversion of 1 UFV = 1.76 Mcal/kg DM was used. Regarding protein values, protein digestible in the intestine from dietary origin (PDIA), protein digestible in the intestine from microbial origin (PDIM), protein digestible in the intestines (PDI) as sum of (PDIA + PDIM) were expressed as g/kg DM. Additionally, rumen protein balance (RPB, g/kg DM) was calculated using the equation $RPB = -84.5 + 0.61 \times CP$ according to INRA (2018).

Statistical Analysis

Data were analyzed by the PROC MIXED procedure for repeated measurements of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Normality was visually tested in all continuous variables using the UNIVARIATE procedure of SAS and tested for homogeneity of variance by the Kolmogórov-Smirnov procedure (Flury & Riedwyl, 1986). Next, different structures of the variance-covariance matrix were tested, and the compound symmetry structure was chosen based on the smallest Bayesian information criterion (Pinheiro & Bates, 1996).

Analysis of variance (ANOVA) was performed using the MIXED procedure of SAS (Viera-torres, Sinda-gonz, Gil-docampo, Bravo-Yaandum, & Toulkeridis, 2020; Villacís, Ruiz, Powney, Guzmán, & Toulkeridis, 2020). The statistical model considered, grazing frequency, cutting heights, Season (rainy season, HP and dry season, LP) and their interactions as fixed effects, the block and residual error were taken as random effects. Least square means differences were determined by t-tests using the PDIFF option of SAS and Bonferroni adjustment was used to allow for planned comparisons among treatments (Green & Britten, 1998). Main effects, least squares mean differences, and interaction terms were considered significant when $P \leq 0.05$ and tendencies when $P < 0.10$.

Results

Agronomic measurements

The effects of grazing frequencies and cutting heights on agronomic measurements are listed in Table 1. With the exception for plant cover score ($25 \pm 2.4\%$, on average; $P = 0.74$) differences by grazing frequency (GF) effect on agronomic variables were observed ($P < 0.001$) indicating the cutting heights (CHs) only a tendency on DM contents ($P = 0.073$) and no differences by season effect ($P = 0.24$ to 0.82). The Tanzania grass managed at GF60 yielded higher plant height (86.27 ± 1.95 cm) compared to GF45 (69.5 ± 1.95 cm) and GF30 (49.16 ± 1.95 cm), respectively. In addition, GF \times CHs interaction was detected ($P = 0.040$; Table 1), the GF60 with either 30 or 45 cm CHs demonstrated a higher plant height when compared to GF30 and GF45 at same CHs. No significant differences were

observed by GF × CHs × S interaction ($P = 0.15$ to 0.57). The tiller population per m^2 were higher for GF30 compared to GF45 and GF60 (304 vs. 245 ± 10.8 tiller/ m^2 , respectively), although without differences between CHs (174 ± 10.8 tiller/ m^2 , on average; $P = 0.18$) or interaction ($P = 0.23$; Table 1). The DM obtained per ha^{-1} was higher in GF60 (7731 kg/DM ha^{-1}) in comparison to GF45 (4817 kg/DM ha^{-1}) and GF30 (2131 kg/DM ha^{-1}), respectively, as listed in Table 1. Contrary to the observed for CHs ($P = 0.073$), DM content tended to be higher at 30 than 45 cm HCs (5565 vs. 4221 ± 603 kg/DM ha^{-1}).

Table 1 - Agronomic measurements of Tanzania grass with regard to grazing frequencies (GF30, GF45 and GF60), two CHs and season (rainy and dry) from January 2019 to January 2020.

Item	Grazing frequency			CHs ¹		Season ²		SEM	<i>P</i> < value			
	GF30	GF45	GF60	30	45	RS	DS		GF	CHs	S	GF × CHs ³
Pasture height, cm	49.16 ^c	69.5 ^b	86.27 ^a	68.9	67.7	69.7	67.0	1.95	0.001	0.57	0.24	0.040
Tiller population, m^2	304 ^a	252 ^b	241 ^b	183	165	172	175	10.8	0.001	0.24	0.82	0.23
Plant cover score, %	26	24	24	24	25	22 ^y	28 ^x	2.4	0.74	0.67	0.74	0.020
DM, kg ha^{-1}	2131 ^c	4817 ^b	7731 ^a	5565 ^x	4221 ^y	5248	4539	603	0.001	0.073	0.31	0.10

¹CHs, Cutting heights, 30 and 45 cm; ²RS, rainy season and DS, dry season; ³GF × CHs, interaction; a-c Mean values with different letter in the same row differ for grazing frequency ($P < 0.05$); x-y Mean values with different letter tended to differ ($P < 0.10$); SEM, standard error of the mean.

Chemical composition and forage quality

Table 2 indicates the chemical composition and forage quality of Tanzania grass (*Megathyrsus maximus*). All chemical components yielded significant differences by GF ($P < 0.001$) and CHs ($P < 0.001$) effects, although without differences on ADF contents (468 ± 0.15 , on average; $P = 0.50$; Table 2). Whereas by season effect, only differences on ADF contents ($P < 0.001$) were observed (489.3 vs. 447.4 ± 0.15 g/kg-1). The Tanzania grass managed at GF60 had greater DM, OM and CF contents compared to GF30 and GF45, respectively, as listed in Table 2, although with low ash and EE contents. Furthermore, the Tanzania grass at 45 cm of CHs indicated greater contents than at 30 cm for DM (234 vs. 213 ± 3.4 g/kg-1), OM (904.9 vs. 896.6 ± 0.13 g/kg-1) and EE (16.2 vs. 14.6 ± 0.16 g/kg-1) but with lower ash (95.2 vs. 103.4 ± 1.50 g/kg-1) and CF (372.9 vs. 385.4 ± 0.17 g/kg-1) contents. Differences by GF × CHs interaction were observed ($P = 0.001$ to 0.004), but not for GF × CHs × S interaction ($P = 0.20$ to 0.69).

The CP contents varied among GF ($P < 0.001$). The Tanzania grass managed at GF30 had a greater CP mean value (146.8 g/kg-1) than GF45 (141.3 g/kg-1) and GF60 (105.5 g/kg-1), respectively. Also, CP contents were affected by CHs effect ($P < 0.001$), the Tanzania grass at 45 cm demonstrated a 15% greater CP content than at 30 cm CHs (141.8 vs. 120.6 ± 1.40 g/kg-1; Table 2) and these values were greater in RS than DS (137.4 vs. 124.9 ± 1.40 ; $P = 0.011$). Detecting a significant interaction between GF × CHs ($P < 0.001$), the Tanzania grass managed at GF30

or GF45 and combined with 45 cm of CHs had greater CP contents (ranged from; 151.5 to 160.5 g/kg-1) than the other combinations of grazing strategies which ranged from (98.0 to 142.0 g/kg-1).

Regarding fibre contents, Tanzania grass managed at GF30 had lower NDF content than GF45 and GF60 (639.5 vs. 739.5 ± 0.19 g/kg-1; $P < 0.001$) whilst at 45 cm was observed a slightly lower NDF content compared to at 30 cm CHs (701.7 vs. 710.6 ± 0.19 g/kg-1; $P < 0.001$; Table 2). With clear differences by GF \times CHs interaction ($P < 0.001$), the NDF contents of Tanzania grass managed at GF30 with either 30 or 45 cm CHs were lower than the other grazing combinations. Similar results were observed on ADF contents. The Tanzania grass managed at GF30 had a lower ADF content (353.9 g/kg-1) with respect to GF45 (494 g/kg-1) and GF60 (557.3 g/kg-1), respectively, as listed in Table 2. Meanwhile, significant differences by GF \times CHs interaction were declared ($P < 0.001$). The Tanzania grass in both CHs (i.e., 30 and 45 cm) managed at GF30 had lower ADF values than GF45 and GF60, respectively.

On the other hand, the forage quality expressed as RFV was affected by grazing frequency effect ($P < 0.001$), those values in GF60 ($57 \pm 0.4\%$) were lower when compared to GF45 ($64 \pm 0.4\%$) and GF30 ($89 \pm 0.4\%$), respectively. There were no differences between HCs ($70 \pm 0.4\%$; $P = 0.45$) or GF \times CHs interaction ($P = 0.62$). Furthermore, the RFQ index yielded differences among GF ($P < 0.001$). The Tanzania grass managed at GF30 had a higher index ($54 \pm 1.1\%$) with regard to GF45 ($27 \pm 1.1\%$) and GF60 ($22 \pm 1.1\%$), respectively. Differences between CHs (30 vs. 45 cm; 36 vs. 33 ± 1.1 ; $P = 0.010$; Table 2) and GF \times CHs interaction ($P = 0.010$) were declared. In addition, forage quality varied according to season effect ($P < 0.001$), as shown in Table 2.

Table 2 - Chemical composition (g/kg⁻¹) and forage quality expressed as (%) of Tanzania grass with regard to grazing frequencies (GF30, GF45 and GF60), two CHs (30 and 45 cm) and season (rainy and dry) from January 2019 to January 2020

Item	Grazing frequency			Cutting heights ¹		Season ²		SEM	<i>P</i> < value				
	GF30	GF45	GF60	30 cm	45 cm	RS	DS		GF	CHs	S	GF × CHs ³	GF × CHs × S ⁴
DM at 60°C	208 ^c	227 ^b	235 ^a	213 ^k	234 ^j	225	217	3.4	0.001	0.001	0.16	0.004	0.51
Ash	106.6 ^a	102.9 ^b	88.4 ^c	103.4 ⁱ	95.2 ^k	102.0	100.0	1.50	0.001	0.001	0.15	0.001	0.26
OM	893.5 ^c	897.1 ^b	911.6 ^a	896.6 ^k	904.9 ^j	898.3	903.2	0.13	0.001	0.001	0.13	0.001	0.20
EE	17.1 ^a	17.0 ^a	12.0 ^b	14.6 ^k	16.2 ^j	16.0	15.0	0.16	0.001	0.001	0.14	0.001	0.69
CP	146.8 ^a	141.3 ^b	105.5 ^c	120.6 ^k	141.8 ^j	137.4	124.9	1.40	0.001	0.001	0.011	0.001	0.44
CF	329.9 ^c	371.8 ^b	435.9 ^a	385.4 ⁱ	372.9 ^k	390.1	368.3	8.4	0.001	0.001	0.18	0.001	0.27
NDF	639.5 ^b	739.1 ^a	739.8 ^a	710.6 ⁱ	701.7 ^k	717.9	694.3	0.19	0.001	0.001	0.18	0.001	0.44
ADF	353.9 ^c	494.0 ^b	557.3 ^a	468.4	468.3	489.3 ^l	447.4 ^m	0.15	0.001	0.50	0.001	0.001	0.69
Forage quality													
RFV	89 ^a	64 ^b	57 ^c	70	70	66 ^m	73 ^l	0.4	0.001	0.45	0.001	0.62	0.99
RFQ	54 ^a	27 ^b	22 ^c	36 ^j	33 ^k	29 ^m	40 ^l	1.1	0.001	0.010	0.001	0.010	0.21

¹Cutting heights, 30 and 45 cm; ²RS, rainy season and DS, dry season; ³GF × CHs, interaction; ⁴GF × CHs × S, interaction; ^{a-c} Mean values with different letter in the same row differ for grazing frequency (*p* < 0.05); ^{j-k} Mean values with different letter in the same row differ for cutting heights (*P* < 0.05); ^{l-m} Mean values with different letter in the same row differ for season (*P* < 0.05); SEM, standard error of the mean

Table 3 - Protein and energy values of Tanzania grass with regard to grazing frequencies (GF30, GF45 and GF60), two CHs and season from January 2019 to January 2020

Item	Grazing frequency			Cutting height ¹		Season ²		SEM	<i>p</i> < value					
	GF30	GF45	GF60	30	45	RS	DS		GF	CHs	S	GF × CHs ¹	GF × CHs × S	
Energy, Kcal/kg														
DM														
GE	4.72 ^a	4.70 ^b	4.65 ^c	4.67 ^k	4.71 ^j	4.70 ^l	4.68 ^m	0.002	0.001	0.001	0.001	0.001	0.001	0.40
DE	2.51 ^a	1.77 ^b	1.35 ^c	1.85 ^k	1.91 ^j	1.78 ^m	2.0 ^l	0.004	0.001	0.001	0.020	0.001	0.001	0.32
ME	2.48 ^a	1.74 ^b	1.33 ^c	1.82 ^k	1.88 ^j	1.76 ^m	1.94 ^l	0.003	0.001	0.001	0.020	0.001	0.001	0.24
ENV	2.34 ^a	1.48 ^b	1.05 ^c	1.59 ^k	1.65 ^j	1.51	1.72	0.010	0.001	0.001	0.20	0.002	0.002	0.93
UFV	1.33 ^a	0.84 ^b	0.60 ^c	0.90 ^k	0.94 ^j	0.86	0.98	0.005	0.001	0.001	0.19	0.003	0.003	0.71
Protein, g/kg DM														
PDIA	36 ^a	34 ^b	25 ^c	29 ^k	34 ^j	33 ^l	30 ^m	0.3	0.001	0.001	0.010	0.001	0.001	0.40
PDIM	47 ^a	40 ^b	37 ^c	41	41	40 ^m	42 ^l	0.8	0.001	0.001	0.19	0.001	0.001	0.17
PDI	83 ^a	74 ^b	62 ^c	70 ^k	75 ^j	73 ^l	72 ^m	0.3	0.001	0.001	0.017	0.001	0.001	0.37
RPB	5 ^b	2 ^c	-20 ^a	-11 ^j	2 ^k	-7	-8	0.8	0.001	0.001	0.10	0.001	0.001	0.39

¹Cutting heights, 30 and 45 cm; ²RS, rainy season and DS, dry season; ³GF × CHs, interaction; ⁴GF × CHs × S, interaction; ^{a-c} Mean values with different letter in the same row differ for grazing frequency (*P* < 0.05); ^{j-k} Mean values with different letter in the same row differ for cutting heights (*P* < 0.05); ^{l-m} Mean values with different letter in the same row differ for season (*P* < 0.05); SEM, standard error of the mean.

Nutrient values

Energy

Energy values of Tanzania grass (*Megathyrsus maximus*) managed at different grazing strategies are listed in Table 3. Significant differences by GF effect on all energy values were observed ($P < 0.001$), the Tanzania grass managed at GF30 generated higher values than to the obtained in GF45 and GF60, respectively, as documented in Table 3. Furthermore, differences between 45 vs. 30 cm CHs were observed for GE (4.71 vs. 4.67 ± 0.002 Kcal/kg DM; $P < 0.001$), DE (1.91 vs. 1.85 ± 0.004 Kcal/kg DM; $P < 0.001$), ME (1.88 vs. 1.82 ± 0.003 Kcal/kg DM; $P < 0.001$), ENV (1.65 vs. 1.59 ± 0.010 Kcal/kg DM; $P < 0.001$) and UFV (0.94 vs. 0.90 ± 0.005 Kcal/kg DM; $P < 0.001$). Significant GF \times CHs interaction was detected ($P < 0.001$ to 0.003 ; Table 3). Tanzania grass managed at GF30 or GF45 and combined with 45 cm CHs showed greater energy values than other GF \times CHs combinations. In the case of season effect, RS had greater GE values than DS (4.70 vs. 4.68 ± 0.002 Kcal/kg DM), but with lower DE and ME values, as shown in Table 3.

Proteins

The protein values of Tanzania grass (*Megathyrsus maximus*) managed at different grazing frequencies, cutting heights and season are listed in Table 3. The Tanzania grass managed at GF30 had greater contents than GF45 and GF60 as PDIA ($P < 0.001$), PDIM ($P < 0.001$) and PDI ($P < 0.001$), respectively. Contrary to the observed for RPB values, a light difference was observed between GF45 and GF30 (5 vs. 2 ± 0.8 g/kg DM) although compared to GF60 huge differences were observed (-20 ± 0.8 g/kg DM). Regarding CHs effect, the Tanzania grass at 45 cm generated greater contents than 30 cm as PDIA (34 vs. 29 ± 0.3 g/kg DM; $P < 0.001$) PDI (75 vs. 70 ± 0.3 g/kg DM; $P < 0.001$) and RPB (2 vs. -11 ± 0.8 g/kg DM; $P < 0.001$), but without differences for PDIM (41 ± 0.8 g/kg DM; $P = 0.62$). Besides this, a significant GF \times CHs interaction was detected ($P < 0.001$). Apparently, the Tanzania grass managed either GF30 or GF45 at 45 cm CHs had higher protein values than GF60. In contrast, the season effect influenced the PDIA and PDI values ($P = 0.010$ to 0.017 ; Table 3). No differences on protein values were observed by GF \times CHs \times S interaction ($P = 0.17$ to 0.40 ; Table 3).

Discussion

Agronomic measurements

González Marcillo et al. (2021) and Carrillo-Oleas, Mancero-Oñate, & Benavides-Lara (2023) have reported differences on agronomic measurements by season effect when Tanzania grass and *Brachiaria decumbes* were assessment in Orellana Province. So, seasonal variations in temperature and water availability occur during the year. Consequently, the annual forage production is conditioned

to weather conditions, and it should be considered in the planning of livestock farms. According to De Sousa et al. (2019) the cutting interval did not influence the dry mass of harvested forage, most likely because of the compensation of higher cycles at lower cutting frequencies, even with lower amounts of total dry matter produced per cycle. In fact, the same author De Sousa et al. (2019) informed similar plant height values than to the obtained by us in tropical Ecuadorian conditions at grazing frequencies of GF30 (55 vs. 49 cm) or GF60 (70 vs. 86 cm). Whereas the reduction in defoliation intensity allows the retention of greater leaf area photosynthetically active and greater remobilization of nutrients, resulting in greater speed of recovery and shorter interval between grazing (Costa et al., 2021). In this sense, Euclides et al. (2018) mentioned that, despite high DM/ha⁻¹ obtained on forages managed at 30 cm, these forages need more days to reach suitable target height, resulting in fewer grazing cycles compared to forages at > 50 cm of CHs.

Other studies in Tanzania grass evaluated in subtropical conditions by Cedeño-Aristega et al. (2021) obtained similar plant height for GF30 and GF45, respectively.

Contrary to the observed by Onyeonagu and Asiegbu (Onyeonagu & Asiegbu, 2013) and Euclides-Batista (Euclides-Batista et al., 2016) who reported higher pasture height than the obtained results in the current study for GF30 (61 to 82 cm) and GF60 (83 to 121 cm), respectively.

However, the Tanzania grass managed at GF60 and combined with 30 or 45 cm of CHs, detected higher plant height values than other combinations (i.e., GF × CHs). As for tiller population per square meter, Onyeonagu and Asiegbu (Onyeonagu & Asiegbu, 2013) determined higher tiller number than the present study for GF30 (446 vs. 304 tiller/m²) and GF60 (366 vs. 241 tiller/m²).

Although our study, lacked to demonstrate differences between CHs or GF × CHs interaction. Euclides et al. (2018) observed an increase in the number of new tillers for Tanzania grass managed with more intense defoliation (25 cm) when compared to lenient defoliation (50 cm). Therefore, our result agree with De Sousa et al. (2019) who encountered lower tiller population at grazing intervals of 45 days when compared to the frequencies of 32 days. Since, the continuous renewal of dead tillers, increasing the availability of biotic and abiotic factors, guarantees the persistence and perenniality of the pastures (Costa, Paulino, Magalhaes, Rodrigues, & Santos, 2016). Consequently, in our conditions Tanzania grass at 30 cm was the most optimal cutting height, which was highly correlated to the higher tiller population ($r = 0.87$; $P < 0.001$).

On the other hand, the DM/ha⁻¹ was strongly influenced by grazing frequency, which our case at GF60, the Tanzania grass indicated higher DM values with respect to other GF. However, our results were lower than to those obtained by Verdecia et al. (2008) for GF30 (2.1 vs. 3.4 t) and GF45 (6.6 vs. 4.8 t) in the rainy season, although in the Ecuadorian conditions Tanzania grass realized a high drought tolerance. A study conducted by Cedeño-Aristega et al. (2021) in subtropical Ecuadorian conditions obtained similar DM/ha⁻¹ compared to our research for Tanzania grass managed at GF30 (2.1 vs. 2.4 kg DM/ha⁻¹) and GF45 (4.8 vs. 4.9 kg DM/ha⁻¹).

As a result, for our conditions, the best combination regarding DM/ha⁻¹, the Tanzania grass should be managed at GF30 and 45 cm of CHs, to restore their organic reserves, especially non-structural carbohydrates and the formation of new leaves, which would favour the appearance of a new generation of tillers (Costa et

al., 2021). In summary, an adequate management of pastures, defoliation intensity and frequency need to be reconciled, in order to ensure forage productivity and quality, and mainly its persistence.

Nutritional composition and forage quality

Cedeño-Aristega et al. (2021) reported a slight lower CP content in Tanzania grass than our study at GF30 (124.6 vs. 146.8 g/kg⁻¹) but similar at GF45 (143.8 vs. 141.3 g/kg⁻¹). However, our CP contents differed to those reported by Verdecia et al. (62; GF30, 116 g/kg⁻¹ and GF45 97 g/kg⁻¹) and Patiño-Pardo et al. (64; GF30, 118 g/kg⁻¹ and GF45, 65 g/kg⁻¹). In addition, Patiño-Pardo et al. (2018) reported a similar CP content than our study for Tanzania grass at 30 cm (123 vs. 120 g/kg⁻¹) and different to those realized by Da Silva et al. (65; 30 cm, 144 g/kg⁻¹) and Euclides-Batista et al. (43; 30 cm, 116 g/kg⁻¹). Nevertheless, our CP contents of Tanzania grass at 45 cm (141.8 g/kg⁻¹) were higher than to the obtained by Euclides et al. (2016; 123 g/kg⁻¹), Da Silva et al. (2019; 119 g/kg⁻¹), Patiño-Pardo et al. (2018; 101 g/kg⁻¹) and Aganga and Tshwenyane (2004; 88 g/kg⁻¹), respectively. Additionally, this study had a similar CP value in the rainy season such to the reported by González Marcillo et al. (2021).

The CP concentrations were negatively and linearly affected by grazing frequencies ($P < 0.001$). It might be explained for a dilution of the CP contents due to greater forage accumulation, due to the stage of maturity influences forage quality. In this sense, Costa et al. (2021) stated that pastures of *Panicum maximum* provided significant reductions in N contents, by increasing the rest periods, as a consequence of the increase on NDF and ADF contents, leading to a greater proportion of lignified tissues and lower protein content, conferring greater values of poorly digestible plant components (Da Silva et al., 2019). Furthermore, forages with low frequency of defoliation has a high proportion of senescent leaves, which contain low nutrient content (Carnevalli et al., 2006), due to the self-shading of the basal leaves by the leaves positioned in the upper portion of the plant. According to Costa et al. (2021), leaf senescence expresses the process of competition for metabolites and nutrients between old and young leaves in growth, which reduces the availability of good quality forage. However, our results yielded that Tanzania grass managed at 45 cm of CHs had sufficient leaf area to ensure fast regrowth, avoiding stem accumulation and so greater nutritional composition. Therefore, the nutritive values of the forages are associated with the ratio between structural and metabolic tissues and is closely related to the morphological traits. Of the nutrients required for animal production, protein is the costliest and usually is the first limiting for fibrous feeds. Besides, according to Coleman and Moore (Coleman & Moore, 2003) when dietary CP is below about 8% of the diet on DM basis, CP content has a strong relationship with intake. Consequently, in terms of chemical composition, the Tanzania grass managed either at GF30 and GF45 and combined with 45 cm of CHs might supply greater nutritional components (i.e., high CP and low NDF and ADF contents) for feeding ruminants. Waghorn & Clark (2011), stated that diets containing high fibre concentrations and very low CP contents are not optimal for high-producing ruminants. Since, it implies a longer retention time in the rumen owing the lower rate of passage, thus leading to greater CH₄ emission (Meister et al., 2021).

Forage quality determines the among of nutrients that herbivores are able to acquire from ingested forages (Briske et al., 2008). In the present study, the RFV index estimated in base to chemical composition indicated differences for the different planned grazing strategies (Table 3). Although, Oppong et al. (2008) argue that cutting height usually lacks to influence the quality of forages. Keba et al. (2013) and Ogoukayode et al. (2021) reported high RFV index for guinea grass *Panicum maximum* (55 to 82), being similar to our results found in Tanzania grass (54 to 89). However, the RFV index estimated in diet involving roughage, has not achieved general acceptance by nutritionist (Zinn & Ware, 2007). This combines into a single number the digestibility of the forages and an estimate of how much forage will be consumed (Horrocks & Vallentine, 1999; Winkler, 2000). Therefore, such calculations can be misleading when forage is the sole source of livestock nutrition, and such index should be carefully interpreted. On the other hand, the Tanzania grass managed at different grazing strategies realized a lower RFQ index compared to RFV. This might be explained as RFQ used chemical components such as NDF, ADF and CP, NFC as well as ash contents. Consequently, RFQ will give a more accurate prediction of animal performance as it is based on more accurate prediction equations. Nevertheless, these adopted laboratory approaches (RFV and RFQ, respectively) for assessing quality are not well accepted by nutritionist (Dan Undersander, 2015) since it is a simple empirical prediction system that fundamentally relies on linear equations (Dereje, Mulugeta, & Geberemariam, 2017).

Feeding values

Energy values

Forages supply energy mostly in the form of structural carbohydrates (i.e., cellulose and hemicellulose). However, increased fibre concentrations with maturation understates their impact on feeding values (FV) because bond strength between lignin, cellulose and hemicellulose intensifies with maturity (Waghorn & Clark, 2011). This might support our results in which the energy contents in grass Tanzania decreased when increase the grazing intervals, as indicated in Table 3, tending to be negatively correlated CP with NDF ($r = -0.39$; $P = 0.070$) and ADF ($r = -0.49$; $P = 0.020$) contents, respectively. In this sense, the Tanzania grass managed at GF30, and 40 cm of CHs realized greater GE contents than other planned combinations and higher than referential values in the literature (Aumont, Caudron, Saminadin, & Xande, 1995; INRA, 2018) due to our greater CP contents, being positively correlated (CP and GE contents, $r = 0.99$; $P < 0.001$). In addition, Ogoukayode et al. (2021) reported lower referential ME contents for *Megathyrus maximus* (ranged from: 1.23 to 1.28 Mcal/kg DM) than to the obtained in this study (1.33 to 2.48 Mcal/kg DM), which might be explained by our modest NDF contents of Tanzania grass managed at GF30 and GF45. Although Aganga and Tshwenyane (Aganga & Tshwenyane, 2004) informed a greater ME content at 40 cm of CHs than our study (2.23 vs. 1.88 Mcal/kg DM).

Organic matter digestibility (OMD) is a crucial factor determining nutritive value and ED due to its multiplicative effect on both net energy concentration and ingestibility (Peyraud & Delagarde, 2013). Consequently, the forages with high fibre contents have low OMD as well as minor ME contents for the destination to milk or meat production, leading within our study to negative correlations

(ME/NDF, $r = -0.45$; $P = 0.030$). Thus, dietary ME content is able to provide a guide to feeding value as animals will usually have high intakes of forages that have a high ME content (Waghorn & Clark, 2011). Finally, the UFV values estimated in this study for GF (0.60 to 1.33 Mcal/kg DM) and CHs (0.94 Mcal/kg DM, on average) were different from the referential feed tables of INRA (36; 0.41 to 0.70; Mcal/kg DM) as a consequence of our richer chemical composition. In the same sense, the GE, DE and ME contents were conditioned by weather conditions, but unfortunately, there are not more studies in Orellana Province, so these results could be considered as referential.

Protein values

The Tanzania grass managed at different grazing strategies under tropical Ecuadorian conditions demonstrated variations on the estimated protein contents. Referential PDI values for guinea grass *Panicum maximum* reported by Aumont et al. (1995) were lower than our study (28 vs. 73 g/kg DM). Nevertheless, these values dependent on maturity stage, due to protein from dietary origin are obtained from forages (Waghorn & Clark, 2011). Consequently, in the feed tables of INRA (INRA, 2018) the PDI values varied according to growth age and the contents ranged from (71 to 86 g/kg DM). Supporting our high correlation found between CP and PDIA contents ($r = 0.99$; $P < 0.001$). Therefore, the lower proportion of CP in forages becomes the first limiting factor to intake, as a result of the lower activity of microorganisms in the rumen because of the low availability of nitrogen substrate for synthesis of microbial protein, especially cellulolytic microorganisms (Bach, Calsamiglia, & Stern, 2005; Garcez, Alves, & Macedo, 2020).

On the other hand, the amount of RPB obtained in this study according to equations proposed by INRA (2018) varied among treatments, which also decreased according to regrowth age and CHs, although there is no official data in the context of Ecuadorian conditions. In the feed tables of INRA (2018) have been reported RPB contents for guinea grass *Panicum maximum* ranging from (-5 to -44 g/kg DM) but these contents depend of its regrowth age. Therefore, CP contents were positively correlated with RPB ($r = 0.98$; $P < 0.001$). The RPB is a new trait that reflects the difference between the microbial protein synthesis allowed by degraded proteins in the rumen and that allowed by the energy from fermentable organic matter (MOF) in the rumen (Sauvant & Nozière, 2016). Also, it has been demonstrated a decrease of OM digestibility when RPB decrease under zero (INRA, 2018). Consequently, higher nutritive values for Tanzania grass may be obtained through suitable grazing strategies.

Conclusions

Based to our results, the INRA (2018) as a feed evaluation system allowed to estimate with very precision the feeding values of *Megathyrsus maximus* under Ecuadorian conditions. Even though, the agronomic data did not significantly vary by season effect, the Tanzania grass managed with a high defoliation frequency (i.e., GF60) had higher agronomic measurements than to those observed in GF30 and GF45. However, it does not necessarily ensure higher nutritional components due to more stem and dead material relative to leaf compared to shorter rest periods in which increased the distribution of leaves in basal stratum. Therefore, the findings of this study accentuate that forage feeding values were primarily

conditioned by grazing management and weather conditions. Consequently, the Tanzania grass within Ecuadorian tropical conditions might be managed at grazing frequencies from 30 or 45 days and cutting heights of 45 cm above the ground, in order to reconcile production, regrowth vigour and nutritional values for raising cattle. Finally, we suggest further long-term field experiments and preferable with the use of animals in order to endorse the recommended defoliation regimens managements for Tanzania grass (*Megathyrsus maximus*).

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Conflict of interest

The authors declare no conflict of interest.

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