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Rumen degradation kinetics of maralfalfa grass (*Pennisetum* sp.) at different maturity stages

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ABSTRACT

Objective: To evaluate the chemical composition, *in situ* dry matter degradability (ISDMD) and potential degradability of dry matter (PDDM) of maralfalfa grass.

Design/Methodology/Approach: Maralfalfa grass was harvested at four maturity stages (45, 60, 90 and 120 days). The *in situ* dry matter degradability was determined by the nylon bag technique using two Holstein bulls provided with a ruminal cannula. Data from chemical composition, ISDMD and PDDM were analysed in a completely random design.

Results: Dry matter, organic matter, ether extract, neutral detergent fiber, acid detergent fiber, crude fiber and hemicellulose increased as plant maturity advanced, whereas crude protein (13.69 to 9.73%) and total ash (17.44 to 11.20%) decreased. The *in situ* dry matter degradability and PDDM at 45 d, showed the highest values in relation to other maturity stages at all incubation periods ($P<0.05$), however, effective dry matter degradable was similar at 45 and 60 d ($P>0.05$). The results showed a high availability of nutrients in maralfalfa grass as indicated by the high crude protein, ISDMD, PDDM values, low neutral detergent fiber, and hemicellulose content.

Study Limitations/Implications: The evaluation of the in situ dry matter degradability of maralfalfa grass allows the identification of its optimal use point in order to implement feeding strategies for based on forage or cut pastures ruminant productions systems.

Findings/Conclusions: Based on changes in chemical composition, ISDMD and PDDM, 45 d of growth was considered as optimum stage of harvesting the *Pennisetum* sp. as a forage resource for maximum utilization of nutrients by the ruminants.

Key words: forage; ruminal degradation; tropical grass.

INTRODUCTION

In 2023, the ruminants provided more than 175 million tons of meat and 950 million tons of milk (FAO, 2023ab), as important source of protein to human diets worldwide, nevertheless, in the next 25 years, it will be necessary to increase the production of animal protein twice derived from ruminants to ensure the demand of a growing world population (Barahona and Sánchez 2005; Gerber *et al.*, 2013; Guyander *et al.*, 2016). Forage resources play a fundamental role in ruminant nutrition and provide over 90% of the energy consumed by them around the world (Wilkins, 2000; Humphreys *et al.*, 2014). Ruminants can convert low-quality forages or vegetal material produced on land unsuitable for crops production for human consumption into high quality protein (Varga and Kolver 1997; Guyader *et al.*, 2016). The above is possible because the rumen microorganisms synthesize and secrete an enzyme complex of β-1-4 cellulases that allow the hydrolysis of forage cell walls (Kobayashi *et al.*, 2004).

In the tropics, forage is the main source of animal feed for milk and meat production, but with low production rates, it is very important to increase productivity and profit-earning capacity in these areas. Currently, the intensive use of cut forages is an alternative that minimizes waste of fodder by trampling, avoids energy expenditure during grazing and even avoids the residue generated by the selection of forage by the animals, thus achieving greater efficiency in animal production (Castillo-Aguilar *et al.*, 2019; López *et al.*, 2020).

Maralfalfa grass (*Pennisetum* sp.), is a perennial tropical grass with high productivity, it has been introduced to Latin-American countries (Colombia, Brazil and Venezuela, among others), due to its potential as forage for ruminants, with a productivity around 60 t ha^{-1} (Márquez *et al.*, 2007; López *et al.*, 2020), and a great adaptability from sea level to elevations over of 3000 m above sea level, high crop yield in soils with medium or high fertility and low pH (Clavero, 2009). In contrast, it should be considered that it does not tolerate floodable soils. Also, feeding dairy cows with maralfalfa grass was indicated by the smallholder dairy farmers that the milk yields increase twofold (Maleko *et al.*, 2018).

A decrease in the nutritional quality of the maralfalfa grass has been reported as maturity increases (Márquez *et al.*, 2007; Palacios *et al.*, 2013); however, in recent years and due to its characteristics as cutting pasture, the use of this forage has increased in the feeding of dairy, meat and dual-purpose cattle production systems in the tropics, however, the information available on this forage for ruminant feeding is lack and consistent. Evaluate the chemical composition and in situ dry matter degradability (ISDMD), determine the rumen kinetics of the animals that consume this forage is essential to estimate the availability rumen of the dry matter, as well as, variables of rumen kinetics such as the degradation rate the degradable dry matter or potential ruminal degradation of maralfalfa grass at different maturity stages to determine the optimum stage of harvest, information that allows us to infer how to use and integrate this forage as a source of replacement or supplementation of ruminants feeds, object of study of the present experimental work.

MATERIALS AND METHODS

Location of the study

Maralfalfa grass was cultivated in an experimental site of Zapotlán, El Grande, Jalisco, Mexico, with geographic coordinates of 19° 27' 13" north latitude and 103° 27' 57" west longitude, with an elevation of 1,520 m. Chemical determinations were carried out in the laboratory of nutrition of the Centro Universitario del Sur of the Universidad de Guadalajara located in the same county (IIEG, 2024).

Chemical analysis

Plant material was harvested at growth intervals of 45, 60, 90 and 120 days from a 4 m^2 area in triplicate at each stage of harvested. The samples were dried following the procedure AOAC (2016) and ground by using Wiley mill (Thomas Scientific® Swedesboro, NJ, USA) through 2 mm sieve for further analysis. Crude protein (CP) was determined by the Kjeldahl method, crude fibre (CF), ether extract (EE), ash and organic matter (OM) were measured by incineration in an electric muffle furnace at 550 °C as described by AOAC (2016). The determination of the neutral detergent fiber (NDF) and acid detergent fiber (ADF) was performed using α-amylase without a correction, as specified by Van Soest *et al.*, (1991). The total carbohydrates (TC) content was determined using the following equation: $TC = OM - (EE + CP)$, described by Sniffen *et al.* (1992). The non-fiber carbohydrates (NFC) content was determined by the following equation: $NFC = 100 - (CP + NDF + EE + ash)$ according to Weiss (1999) and metabolizable energy (ME) per kilogram of dry matter was calculated according to Weiss (1993).

In situ dry matter degradability

The *in situ* dry matter degradability (ISDMD) was determined using a two 4-year-old Holstein bulls (325±23 kg) provided with a ruminal cannula with a core diameter of 10 cm (Bar Diamond Lane, Parma, ID, USA) and housed in individual pens. The experimental conditions of the animals adhered to the official Mexican standard of technical specifications for the production, care and use of laboratory animals (NOM-062-ZOO-1999). The ISDMD, was determined by the procedure reported by Vanzant *et al.* (1998). Nylon bags (10×15 cm, pore size 40-60 μm) with 5 g of sample were used. Each sample was incubated in triplicate for 0, 16, 24, 36, 48, 72, and 96 h in the rumen. The zero-hour samples were processed in the same way as the incubated samples, excluding the rumen incubation step. Upon removal from the rumen, bags were rinsed 3 times with cold water in a washing machine set to agitate for 6 min each rinse. Additionally, blanks without substrate were incubated in each run, blanks were secured with nylon thread to a piece of string (length: 30 cm; weight: 150 g) and left suspended in the rumen. Subsequently, the bags were removed from the rumen according to the incubation times along with the zero hour and washed with running tap water at low pressure until the water came out clear. Bags were dried in a circulating air oven (48 h at 60 °C). The DMD for the experimental material from each incubation time was calculated by the weight loss of the samples in bags during ruminal incubation. The dry weight was recorded, and the degradability of the dry matter was determined.

Non-linear degradation characteristics ruminal (rapidly soluble fraction, degradable dry matter fraction potentially digestible, rate of degradation of the degradable fraction of the dry matter) and the effective degradability of the DM were calculated using the model described by Ørskov and McDonald (1979) and modified by McDonald (1981): $P = a + b(1 - e - ct)$; Where; *a*, is the washing loss or soluble (%); *b*, is the insoluble, but potentially digestible fraction (%); *P*, is the degradation of DM (%); $a+b$, potential degradability (%); *c*, is the fractional degradation rate (h^{-1}); and *t* is the time (h). Ruminal turnover constants (k) at 2, 5, and 8% h^{-1} which are representative of low, medium, and high consumption respectively (ARC, 1984), were used to model effective degradation (ED; Ørskov and McDonald, 1979):

$$ED = a + (b * c)/(c + k)$$

Statistical analysis

The fractions of Non-linear degradation characteristics ruminal were calculated using the NLIN procedure of SAS. Data from chemical composition, ISDMD and potential degradability of DM were analyzed in a completely random design using the PROC GLM procedure of SAS statistical package (Statistical Analysis System - SAS, version 9.1.3, 2006) with the following model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij}= the value of each observation, μ=overall mean, T_i=treatment effect and e_{ij}=experimental error.

Significant effects of the treatment were declared at $P<0.05$. Significant differences in the means among treatments were evaluated by Tukey's multiple range tests.

RESULTS AND DISCUSSION

The content of DM, OM, NDF, ADF, CF and hemicellulose (HC), increased as the cuts progressed, higher values were found at 120 d. No changes were recorded between 45 and 90 d on the crude protein and ash contents, while showed the lowest content at 120 d harvest period. Ether extract, did not show a consistent trend through the different harvest days (Table 1). In overall, the content of DM and ADF were lower, the ash and CP values were higher and the NDF content were similar to lower than those reported by Turano *et al.* (2016), in some *Pennisetum purpureum* hybrids and Napier grass varieties under rain-grown and different irrigated conditions.

The recorded DM, CP, and fiber fractions concentrations higher than those reported by Carulla *et al.* (2004), at cutting ages of 47 to 120 d, but are similar in CF and EE, at 45 and 65 d of maturity to the concentrations reported by Ramírez and Pérez (2006) and Gómez *et al.* (2020) on maralfalfa grass, although these authors reported a lower CP content. Márquez *et al.* (2007) and Correa (2006), reported values of 21.8% of CP and 2.5% of EE, at 56 d of age, which are higher than those recorded in this study. Cerdas (2015) reported DM and CP values similar to those found in this study for maralfalfa grass at 49 d. Correa (2006), Marquez *et al.* (2007) and Cerdas (2015), reported that CP content decreases as maturity advances; Citalán *et al.* (2012), reported that the CP content is higher between 45 and 60 days of maturity and recommend harvest at these maturity stages. The *Pennisetum* genus showed similar results that those obtained in this study (González *et al.*,

Table 1. Chemical composition (%) of maralfalfa grass harvested at different stages of growth.

Variables	Maturity stage (days)				
	45	60	90	120	SEM
DM	14.04^{bc}	16.48^{b}	21.05^{a}	22.13^{a}	0.30
OM	82.56^{c}	84.89^{b}	87.13^{a}	88.80^{a}	0.24
CP	13.69^{a}	13.78^{a}	12.59^{a}	9.73^{b}	0.14
EE	1.48^{a}	1.14^{a}	1.94^{a}	2.44^{a}	0.02
CF	39.89^{b}	40.76^{b}	44.25^{a}	46.34^{a}	0.12
NDF	52.74^{c}	59.93^{c}	64.27^{b}	72.12^{a}	0.15
ADF	28.31^{c}	33.65^{b}	35.66^{b}	46.57^{a}	0.18
HC	17.08^{c}	26.28^{b}	35.96^{a}	25.55^{b}	0.15
Ash	17.44^{a}	15.11^{a}	12.87^{b}	11.20^{b}	0.19
TC	67.39^{b}	69.97^{b}	72.60^{ab}	76.63^{a}	0.29
NFC	14.65^{a}	10.04^{b}	8.33^{b}	4.51^{c}	0.32
ME (Mcal/kg)	2.76^{a}	2.75^{a}	2.84^{a}	2.83^{a}	0.02

$^{a\text{-}c}$ Different letters in the same row indicate differences ($P<0.05$). DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; CF: Crude fibre; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; HC: Hemicellulose; TC: Total carbohydrates; NFC: Non-fiber carbohydrates; ME: Metabolizable energy; SEM: Standard error of the mean.

2011; Ramos, 2013); however, the differences found may be associated with the variety and growth time, the fertility of the soils and specially the weather where that research were conducted (Correa *et al.*, 2006; Clavero and Razz, 2009).

According to Gómez *et al.* (2020), DM content increased with age, same results were found by González *et al.* (2011), that reported changes in DM of green and purple elephant grass (*Pennisetum purpureum*) starting at 42 d, reaching the highest DM concentration 70 d. These changes in DM may be due to the genotype, variety, and age, since the maturity of the shoots increases; in the same manner the DM content increases in stems and leaves (Márquez *et al.*, 2007).

Metabolizable energy is the energy available in feed for intermediary metabolism, which is retained in the animal's tissues and used for maintenance and growth or for the formation of animal products. Therefore, determining its content in feed allows for more precise animal diet formulations (Geren *et al.*, 2020). Likewise, Mertens (1996) showed the energy content of pastures decreases with maturity, limiting its consumption and use by the animal. The energy content in maralfalfa may vary depending on the cutting age, however, in this study, no significant differences have been found in EM among different cutting ages, probably because it reached physiological maturity relatively quickly, meaning that energy content can be stabilized at an early stage of growth and also or even the moisture content of the plant (Buxton, 1996; Ventura, 2019). The chemical composition results reported herein showed that the nutritional value of the forage progressively decreases with the maturity of the plant, due to an increased cell wall concentration and lower cell content percentage (Ramírez *et al.*, 2011; Herrera, 2015; Ledea *et al.*, 2017).

The *in situ* dry matter degradability (ISDMD) of the maralfalfa grass was different among the four cutting days of harvested ($P<0.05$). At 45 d harvest period, the highest

ISDMD values of all incubation times were found (Table 2). The lower ISDMD values after 45 d harvest period, may be attributed a higher content of non-fibre carbohydrates (NFC) that consist in starches, simple sugars and soluble fiber (Turano *et al.*, 2016), which are the immediate source of energy for rumen microorganisms, which causes an increase in the degradability of dry matter, and explains the results of this study (Jung and Allen, 1995; Agnanga *et al.*, 2005; Jagadeesh *et al.*, 2017) and a high content of cell wall, which indicate a higher concentration of structural carbohydrates, with a lower concentration of NDF.

In the early stages the leaves of maralfalfa grass contributed >50% total herbage yield. Consequently, the percentage of stem and dead material increased, and leaf contribution decreased (Calzada *et al.*, 2014). Similar results were reported by Correa (2006), the dry matter degradability in rumen decreased as the harvest periods advanced, also, these authors reported values of 56.9% to 49.1% at 56 and 105 d of cutting age, respectively, which are lower than those reported herein.

Ruminal degradability of the maralfalfa is shown in Table 3. All dry matter fractions (a, b and c) were different among the maturity stages of the maralfalfa grass (P<0.05; Table 3), at 60 and 120 d harvest period there was the highest amount of soluble DM, and at 45 and 90 d the lowest amount. Degradable dry matter fraction (b) shows the higher values at 45 and 90 d and lowest at 60 and 120 d and was similar for non-degradable fraction.

The potential degradability of dry matter (PDDM) was different between the maturity stages of the maralfalfa grass (P<0.05; Table 4), at 120 d the highest values of PDDM were found, while at 90 d the lowest were registered.

Table 2. *In situ* dry matter degradability (%) of maralfalfa grass harvested at different stages of growth.

Incubation time, h	Stage of maturity (days)				
	45	60	90	120	SEM[1]
96	81.74[a]	72.62[b]	67.82[c]	60.63[d]	0.85
72	79.59[a]	73.71[b]	63.59[c]	60.00[c]	0.90
48	76.47[a]	69.59[b]	61.33[b]	53.49[c]	0.81
36	74.80[a]	65.85[b]	58.87[c]	51.72[d]	1.14
24	61.47[a]	54.20[b]	49.44[b]	50.94[b]	1.08
16	58.85[a]	58.47[a]	45.07[b]	45.07[b]	2.37

[a-d] Different letters in the same row indicate differences (P<0.05). SEM: Standard error of the mean.

Table 3. Effect of stage of maturity of maralfalfa grass on ruminal degradability parameters (%).

	Stage of maturity (days)				
Variables	45	60	90	120	SEM
Soluble (a)	31.70[b]	42.89[a]	24.30[c]	39.06[a]	0.58
Degradable (b)	50.68[a]	33.31[c]	43.54[b]	25.83[d]	0.62
Non-degradable	17.62[a]	23.80[b]	32.16[c]	35.11[c]	0.45
Constant of degradation (c)	0.044[a]	0.029[b]	0.039[a]	0.019[c]	0.08

[a-c] Different letters in the same row indicate differences (P<0.05). SEM: Standard error of the mean.

According to Sanchês *et al.* (2018), reduction in fraction a, is due to plants has the tendency in accumulating supporting structures that collaborate with cell-wall densification. Fraction a is composed of amides, amines, and nitrogenous bases, as well as components of the cell wall such pectin, and corresponds to the DM that is lost or solubilized during washing and represents the first contribution of nitrogen and energy for the microbial activity of the rumen (Van soest, 1994). The degradable fraction in the rumen (b) and non-degradable fraction of dry matter affect the ruminal degradability of the DM and works as direct indicators of the quality of the forage. The higher values of these proportions were at 45 d harvest period ($P<0.05$), which coincides with the ruminal degradability coefficients of DM found in this study. The fraction b presented lower values at the advanced cutting days (Sanchês *et al.*, 2018). The rate of dry matter degradation differed among cutting ages ($P<0.05$), being the slowest at 120 d, which agrees with the ruminal degradability percentages and the different DM proportions found at this harvest period in the study. Correa (2006) reported similar values in maralfalfa grass at 56 and 102 d harvest periods.

The sum of fractions a and b corresponds to the potentially digestible DM, this being the maximum proportion of DM available for rumen degradation, a decrease in potential degradability at 60, 90, and 120 cutting days, can be justified by the increase in the proportion of lignified tissue (Sanchês *et al.*, 2018). The effective degradability of dry matter is a value that allows to know the real degradation of DM, considers the speed and onset of degradation, potential degradability, and other relevant aspects of ruminal kinetics (Singh *et al.*, 1989). Differences were found in the potential and effective degradability of the DM ($P<0.05$) of maralfalfa grass at four stages of maturity (Table 4), where at lower ages of maturity, there is a greater amount of DM that can be potentially degraded.

The tropical grasses in the first stages of growth present a thin cell-wall with low content of fiber, this allows an easy breakdown and short digestion times. When maturity increases, the vascular structures of the leaves tend to get thicker, likewise the vascular tissue and sclerenchyma of both leaves and stems lignify, becoming physically stronger and difficult to degrade (Barahona and Sánchez 2005; Cerdas, 2015). Dry matter production and crude protein are the two most studied variables in pasture evaluation, both variables are negatively correlated. If a grass is selected for its forage production, this can be detrimental to the CP value and vice versa. Hence, the importance of offering alternatives that allow the simultaneous evaluation of DM production and CP content in tropical grasses. The

Table 4. Effect of stage of maturity of maralfalfa grass on potential degradability and effective degradable DM.

Stage of maturity (days)	Potential degradability %	Effective degradation		
		2	5	8
		%/h		
45	82.38^a	66.70^a	55.50^a	49.80^a
60	76.20^b	67.80^a	55.20^a	51.90^a
90	67.84^b	59.00^b	43.50^b	38.60^b
120	64.89^b	56.20^b	46.40^b	44.20^b

$^{a, b}$ Different letters in the same row indicate differences ($P<0.05$).

nutritional value of maralfalfa grass, reduces as the regrowth age increases, mainly due to the reduction in CP contents, ash and ISDMD. Therefore, is recommended to harvest maralfalfa grass between 30-45 d since it showed the best nutritional value (Bacorro *et al.*, 2019), which in may result in improved of the animal performance and productivity.

CONCLUSION

The results showed high nutrient availability in maralfalfa grass as indicated by the high CP, ISDMD, potential degradability values, low NDF, and hemicellulose content registered at 45 d of cutting age. Furthermore, the DM degradability declines with maturity, appropriate management and harvesting at the optimal balance between nutritional quality and dry matter production are required to take advantage of the greater contents of nutrients. At the ruminal level, degradable DM in maralfalfa is higher at 45 d, so it begins to degrade earlier and at a higher degradation rate and with a lower proportion of non-degradable DM, which indicates a high nutritional quality at that cutting age. At 90 d of age, it also shows an acceptable degradable DM, although higher non-degradable DM but with a degradation rate similar to that of 45 d. The potential degradability of DM at 45 d is almost 20% higher than the cut-off age of 120 days, but only 6% at 60 d. In general, the results found indicate that the ruminal availability of maralfalfa DM is high at 45 to 60 d of maturity, with a higher digestion rate at 45 d. It is important to highlight that we should not only consider the characteristics of the forage such as the chemical composition, but also the onset and speed of degradation, potential degradability, among others, since these parameters have a significant effect on the amount of dry matter and nutrients that may be available to the animal that consumes this forage.

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