RESEARCH ARTICLE

Determining the pre-grazing sward height of Kikuyu grass (*Cenchrus clandestinus* - Hochst. ex Chiov.) for optimizing nutrient intake rate of dairy heifers

Alejandra Marín Gómez[1,2]*, Emilio A. Laca[3], Tiago Celso Baldissera[4], Cassiano Eduardo Pinto[4], Fábio Cervo Garagorry[4], Angel S. Zubieta[1], Carolina Bremm[1], Jerôme Bindelle[5], Paulo César de Faccio Carvalho[1]

1 Grazing Ecology Research Group, Federal University of Rio Grande do Sul, Porto Alegre, Brazil,
2 Facultad de Ciencias Agrarias, Departamento de Producción Animal, Universidad Nacional de Colombia, Medellín, Colombia, 3 Department of Plant Sciences, University of California, Davis, California, United States of America, 4 Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Lages, Santa Catarina, Brazil, 5 Precision Livestock and Nutrition Unit, AgricultureIsLife, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, Liège University, Gembloux, Belgium

* amaring@unal.edu.co

Abstract

Understanding the grazing process and animal response to sward structures (e.g., sward height) is key to setting targets for efficient grazing management. We hypothesized that the short-term intake rate (STIR) of dry matter (DM) and digestible organic matter (OM) by dairy heifers is maximized with Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov.) of intermediate sward heights. The treatments consisted of five pre-grazing sward heights (10, 15, 20, 25, and 30 cm) randomly assigned to two of ten paddocks. The experimental design included two measurements of each paddock at different periods and times of day. Three Holstein heifers (440 ± 42 kg body weight) were used to determine the STIR, which was estimated using the double-weighing technique with correction for insensible weight losses. The bite mass (BM), bite rate (BR), sward structural characteristics, and nutritional value of herbage samples were assessed. The data were analyzed using mixed models with a factorial arrangement of five sward heights, two times of day, and two evaluation periods. The sward height of Kikuyu grass that maximized both STIRs was approximately 20 cm. The STIR of the DM was 30% and 15% lower than the maximum in the shortest and tallest swards tested, respectively. In swards shorter than 20 cm, the STIR was lower because the BM decreased with sward height, whereas in those greater than 20 cm, the lower BM and STIR of DM was explained by a decrease in bulk density and bite volume. The top stratum was composed mainly of highly digestible leaf blades with similar nutrient content across sward heights; therefore the STIR of digestible OM was also maximized at 20 cm. Hence, the optimal pre-grazing sward height of Kikuyu grass should be managed at 20 cm under rotational stocking systems to maximize nutrient intake rate of dairy heifers.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The intake of dry matter (DM) and digestible nutrients is an essential driver of livestock productivity [1]. In dairy production systems where cows are stall-fed, the quantity of nutrients offered is highly controlled and intake per unit of feeding time is greater and more efficient than pasture-based systems because feeds are nutrient and energy-dense [2,3]. In pasture-based dairy systems, forage is spread over large areas, and the rate of food intake is determined by bite mass (BM) and the time required to find, gather, chew, and swallow each bite [4,5]. The instantaneous intake rate during grazing is the product of the BM and bite rate (BR) [6]. On a daily scale, intake rate is also affected by the time required for rumination and digestion, social interactions, rest, and milking [7–9]. In this context, intake per unit of feeding time, hereafter referred to as short-term herbage intake rate (STIR), generally limits daily intake, and increases in STIR translate into greater productivity [10]. Therefore, if grazed herbage is the main source of nutrients for dairy cows, it is pivotal that animals have continuous access to pastures with structures that allow for the maximum STIR.

Sward structure plays an important role in the grazing process and determines the STIR [11–13]. Among the various sward structural characteristics (e.g., sward height, bulk density, herbage mass, and leaf: stem ratio), sward height has been identified as the main factor determining bite mass (BM) and STIR [12,14,15]. Therefore, to sustain high DM and nutrient intake rates, optimal sward height should be a grazing management target [10]. *Rotatinuous* stocking was proposed as a grazing concept to optimize and sustain STIR by maintaining swards at an optimal height for animal grazing that differs among forage species [10,14,16–18].

A high herbage intake rate combined with a high quality diet is key in pasture-based dairy production systems, especially in the tropics [19,20], where low levels of performance are partly attributed to the intrinsic lower quality of C4 compared to C3 grasses [21], among other things. Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov) is a subtropical species from East Africa that is widely used in the dairy systems of some regions, including Africa, Latin America, Australia, and New Zealand [22–24]. As optimal heights differ among forage species, farmers need to know the targets for their particular species. Hence, we aimed to determine the Kikuyu grass sward height that maximizes DM and digestible OM intake rate for dairy heifers and identify the mechanisms that explain the existence of such optimal height. We tested the hypotheses that 1) the short-term intake rate (STIR) of DM by dairy heifers is maximized at intermediate sward heights of the Kikuyu grass, and that 2) the maximum STIR of digestible OM occurs at the same sward height as that of DM. This study contributes to a better understanding of the grazing process and how animals respond to the sward height of subtropical grass species such as Kikuyu grass, and it provides a promising pre-grazing management sward height target for optimizing the nutrient intake rate of dairy heifers under rotational stocking.

Materials and methods

Ethics statement

This study was conducted in accordance with the recommendations of the Ethical Review Committee on the Use of Animals at the Federal University of Rio Grande do Sul, Brazil (approved project no. 33970) for separate experiments using the same non-invasive ingestive behavior protocol, because at the time of this study in 2017, the Animal Ethics Committee at the Santa Catarina Agricultural Research and Rural Extension Company was still being formed. The experimental animals were used in accordance with the Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching for Scientific Purposes [25].

Experimental site

The study was conducted at the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), municipality of Lages, S. C, Brazil (27˚47´10.5″ S, 50˚18´20.5″ W, 937 m. a.s.l.). According to the Köppen climate classification, the regional climate is Cfb-type. The mean annual temperature is 16.8˚C and the mean annual average precipitation is 1460 mm [26]. According to the USDA Soil Taxonomy [27], the soil of the experimental area was classified as humudept (with an umbric epipedon) [28].

The experiment was performed in a 5000 m^2 permanent pasture of Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov), established in the early 1990s that has been grazed by dairy and beef cattle since inception. The entire area was mowed to 5 cm of height (all cuttings were removed) and divided into ten paddocks of 500 ± 5 m^2 on January 15, 2017. The entire pasture received one application of 250 kg/ha of fertilizer (N-P-K, 9–33–12) and 135 kg/ha of urea on January 26, 2017 (first evaluation period). On March 22, 2017, 67.5 kg/ha of urea was applied (second evaluation period). Due to frost events and low temperatures in winter and possibly spring, Kikuyu dies at the end of autumn and regrows at the end of spring [29]; therefore, the data collection in this study lasted from February 28 to April 15, 2017.

Treatments and experimental design

Treatments consisted of five pre-grazing sward heights (10, 15, 20, 25, and 30 cm) of Kikuyu grass. Each treatment was randomly assigned to two out of ten paddocks. One paddock of each sward height was randomly assigned to a morning grazing session, and the other was assigned to an afternoon session. This resulted in a factorial of sward height and time of day, in which each treatment was applied to one experimental unit. Each paddock was grazed and the corresponding treatment was observed once during the first experimental period. Subsequently, each paddock was mowed to a height equal to half of its nominal height treatment and allowed to grow back until it reached the same target height used in the first period. The time-of-day factor was reversed for each paddock, and all paddocks were grazed for a second time during the second experimental period. Due to time constraints, only two paddocks were grazed and observed per day, once in the morning and once in the afternoon. The order in which the treatments were observed was randomized for each period. This resulted in 20 short-term grazing sessions.

The experimental design included two measurements of each physical paddock, albeit in different periods and times of the day. Therefore, data were analyzed with mixed models, including a random effect for the paddock, to account for the possible intraclass correlation of errors.

Animal measurements and grazing sessions

Three Holstein Friesian heifers with an average body weight of 440 ± 42 kg and 22 ± 2 months of age were used to determine the STIR. Short-term intake rates were assessed by weighing the animals before and after grazing and correcting for insensible weight losses (water evaporation, respiratory losses, carbon dioxide, and methane) as described in the double-weighing technique [30]. Heifers were familiarized with the experimental protocol one month before beginning the grazing trial and maintained in an adjacent area similar to the experimental paddocks with free access to Kikuyu grass. The same group of three dairy heifers grazed each paddock once in the morning and in the afternoon during each experimental period.

The animals were allowed to graze for 45 min during peak grazing times at 7:30 and 16:00 h (the first and last grazing meals, respectively [31]). The grazing session lasted 45 ± 5 min, which was considered the minimum time to detect body weight fluctuations [9]. The paddock

area (500 ± 5 m^2) was calculated to result in a less than a 10% change in average sward height during the grazing session, ensuring that the same sward structure at the bite level was available for the animals to graze over the entire course of each grazing session.

Before each grazing session, the three heifers were fitted with a feces and urine collection bag and an Institute of Grassland and Environmental Research (IGER) Behaviour Recorder (Ultra Sound Advice, London, UK) [32], which records the effective eating time (ET, the length of time that an animal spends eating during grazing) and number of grazing (biting and non-biting) jaw movements. The animals were weighed (W1) on an electronic scale (10 g precision), and turned into the appropriate paddock. The precise pre-grazing time (t1, min: s) was recorded with a timepiece synchronized to the IGER Behavior Recorder clock. After each grazing session, the animals were returned to the handling area and weighed again (W2). The precise time was recorded (t2) and the IGER Behaviour Recorder was removed. Heifers were weighed (W3) and the time was recorded (t3); they were left in the handling area without feed or water for 45 ± 5 min to measure insensible weight losses. The heifers were weighed again and the precise time (t4) was recorded. During grazing sessions, feces and urine were collected totally without leakage. The animals were not fasted at any time to avoid any alteration in their ingestive behavior [33,34] and diet selection [35]. Finally, all equipment was removed, and the animals were released in an adjacent area with free access to Kikuyu grass. The ET was determined by analyzing the grazing behavior recording using the software "Graze" (Ultra Sound Advice, London, UK) [36]. Thus, the STIR on a fresh-matter basis was calculated as follows:

$$STIR = \left\{ \left[\frac{(W_2 - W_1)}{(t_2 - t_1)} \right] + \left[\frac{(W_3 - W_4)}{(t_4 - t_3)} \right] \right\} \left[\frac{(t_2 - t_1)}{ET} \right] \qquad (1)$$

where W1 and W2 are pre- and post-grazing animal weight (kg), respectively; t1 and t2 pre- and post-grazing time (min), respectively; W3 and W4 are pre- and post-insensible weight losses (kg), respectively; t3 and t4 are the pre- and post-insensible weight losses time (min), respectively; and ET is effective eating time (min). ET was calculated as the total session time excluding intervals of jaw inactivity greater than 3 s [31]. The STIR of DM for each treatment was calculated as the STIR of fresh matter multiplied by forage DM content, which was estimated based on the hand-plucked herbage samples (as described below).

The STIR of digestible OM (g/min) was calculated as the product of the STIR of DM and *in vitro* OM digestibility (g/kg) obtained from grazing herbage samples simulated by hand-plucking. The bite mass (BM, g DM) was calculated as the product of the STIR and ET divided by the total number of bites. The bite rate (BR, bites per min) was determined for each animal and each grazing session by dividing the total number of bites by effective eating time.

Sward measurements

Sward height was measured at 150 haphazardly selected points per paddock, distributed over the entire paddock before and after each grazing session using a Hill Farming Research Organization type sward stick [37]. Pre-grazing herbage mass was assessed using three random herbage samples clipped at ground level using a metallic quadrat of 0.25 m^2. Each fresh herbage sample was immediately separated into leaf laminae, stems + sheaths, and dead material, and dried in a forced-air oven at 55˚C for 72 h. The total herbage mass (kg DM/ha) was the sum of the masses of all components. Bulk density (g DM/m^3) of the individual sward components was calculated as the corresponding clipped dry mass divided by sward volume clipped, which was the product of the quadrat area (0.5×0.5 m) and average sward height. Four representative herbage samples (~200 g fresh weight) were hand-plucked [38], two at the beginning

and two at the end of each grazing session, mimicking the closely observed grazing behavior of the heifers.

Herbage chemical analysis

Hand-plucked herbage samples were analyzed in duplicate for dry matter (DM; method 930.04; [39]), ash (method 930.05; [39]), and neutral detergent fiber (NDF), and acid detergent fiber (ADF) [40] by using an ANKOM 200 fiber analyzer without heat-stable alpha-amylase. NDF and ADF were expressed including residual ash. Samples were also characterized for N content by the Kjeldahl digestion (method 984.13; [39]). Crude protein (CP) was calculated as N concentration × 6.25. A two-stage [41] technique (incubation with rumen fluid followed by acid-pepsin digestion) was used to estimate the *in vitro* OM digestibility (IVOMD).

Statistical analyses

Statistical analyses were performed using R software version R 3.5.3 [42]. The relationship between ingestive behavior data (STIR of DM, BM, BR, and STIR of digestible OM) and sward height (SH) was analyzed as described by [17], which consists of fitting a double linear model or broken line model, as follows: $y = f\{p+a1(SH—v), p + a2(SH—v)\}$, where y is the STIR of DM, BM, BR, and STIR of digestible OM, f is the min (STIR of DM, BM, and STIR of digestible OM) or max (for BR) function; v and p are the coordinates of the point where lines cross; SH is the observed average value of sward height; and a1 and a2 are the slopes of the component lines. Before assessing treatment effects, values were corrected by subtracting the animal effects as follows: corrected Y = (original observation − animal average) + overall average. Ingestive behavior models were fitted to the data by deviance minimization using the optim {stats} function of the stats package in the R 3.5.1 [42]. We also used a quadratic equation to model the relationship between ingestive behavior data and sward height, and compared it to the broken line model using Akaike's information criterion (AIC).

Nutrient content and sward variables were analyzed using linear mixed-effects models with the lmer function of the lme4 package [43]. Treatment, time of day, period and their two-way interactions were the fixed effects and paddock was the random effect in the model: $y \sim (\text{treatment} + \text{time of day} + \text{period})^2 + (1 | \text{paddock})$. Significance was declared at $P < 0.05$ and tendencies at $0.05 < P \leq 0.10$. Residuals of the analyses were checked for normality using the Shapiro-Wilk normality test with the shapiro. test function in R [42].

Results

Sward structure and nutritional value

The pre-grazing sward heights obtained were close to the nominal treatment heights, and the differences between the pre-and post-grazing sward heights did not exceed the predetermined maximum of 10% of the initial height (Table 1). There were interactions among the treatments with the time of day and period of evaluation for the actual pre-and post-grazing sward heights ($P \leq 0.001$, S1 Text), showing slight reductions in 20 and 25 cm sward heights from the morning evaluation and Period 1 (Table 1).

Overall, there were interactions between treatments and the time of day and period for all the sward structure variables (S2 Text). No significant interactions were found between the time of day and period for any sward variable (S2 Text).

The total herbage mass and bulk density of the treatments are shown in Fig 1. Herbage mass increased linearly when sward height was assessed in the morning, but in the afternoon, it increased up to 20 cm and then decreased for taller sward heights (Fig 1A), which resulted in

Table 1. Pre- and post-grazing sward heights of Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov) at two times of day in two evaluation periods.

	Treatment					
	10	15	20	25	30	
	Pre-grazing Sward height (cm)					SEM
Time of day (AM)	10.0	15.9	19.7	23.7	32.3	0.34
Time of day (PM)	9.6	14.4	20.5	24.8	30.3	0.34
Period 1	10.2	15.7	19.6	24.5	30.8	0.34
Period 2	9.4	14.4	20.6	24.0	31.8	0.34
	Post-grazing Sward height (cm)					SEM
Time of day (AM)	9.5	15.1	17.8	22.1	30.0	0.56
Time of day (PM)	9.1	13.7	19.4	23.2	28.6	0.56
Period 1	9.4	15.2	17.8	22.6	29.5	0.56
Period 2	9.2	13.6	19.3	22.7	29.1	0.56

The times of the day AM and PM correspond to the morning and afternoon assessment respectively. SEM: Standard error of the mean.

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a treatment × time-of-day interaction (P < 0.001, S2 Text). The 20 cm treatment resulted in a reduction in herbage mass of approximately 25% from Periods 1 to 2 (Fig 1B), which lead to a treatment × period interaction (P< 0.001, S2 Text). Total herbage bulk density decreased with the sward height, with a significant interaction between treatment and time of day (P < 0.001, S2 Text) and between treatment and period (P < 0.001, S2 Text). The interactions indicated that swards from 15 and 25 cm were denser than other sward heights in the afternoon (Fig 1C), and that 20 cm sward was denser and more abundant in Period 1 than in Period 2 (Fig 1D).

The chemical composition and IVOMD of the hand-plucked forage at different sward heights are shown in Table 2. The NDF values ranged from 48% to 53% of DM, ADF between 17% and 23% of DM, and IVOMD between 58% and 75% of OM (Table 2). The CP values were high and ranging from 27% to 33% (Table 2). The time of day had a strong influence on the fiber content of the collected forage, showing that in the afternoon (PM), swards had lower NDF and ADF than in the morning (Table 2 and S3 Text). Consequently, IVOMD was higher in the afternoon than in the morning, although significant interactions between treatment and time of day and period were observed for IVOMD (P < 0.001, S3 Text). The treatment and time-of-day interaction for IVOMD showed a reduction in taller height mainly in the afternoon assessment, but it was established in Period 2, which also resulted in a significant interaction of treatment by period (P < 0.001, S3 Text). The CP content was lower in the afternoon than in the morning in hand-plucked herbage samples (treatment × time-of-day, P < 0.05, S3 Text). In period 2, the CP content in the 25 cm sward height decreased subtly (treatment × period, P = 0.06, S3 Text). No interactions were found between the time of day and period for any nutritive variable.

Components of ingestive behavior

The STIR of the DM model indicated that a maximum STIR of 44 g DM/min was reached with a sward height of 19.3 cm (Fig 2A). The increasing slope (a = 1.36 g DM/min/cm, P < 0.01) was steeper than the decreasing slope (a2 = – 0.44 g DM/min/cm, P < 0.04), (Fig 2A). The BM model achieved a maximum of 0.77 g DM/bite at 20.9 cm of sward height; BM first increased linearly (a1 = 0.023 g DM/cm, P < 0.01) with increasing sward heights up to
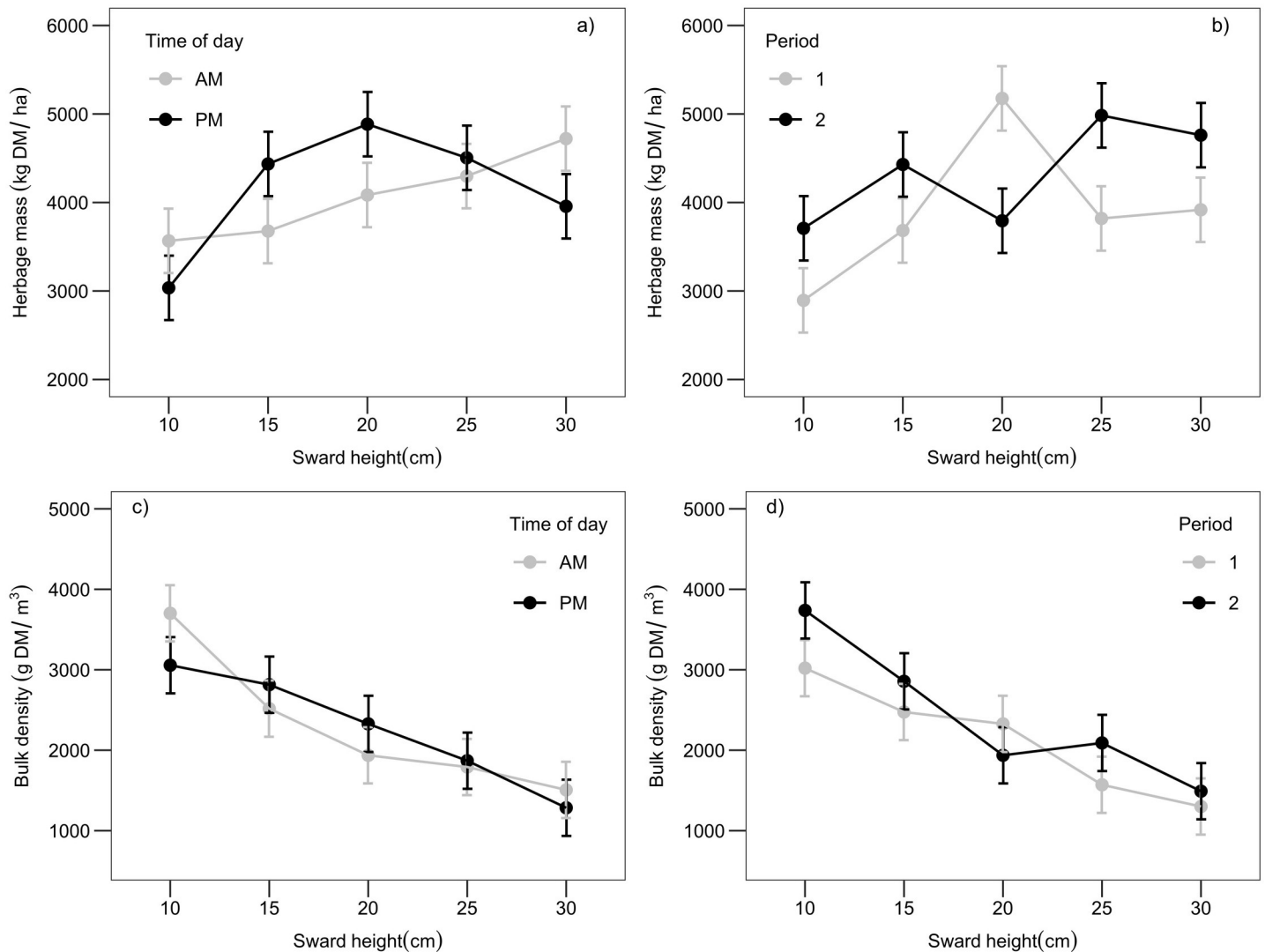
Fig 1. Interactions between sward heights of Kikuyu grass and time of day and period of evaluation. Interactions between sward heights (10, 15, 20, 25, and 30 cm) of Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov) and time of day (morning, AM, and afternoon, PM) on herbage mass (a) and bulk density (c), and interactions between sward heights and period of evaluation (1 and 2) on herbage mass (b) and bulk density (d). The bars represent the standard errors of the mean.

20.9 cm, and then decreased (a2 = –0.016 g DM/cm, P < 0.01) (Fig 2B). The minimum BR (57.71 bites/min) occurred at 20.3 cm (Fig 2C). The BR model showed an opposite relationship to BM, first decreasing (a1 = –0.44 bites/min/cm, P = 0.016) up to 20.3 cm, and then increasing (a2 = 0.53 bites/min/cm, P = 0.003) with increasing sward height. The STIR of digestible OM had a similar response to that of DM with a maximum value of 29.16 g of digestible OM/min at 20.3 cm, an increasing slope (a1 = 0.68 g digestible OM/min/cm, P < 0.01) and decreasing slope (a2 = –0.34 g digestible OM/min/cm, P = 0.04), (Fig 2D).

Discussion

This study showed that an intermediate sward height of approximately 20 cm for Kikuyu grass maximized the STIR of DM and digestible OM by dairy heifers. The intake rate of DM was 30% lower than the maximum in 10 cm swards and 15% lower than the maximum in the 30

Table 2. Nutritional value of hand-plucked herbage samples of five sward heights of Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov) at two times of day in two periods of evaluation.

	Treatment					
	10	15	20	25	30	
	NDF (g/kg of DM)					SEM
Time of day (AM)	519.0	554.1	526.0	539.4	536.0	16.2
Time of day (PM)	495.1	490.1	482.0	498.5	485.0	16.2
Period 1	489.0	516.0	511.5	505.0	525.0	16.2
Period 2	525.5	528.2	496.4	533.1	496.0	16.2
	ADF (g/kg of DM)					SEM
Time of day (AM)	199.4	229.1	210.1	207.5	210.0	9.43
Time of day (PM)	179.2	182.3	181.13	188.0	183.1	9.43
Period 1	179.4	205.0	193.0	194.5	205.2	9.43
Period 2	199.2	206.5	198.2	201.0	188.0	9.43
	CP (g/kg of DM)					SEM
Time of day (AM)	331.0	294.0	316.0	307.0	292.0	11.0
Time of day (PM)	301.4	272.0	284.0	278.0	290.0	11.0
Period 1	318.1	273.0	294.2	296.0	287.0	11.0
Period 2	314.0	293.0	305.13	289.0	295.0	11.0
	IVOMD (g/kg of OM)					SEM
Time of day (AM)	672.2	624.4	588.4	659.1	604.0	12.2
Time of day (PM)	753.2	756.1	714.0	705.4	684.0	12.2
Period 1	733.1	698.2	671.0	706.0	628.1	12.2
Period 2	692.4	682.2	631.4	659.0	660.0	12.2

The times of the day, AM and PM correspond to the morning and afternoon hand-plucked herbage sampling, respectively. SEM: Standard error of the mean.

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cm swards. The BM and STIR constraints in the range below 20 cm are explained by the widely accepted relationships between sward height and bite dimensions (bite area and bite depth) [12,44,45] and the low amount of herbage that the animal can harvest [46,47]. However, for swards greater than 20 cm, these relationships do not explain the decline in the intake rate.

The increase in BR due to the low bite mass [9,11,48] or the reallocation of grazing jaw movements [49,50] was insufficient to maintain the intake rate in the range of short sward heights (<20 cm). Although at taller sward heights (>20 cm), the STIR of DM did not decline as fast as the BM with increasing heights because the increase in BR was steeper than expected based on the increasing side of the response (Fig 2C). This increase was not sufficient to compensate for a small bite mass. We argue that the only possible mechanism for the decline in intake rate is that bite volume declines with increasing sward height, in addition to the observed decline in herbage bulk density.

To elucidate which BM components were involved in the declining phase of the STIR of DM model, we calculated bite dimensions based on mechanistic-empirical models (calculated values, Table 3). Bite volume was calculated as (A) measured bite mass/sward bulk density at the top stratum for each observation averaged over treatments and (B) bite depth × bite area, where bite depth was 0.5 × sward height and bite area was calculated with an empirical model that included the effects of sward height and herbage bulk density of the top stratum [51], and an allometric relationship between dental arcade (DA) breadth and body mass in ruminants [52]. Bite Volume A represents the best approximation of the actual bite volume based on measured quantities, whereas Bite Volume B represents the expected volume based on the generally accepted effects of sward height and density [12,53].
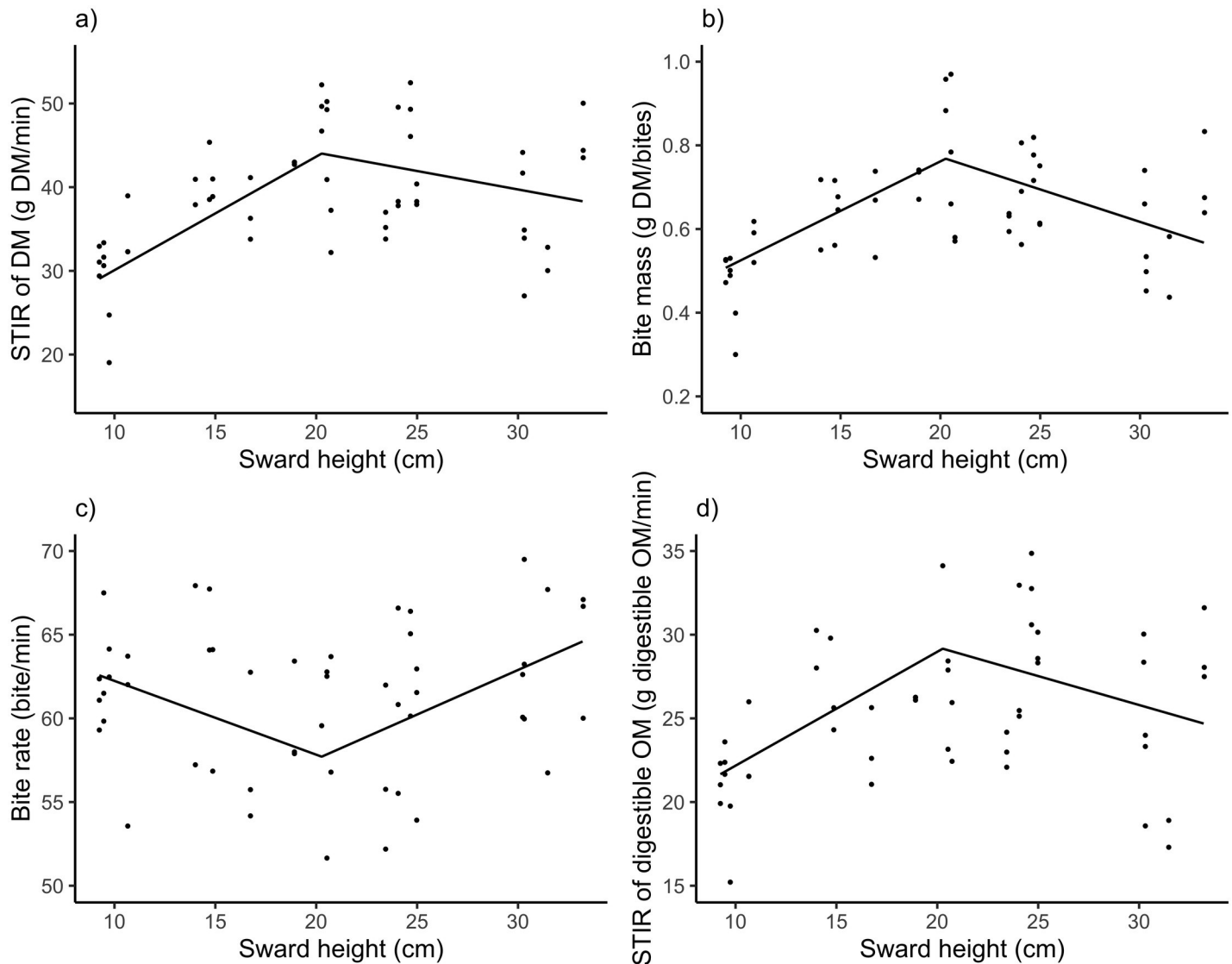
Fig 2. Relationships between ingestive behavior variables and sward height of Kikuyu grass. (a) Relationships between short-term intake rate of the dry matter (STIR of DM), (b) bite mass (BM), (c) bite rate (BR), and (d) short-term intake rate of digestible organic matter (STIR of digestible OM) of dairy heifers as a function of sward height (SH) in monoculture of Kikuyu grass (*Cenchrus clandestinus*—Hochst. ex Chiov). Equation for STIR of DM = min(44 + 1.36 (SH– 19.3), (44–0.44 (SH– 19.3)), P < 0.001, R^2 = 0.35; BM = min(0.77 + 0.023 (SH– 20.9), (0.77–0.016 (SH– 20.9), P 0.001, R^2 = 0.36; BR = min(57.71–0.44 (SH– 20.3); (57.71 + 0.53 (SH– 20.3), P < 0.009, R^2 = 0.14; STIR of digestible OM = min((29.16 + 0.68 * (SH– 20.3); (29.16–0.34 * (SH– 20.3)), P < 0.001, R^2 = 0.21.

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Bite Volume A was expected to increase with greater sward height because both bite depth and bite area tended to increase at greater sward heights [12,53], as shown by the changes in Bite Volume B, but this did not occur. In the tall swards in the present experiment, bite volume appeared to remain constant or declined with increasing sward height (Table 3), whereas if it had followed the expected response, bite mass would have increased monotonically with sward height up to 1.09 g; therefore, the decline in bulk density alone cannot explain the observed decline in BM[A]. Thus, the decline in BM and STIR of DM for swards taller than 20 cm can be explained by both a decline in bulk density and an unexpected decline, or the lack of the usually observed increase in bite volume.

Table 3. Foraging behavior variables of dairy heifers grazing Kikuyu grass managed under different sward heights (observed and calculated values).

Variable	Sward height (cm)				
	10	15	20	25	30
	Observed values				
Herbage bulk density of top stratum (g/dm^3)	0.95	0.68	0.79	0.76	0.61
STIR of DM (g DM/min)	30.6	39.3	44.3	41.3	38.2
BM^A (g)	0.50	0.65	0.78	0.68	0.60
BR (bites/min)	61.6	61.2	57.5	60.2	63.4
Bite Volume^A (dm^3)	0.52	0.95	0.99	0.89	0.98
Variable	Calculated values				
Bite Volume^B (dm^3)	0.39	0.71	0.99	1.28	1.71
Bite depth (m)	0.050	0.075	0.10	0.12	0.15
Bite area (dm^2)	0.78	0.94	0.99	1.02	1.12
BM^B (g)	0.35	0.49	0.76	0.95	1.09

STIR of DM, bite mass (BM^A), and bite rate (BR) are the mean values obtained during the grazing sessions. Bite volume^A is the ratio of bite mass to bulk density of top stratum. Bite area was calculated using the equation by [51] bite area = $2DA^2 (1 + 50/SH)^{-1} e^{(-0.3(HBd-1))}$, where SH = sward height (cm), HBd = herbage bulk density of the top stratum (g/dm^3), and DA = dental arcade (cm). DA was calculated using the allometric equation DA = $8.6 BW^{0.36}$, published by [52], where BW is body weight (kg). Bite volume^B was the product of bite area and bite depth. Bite depth was a constant proportion of 50% of sward height. Bite mass (BM^B) was calculated as the product of bite volume^B and bulk density of the top stratum.

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Bite mass and STIR constraints by the sward structure, especially sward height and bulk density, are well recognized in research at the plant-animal interface [12,54,55]. However, reductions in bite volume at greater sward heights are less common. Some authors argue that bite mass and STIR reductions at high sward heights are due to increments in time per bite associated with decreasing bulk density in the upper stratum of the sward [10,18,56]. Similarly, it is widely recognized that the presence of stems + sheaths in the grazed stratum constrains the bite mass and herbage intake rate [45,47,57]. According to [17], the BM decreases in tall sward heights of *Cynodon sp* (cv. Tifton 85) because of the increasing proportion of less desirable plant parts, such as stems and sheaths, which reduces the bite volume. In this study, the stems + sheath mass had a similar response to the total herbage mass, increasing with sward height and showing an interaction between sward height and time of day and period of evaluation. Previous studies on three-dimensional sward structures at the bite scale have shown a greater dispersion of leaf blades in the upper stratum with increasing sward height in tall pastures [58–60]. We hypothesize that the influence of the stem and sheath mass and the declines in total bulk density and leaf bulk density with sward height resulted in changes in the spatial distribution of the leaves in the top stratum, which resulted in reductions in the bite volume, and thus in smaller bites in tall swards.

The time of day for hand-plucked herbage had a marked effect on NDF and ADF. In contrast, the interaction between sward height, time of day, and evaluation period influenced both CP and IVOMD. Variations in herbage chemical composition due to time of day, grazed stratum, evaluation period, or interactions have previously been described and discussed in the literature [20,61,62]. Our results concerning the NDF, ADF, CP, and IVOMD were consistent with the values found in the upper stratum of Kikuyu sward [20,63]. However, CP exhibited higher values than those usually reported for the grazing layer of Kikuyu grass pastures [22,64].

In the morning, the swards had higher CP content, lower IVDMD, and higher NDF and ADF than those in the afternoon. Studies on the vertical distribution of biomass and chemical composition have suggested that concentrations of photosynthates in leaves have greater diurnal fluctuations than that of stems and pseudostems; therefore, NDF and CP concentrations may be diluted in the DM as the day progresses [61,65,66]. Consistently, other studies have shown that the CP content of leaves changes significantly with the stage of regrowth of the pasture [67] and even with anatomical characteristics along the length of the leaf blades [68]. The high CP values can also be explained by the higher N content in leaf blades than in stems and sheaths due to the N fertilization [69].

Even considering the fluctuation of time of day and period in herbage chemical composition, as the grazed stratum contained mainly highly digestible leaves with similar nutrient content between sward heights, the maximization of the STIR of DM and digestible OM occurred concomitantly in this study. The similarities in nutritive value between sward heights can be attributed to the fact that all pastures were still in their vegetative stage. For a given stratum of the sward, the differences between regrowth ages were commonly more marked between vegetative and reproductive stages [20,64]. In the vegetative stage, the nutritive value differs little among the plant parts [20,58]. Although additional work is required to fully understand the differences and relationships between the intake rate of DM and nutrients, our results concerning the intake rate of nutrients are consistent with findings reported in the scientific literature in which DM intake rate maximization has been incorporated into the maximization of nutrient intake rate [14,16–18]. Finally, if grazed herbage is to be an important source of nutrients for a dairy cow to meet its nutritional requirements, it is reasonable to set grazing management targets that provide the longest pasture access time of Kikuyu grass of 20 cm.

Conclusions

To maximize the short-term intake rate (STIR) of DM and digestible OM of dairy heifers under rotational stocking, the pre–grazing sward height of the Kikuyu grass should be managed at 20 cm. Very low (10 cm) or high (30 cm) sward heights of Kikuyu grass as grazing management targets would constraint the BM, and thus, the STIR.

Supporting information

S1 Text. Summary of Anova results for the fitted linear mixed-effects of pre- and post-grazing sward heights of Kikuyu grass.
(TXT)

S2 Text. Summary of Anova results for the fitted linear mixed-effects of sward structure characteristics of Kikuyu grass.
(TXT)

S3 Text. Summary of Anova results for the fitted linear mixed-effects of Nutritional value of hand-plucked herbage samples of Kikuyu grass.
(TXT)

Acknowledgments

Author Contributions

Conceptualization: Alejandra Marín Gómez, Emilio A. Laca, Carolina Bremm, Jerôme Bindelle, Paulo César de Faccio Carvalho.

Data curation: Alejandra Marín Gómez, Carolina Bremm.

Formal analysis: Alejandra Marín Gómez, Emilio A. Laca, Carolina Bremm.

Funding acquisition: Alejandra Marín Gómez, Tiago Celso Baldissera, Cassiano Eduardo Pinto, Fábio Cervo Garagorry, Paulo César de Faccio Carvalho.

Investigation: Alejandra Marín Gómez.

Methodology: Alejandra Marín Gómez, Tiago Celso Baldissera, Cassiano Eduardo Pinto, Fábio Cervo Garagorry, Angel S. Zubieta.

Resources: Tiago Celso Baldissera, Cassiano Eduardo Pinto, Fábio Cervo Garagorry, Paulo César de Faccio Carvalho.

Supervision: Emilio A. Laca, Jerôme Bindelle, Paulo César de Faccio Carvalho.

Validation: Paulo César de Faccio Carvalho.

Writing – original draft: Alejandra Marín Gómez.

Writing – review & editing: Emilio A. Laca, Tiago Celso Baldissera, Angel S. Zubieta, Jerôme Bindelle, Paulo César de Faccio Carvalho.

References

1. Boval M, Dixon RM. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. Animal. 2012; 6: 748–762. https://doi.org/10.1017/S1751731112000304 PMID: 22558923

2. Carvalho PC de F, Dewulf AKMY, Moraes A, Bremm C, Trindade JK da, Lang CR. Potencial do capim-quicuio em manter a produção e a qualidade do leite de vacas recebendo níveis decrescentes de suplementação. Revista Brasileira de Zootecnia. 2010; 39: 1866–1874. https://doi.org/10.1590/S1516-35982010000900002

3. Dillon P. Achieving high dry-matter intake from pasture with grazing dairy cows. Pages 1–26 in Fresh Herbage for Dairy Cattle, The Key to a Sustainable Food Chain. Pages 1–26. In: Elgersma A, Dijkstra J, Tamminga S, editors. Fresh Herbage for Dairy Cattle, The Key to a Sustainable Food Chain. Pages 1–26. Dordrecht, the Netherlands: ed. Spring; 2006. pp. 1–26.

4. Owen-Smith RN. Adaptive herbivore ecology: from resources to populations in variable environments. Student ed. Cambridge University Press; 2002.

5. Bailey DW. Daily selection of feeding areas by cattle in homogeneous and heterogeneous environments. Applied Animal Behaviour Science. 1995; 45: 183–200. https://doi.org/10.1016/0168-1591(95)00586-H

6. Allden WG, Whittaker IAMcD. The determinants of herbage intake by grazing sheep: the interrelationship of factors influencing herbage intake and availability. Australian Journal of Agricultural Research. 1970; 21: 755–766.

7. Beggs DS, Jongman EC, Hemsworth PE, Fisher AD. Implications of prolonged milking time on time budgets and lying behavior of cows in large pasture-based dairy herds. Journal of Dairy Science. 2018; 101: 10391–10397. https://doi.org/10.3168/jds.2018-15049 PMID: 30219427

8. Chilibroste P, Gibb MJ, Soca P, Mattiauda DA. Behavioural adaptation of grazing dairy cows to changes in feeding management: do they follow a predictable pattern? Animal Production Science. 2015; 55: 328. https://doi.org/10.1071/AN14484

9. Gibb MJ, Huckle CA, Nuthall R, Rook AJ. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Science. 1997; 52: 309–321. https://doi.org/10.1111/j.1365-2494.1997.tb02361.x

10. Carvalho PC de F. Harry Stobbs Memorial Lecture: Can grazing behaviour support innovations in grassland management? 22nd Trop Grassl. Sidney, Australia; 2013. pp. 137–155.

11. Forbes TD. Researching the plant-animal interface: the investigation of ingestive behavior in grazing animals. J Anim Sci. 1988; 66: 2369–2379. https://doi.org/10.2527/jas1988.6692369x PMID: 3049496

12. Laca EA, Ungar ED, Seligman N, Demment MW. Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. Grass and Forage Science. 1992; 47: 91–102. https://doi.org/10.1111/j.1365-2494.1992.tb02251.x

13. Hodgson J, Clark DA, Mitchell RJ. Foraging Behavior in Grazing Animals and Its Impact on Plant Communities. Pages: 796–827. Forage quality, evaluation, and utilization. Forage quality, evaluation, and utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 1994. pp. 796–827. https://doi.org/10.2134/1994.foragequality.c19

14. Mezzalira JC, Carvalho PC de F, Fonseca L, Bremm C, Cangiano C, Gonda HL, et al. Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. Applied Animal Behaviour Science. 2014; 153: 1–9. https://doi.org/10.1016/j.applanim.2013.12.014

15. Hodgson J. Grazing management: Science into practice. London, UK: In Longman Handbooks in Agriculture.; 1990.

16. Fonseca L, Mezzalira JC, Bremm C, Filho RSA, Gonda HL, Carvalho PC de F. Management targets for maximising the short-term herbage intake rate of cattle grazing in Sorghum bicolor. Livestock Science. 2012; 145: 205–211. https://doi.org/10.1016/j.livsci.2012.02.003

17. Mezzalira JC, Bonnet OJF, Carvalho PC de F, Fonseca L, Bremm C, Mezzalira CC, et al. Mechanisms and implications of a type IV functional response for short-term intake rate of dry matter in large mammalian herbivores. Fryxell J, editor. Journal of Animal Ecology. 2017; 86: 1159–1168. https://doi.org/10.1111/1365-2656.12698 PMID: 28542901

18. Szymczak LS, de Moraes A, Sulc RM, Monteiro ALG, Lang CR, Moraes RF, et al. Tall fescue sward structure affects the grazing process of sheep. Scientific Reports. 2020; 10: 11786. https://doi.org/10.1038/s41598-020-68827-0 PMID: 32678270

19. Insua JR, Utsumi SA, Basso B. Estimation of spatial and temporal variability of pasture growth and digestibility in grazing rotations coupling unmanned aerial vehicle (UAV) with crop simulation models. Loor JJ, editor. PLOS ONE. 2019; 14: e0212773. https://doi.org/10.1371/journal.pone.0212773 PMID: 30865650

20. Benvenutti MA, Findsen C, Savian J V., Mayer DG, Barber DG. The effect of stage of regrowth on the physical composition and nutritive value of the various vertical strata of kikuyu (Cenchrus clandestinus) pastures. Tropical Grasslands-Forrajes Tropicales. 2020; 8: 141–146. https://doi.org/10.17138/tgft(8)141-146

21. Sollenberger LE, Burns JC. Canopy characteristics, ingestive behaviour and herbage intake in cultivated tropical grasslands. 19th International grassland congress. São Pedro, SP, Brazil; 2001. pp. 321–327.

22. García SC, Islam MR, Clark CEF, Martin PM. Kikuyu-based pasture for dairy production: a review. Crop and Pasture Science. 2014; 65: 787. https://doi.org/10.1071/CP13414

23. Clark CEF, Kaur R, Millapan LO, Golder HM, Thomson PC, Horadagoda A, et al. The effect of temperate or tropical pasture grazing state and grain-based concentrate allocation on dairy cattle production and behavior. Journal of Dairy Science. 2018; 101: 5454–5465. https://doi.org/10.3168/jds.2017-13388 PMID: 29550132

24. Marín-Santana MN, López-González F, Hernández-Mendo O, Arriaga-Jordán CM. Kikuyu pastures associated with tall fescue grazed in autumn in small-scale dairy systems in the highlands of Mexico. Tropical Animal Health and Production. 2020; 52: 1919–1926. https://doi.org/10.1007/s11250-020-02216-7 PMID: 31960267

25. FASS. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Fed. Anim. Sci. Soc, editor. Champaign, IL; 2010.

26. Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift. 2013; 22: 711–728. https://doi.org/10.1127/0941-2948/2013/0507

27. Soil Survey Staff. Keys to Soil Taxonomy, 12th Edn Washington. 12th ed. USDA-Natural Resources Conservation Service. Washington, DC.; 2014.

28. Rauber LR, Sequinatto L, Kaiser DR, Bertol I, Baldissera TC, Garagorry FC, et al. Soil physical properties in a natural highland grassland in southern Brazil subjected to a range of grazing heights. Agriculture, Ecosystems and Environment. 2021;319. https://doi.org/10.1016/j.agee.2021.107515

29. Sbrissia AF, Duchini PG, Zanini GD, Santos GT, Padilha DA, Schmitt D. Defoliation Strategies in Pastures Submitted to Intermittent Stocking Method: Underlying Mechanisms Buffering Forage Accumulation over a Range of Grazing Heights. Crop Science. 2018; 58: 945. https://doi.org/10.2135/cropsci2017.07.0447

30. Penning P, Hooper GE. An evaluation of the use of short term weight changes in grazing sheep for estimating herbage intake. Grass and Forage Science. 1985; 40: 79–84. https://doi.org/10.1111/j.1365-2494.1985.tb01722.x

31. Gibb, Huckle, Nuthall. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science. 1998; 53: 41–46. https://doi.org/10.1046/j.1365-2494.1998.00102.x

32. Rutter SM, Champion RA, Penning PD. An automatic system to record foraging behaviour in free-ranging ruminants. Applied Animal Behaviour Science. 1997; 54: 185–195. https://doi.org/10.1016/S0168-1591(96)01191-4

33. Greenwood GB, Demment MW. The effect of fasting on short-term cattle grazing behaviour. Grass and Forage Science. 1988; 43: 377–386.

34. Gregorini P, Clark CEF, Jago JG, Glassey CB, McLeod KLM, Romera AJ. Restricting time at pasture: Effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session. Journal of Dairy Science. 2009; 92: 4572–4580. https://doi.org/10.3168/jds.2009-2322 PMID: 19700720

35. Newman JA, Penning PD, Parsons AJ, Harvey A, Orr RJ. Fasting affects intake behaviour and diet preference of grazing sheep. Animal Behaviour. 1994; 47: 185–193. https://doi.org/10.1006/anbe.1994.1021

36. Rutter SM. Graze: A program to analyze recordings of the jaw movements of ruminants. Behavior Research Methods, Instruments, & Computers. 2000; 32: 86–92. https://doi.org/10.3758/bf03200791 PMID: 10758667

37. Barthram GT. Experimental techniques: the HFRO sward stick. Pages 29–30. In: Biennial Report of the Hill Farming Research Organization. Alcock MM, editor. Biennial Report of the Hill Farming Research Organization. Midlothian, UK; 1985.

38. De Vries MFW. Estimating forage intake and quality in grazing cattle: a reconsideration of the hand-plucking method. Journal of Range Management. 1995; 370–375.

39. AOAC. Official Methods of Analysis. 20th ed. AOAC International, editor. Rockville, Maryland, USA; 2016.

40. Van Soest PJ, Robertson JB, Lewis BA. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-starch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science. 1991; 74: 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2 PMID: 1660498

41. Tilley JMA, Terry RA. A Two-Stage Technique for the in vitro Digestion of Forage Crops. Grass and Forage Science. 1963; 18: 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

42. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www R-project org. 2018.

43. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67. https://doi.org/10.18637/jss.v067.i01

44. Flores ER, Laca E a., Griggs TC, Demment MW. Sward Height and Vertical Morphological Differentiation Determine Cattle Bite Dimensions. Agronomy Journal. 1993; 85: 527. https://doi.org/10.2134/agronj1993.00021962008500030001x

45. Gregorini P, Gunter SA, Bowman MT, Caldwell JD, Masino CA, Coblentz WK, et al. Effect of herbage depletion on short-term foraging dynamics and diet quality of steers grazing wheat pastures1. Journal of Animal Science. 2011; 89: 3824–3830. https://doi.org/10.2527/jas.2010-3725 PMID: 21642497

46. Illius AW, Gordon IJ. The Allometry of Food Intake in Grazing Ruminants. The Journal of Animal Ecology. JSTOR; 1987. p. 989. https://doi.org/10.2307/4961

47. Benvenutti MA, Gordon IJ, Poppi DP. The effects of stem density of tropical swards and age of grazing cattle on their foraging behaviour. Grass and Forage Science. 2008; 63: 1–8. https://doi.org/10.1111/j.1365-2494.2007.00609.x

48. Hodgson J. The control of herbage intake in the grazing ruminant. The Proceedings of the Nutrition Society. 1985. pp. 339–346. https://doi.org/10.1079/pns19850054 PMID: 3840260

49. Laca EA, Ungar ED, Demment MW. Mechanisms of handling time and intake rate of a large mammalian grazer. Applied Animal Behaviour Science. 1994; 39: 3–19. https://doi.org/10.1016/0168-1591(94)90011-6

50. Gibb MJ, Huckle CA, Nuthall R, Rook AJ. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science. 1999; 63: 269–287. https://doi.org/10.1016/S0168-1591(99)00014-3

51. Baumont R, Cohen-Salmon D, Prache S, Sauvant D. A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. Animal Feed Science and Technology. 2004; 112: 5–28. https://doi.org/10.1016/j.anifeedsci.2003.10.005

52. Illius AW, Gordon IJ. The allometry of food intake in grazing ruminants. The Journal of Animal Ecology. 1987; 989–999.

53. Cangiano CA, Galli JR, Pece MA, Dichio L, Rozsypalek SH. Effect of liveweight and pasture height on cattle bite dimensions during progressive defoliation. Australian Journal of Agricultural Research. 2002; 53: 541–549. https://doi.org/10.1071/AR99105

54. Black JL, Kenney PA. Factors affecting diet selection by sheep. 2. Height and density of pasture. Aust J Agric Res. 1984; 35: 565–578.

55. Benvenutti MA, Gordon IJ, Poppi DP. The effect of the density and physical properties of grass stems on the foraging behaviour and instantaneous intake rate by cattle grazing an artificial reproductive tropical sward. Grass and Forage Science. 2006; 61: 272–281. https://doi.org/10.1111/j.1365-2494.2006.00531.x

56. Guzatti GC, Duchini PG, Sbrissia AF, Mezzalira JC, Almeida JGR, Carvalho PC de F, et al. Changes in the short-term intake rate of herbage by heifers grazing annual grasses throughout the growing season. Grassland Science. 2017; 63: 255–264. https://doi.org/10.1111/grs.12170

57. Fonseca L, Carvalho PCF, Mezzalira JC, Bremm C, Galli JR, Gregorini P. Effect of sward surface height and level of herbage depletion on bite features of cattle grazing Sorghum bicolor swards1. Journal of Animal Science. 2013; 91: 4357–4365. https://doi.org/10.2527/jas.2012-5602 PMID: 23825342

58. Laca EA, Shipley LA, Reid ED. Structural Anti-Quality Characteristics of Range and Pasture Plants. Journal of Range Management. 2001; 54: 413–419. https://doi.org/10.2307/4003112

59. Orr RJ, Rutter SM, Yarrow NH, Champion RA, Rook AJ. Changes in ingestive behaviour of yearling dairy heifers due to changes in sward state during grazing down of rotationally stocked ryegrass or white clover pastures. Applied Animal Behaviour Science. 2004; 87: 205–222.

60. Gordon IJ, Benvenutti M. Food in 3D: how ruminant livestock interact with sown sward architecture at the bite scale. Feeding in domestic vertebrates: from structure to behaviour. CABI; 2006. pp. 263–277.

61. Delagarde R, Peyraud JL, Delaby L, Faverdin P. Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology. 2000; 84: 49–68. https://doi.org/10.1016/S0377-8401(00)00114-0

62. Gregorini P. Diurnal grazing pattern: Its physiological basis and strategic management. Animal Production Science. 2012; 52: 416–430. https://doi.org/10.1071/AN11250

63. Marín A, Bindelle J, Zubieta ÁS, Correa G, Arango J, Chirinda N, et al. In vitro Fermentation Profile and Methane Production of Kikuyu Grass Harvested at Different Sward Heights. Frontiers in Sustainable Food Systems. 2021;5. https://doi.org/10.3389/fsufs.2021.682653

64. Schmitt D, Padilha DA, Dias KM, Santos GT, Rodolfo GR, Zanini GD, et al. Chemical composition of two warm-season perennial grasses subjected to proportions of defoliation. Grassland Science. 2019; 65: 171–178. https://doi.org/10.1111/grs.12236

65. Griggs TC, MacAdam JW, Mayland HF, Burns JC. Nonstructural carbohydrate and digestibility patterns in orchardgrass swards during daily defoliation sequences initiated in evening and morning. Crop Science. 2005; 45: 1295–1304. https://doi.org/10.2135/cropsci2003.0613

66. Gastal F, Lemaire G, Durand J-L, Louarn G. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. Crop physiology. Elsevier; 2015. pp. 161–206.

67. Reeves M, Fulkerson W, Kellaway R. Forage quality of kikuyu (Pennisetum clandestinum): the effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (Lolium perenne). Australian Journal of Agricultural Research. 1996; 47: 1349. https://doi.org/10.1071/AR9961349

68. Garcia LF, Silva GP, Geremia EV, Goulart LBL, Dias CTDS, da Silva SC. Central rib and the nutritive value of leaves in forage grasses. Sci Rep. 2021; 11: 5440. https://doi.org/10.1038/s41598-021-84844-z PMID: 33686178

69. Delevatti LM, Cardoso AS, Barbero RP, Leite RG, Romanzini EP, Ruggieri AC, et al. Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. Scientific Reports. 2019; 9: 7596. https://doi.org/10.1038/s41598-019-44138-x PMID: 31110320