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Research Paper

Ingestive behavior and dry matter intake of dairy cattle grazing Kikuyu grass (*Cenchrus clandestinus*) pastures

Comportamiento ingestivo y consumo de materia seca de vacas lactantes en pastoreo de pasto kikuyo (Cenchrus clandestinus)

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Abstract

The objective of this study was to evaluate the effect of animal characteristics, grazing management, and supplementation on ingestive behavior and dry matter intake (DMI) of Kikuyu grass in lactating cows. Four trials were conducted with multiparous Holstein dairy cows in non-limiting forage conditions using 9 cows in each trial, 1 cow per paddock. Individual DMI was estimated through forage mass difference (pre- and post-grazing mass), ingestive behavior, and using markers [chromium oxide and undegradable acid detergent fibre (uADF)]. DMI was also estimated using 3 nutritional models (CSIRO, NRC and AFRC). Grazing time and bite mass were positively related to the cow body weight, while bite rate showed a negative relationship with forage mass. The grazing time on a pasture of 42 d regrowth was less than the time spent grazing on a pasture of 28 or 56 d regrowth. DMI estimated by forage mass difference showed a positive relation with forage mass, supplement intake and liveweight. DMI estimated using markers showed a positive relation with milk production and liveweight and a negative relationship with forage height. Forage mass difference and ingestive behavior measurements provided good estimates (R^2>0.8) of DMI associated with forage mass, liveweight and supplement intake in cows grazing Kikuyu grass.

Keywords: Bite mass, bite rate, external and internal markers, grassland systems, grazing time.

Resumen

El objetivo de este estudio fue evaluar el efecto de las características de los animales, el manejo del pastoreo y la suplementación sobre el comportamiento ingestivo y el consumo de materia seca (CMS) del pasto kikuyo en vacas lactantes. Se realizaron cuatro ensayos con vacas lecheras Holstein multíparas en condiciones en que la provisión de forraje no fue una limitante utilizando 9 vacas en cada ensayo, 1 vaca por potrero. El CMS individual se estimó a través de la diferencia de la disponibilidad de forraje (antes y después del pastoreo), el comportamiento ingestivo y el uso de marcadores [óxido crómico y fibra detergente ácida no degradable (uADF)]. El CMS también se estimó utilizando 3 modelos nutricionales (CSIRO, NRC y AFRC). El tiempo de pastoreo y tamaño de bocado se relacionaron positivamente con el peso corporal de la vaca, mientras que el tamaño de bocado mostró una relación negativa con la disponibilidad de forraje. El tiempo de pastoreo fue menor en una pradera de 42 días de rebrote que en las de 28 o 56 días. El CMS estimado por diferencia en la disponibilidad de forraje mostró una relación positiva con la masa de forraje, el consumo de suplementos y el peso vivo. El CMS estimado mediante marcadores mostró una relación positiva con la producción de leche y el peso vivo y una relación negativa con la altura del forraje. Las mediciones de la diferencia en la disponibilidad de forraje y el comportamiento ingestivo proporcionaron buenas estimaciones (R^2>0.8) del CMS asociado con la disponibilidad de forraje, el peso vivo y el consumo de suplementos en vacas que consumen pasto kikuyo.

Palabras clave: Masa de bocado, marcadores externos e internos, sistemas de pastizales, tasa de bocado, tiempo de pastoreo.

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Introduction

The main factor that defines animal performance in ruminants is dry matter intake (DMI) (Sollenberger and Vanzant 2011). Physiological and physical constraints, optimization of oxygen consumption and animal behavior have been used to explain DMI by ruminants in different contexts (NASEM 2016). However, physical rumen gut fill and animal behavior are more related to DMI of ruminants in grassland conditions (Boval et al. 2015; Sollenberger et al. 2020a). Also, supplementation has an associative effect on DMI in ruminants because it may maintain or increase forage intake and increase total DMI (additive effect) or reduce forage intake but increase total DMI (substitutive effect) (Bargo et al. 2003).

Dairy production feeding systems in the Colombian highlands consist of forages, especially Kikuyu grass (*Cenchrus clandestinus*), plus concentrate supplementation (Carulla and Ortega 2016). Kikuyu grass is a C4 species that tolerates acid soils, drought conditions and poor management, resulting in low animal productivity (Vargas et al. 2018). Literature suggests that good management of Kikuyu grasslands and appropriate supplementation may promote high milk production and farm profitability (Fariña et al. 2011). There is interest in understanding the environmental and management factors that modify Kikuyu grass productivity and nutritive value to define management recommendations for increasing ruminant performance (Fonseca et al. 2016; Escobar et al. 2020; Avellaneda et al. 2020).

In Colombia, DMI of dairy cows in Kikuyu grass pastures has been evaluated using external and internal markers (Aguilar et al. 2009; Correa et al. 2009; Mojica et al. 2009; Parales et al. 2016) or by calculating the difference between the forage mass on offer and the forage mass remaining following a grazing event (Gómez-Vega et al. 2019). Studies have evaluated and modelled the effect of different animal characteristics such as milk production or liveweight (NRC 2001; CSIRO 2007), supplementation level (Alderman and Cottrill 1993), forage management such as grazing frequency or time (Abrahamse et al. 2008) or ingestive behavior (Boval and Sauvant 2019) on the DMI. This approach has not been thoroughly evaluated in milk production systems of the Colombian highland tropics or used for development of models specific to the production system and conditions of the region. This research aimed to evaluate the relation between animal characteristics, grazing management and supplementation amount on DMI and animal behavior. We hypothesized that using variables that are easy to measure in the field, such as forage mass, plant height, supplement supply, cow liveweight and grazing time, can be used to make more accurate predictions of Kikuyu grass intake in lactating cows.

Materials and Methods

Animal management and procedures were approved by the bioethics committee of the Corporación Colombiana de Investigación Agropecuaria (Agrosavia) act number 029. Four experiments were conducted in the dairy unit at Tibaitatá research center, Agrosavia, at 2516 masl (latitude 4°35′56′′ N, longitude 74°04′51′′ W) and a mean temperature of 16 °C in Mosquera, Colombia. Two hectares of pre-established Kikuyu grass were used. The area was mowed at 10 cm and lime (2 t lime/ha), urea (100 kg urea/ha) and DAP (50 kg DAP/ha) were applied following the recommendation of ICA (1992). The area was divided into 18 separately fenced paddocks (approximately 1,100 m^2 each), with 9 paddocks used in each of the 4 experiments conducted.

Cow management and experimental design

Multiparous Holstein dairy cows were used in each of the 4 experiments. Kikuyu grass was offered at 3 kg forage dry matter/100 kg liveweight to ensure forage mass was not limiting (Correa et al. 2008). Each cow was assigned to an individual paddock with water *ad libitum*. Supplementation was supplied at the milking parlor twice per day. Each trial was implemented for 15 days. The first 10 days were an adaptation period to management and supplement intake and the last 5 days were the measurement period.

Experiment 1: Effects of cow liveweight and level of milk production. Nine cows with different liveweight (low: 441±14 kg; medium: 502±21 kg; high: 676±38 kg) and milk production (low: 9.0±0.6 L/d; medium: 11.9±1.7 L/d; high: 17.3±0.9 L/d) were allocated, 1 cow per paddock, to 9 paddocks after 43 days of Kikuyu grass regrowth with 3 fence movements throughout the day (06.00, 10.00 and 15.00 h). In addition to grazing, cows received 1 kg supplement per 4.25 kg of milk produced. Measurements were taken per cow per paddock for each combination of liveweight and milk production.

Experiment 2: Effect of different lengths of regrowth period. Nine cows with similar milk production (13.5±2.7 L/d) but different liveweight (low: 435±6 kg; medium: 502±27 kg; high: 657±75 kg) were allocated, 1 cow per paddock, to each of 9 paddocks. Three different regrowth periods (28, 42 or 56 d) of Kikuyu grass were used and cows received 1 kg supplement per 4 kg of milk produced. The experimental unit was a cow in an individual paddock with 3 replicates per treatment. Regardless of treatment, there were 3 fence movements throughout the day (06.00, 10.00 and 15.00 h).

Experiment 3: Effect of number of times cows were moved to a new, ungrazed area in the paddock per day. Nine cows with similar body weight (500±33 kg) and milk production (14.2±1.9 L/d) were allocated, 1 cow per paddock, to each of 9 paddocks. Daily forage availability was varied by using electric-fence movements at 2 (6.00 and 14.30 h), 4 (6.00, 10.00, 12.00 and 14.30 h) or 6 (6.00, 9.00, 10.00, 11.00, 12.00 and 14.30 h) times throughout the day with cows also receiving 1 kg supplement per 4 kg milk produced. The experimental unit was a cow in an individual paddock with 3 replicates per treatment.

Experiment 4: Effect of rate of supplementation and milk production of cow. Nine cows with different milk production (low: 11.9±0.4 L/d; medium: 15.4±1.0 L/d; high: 19.1±1.8L/d) but similar liveweight (578+53 kg) were allocated, 1 cow per paddock, to each of 9 individual paddocks with a regrowth period of Kikuyu grass of 43 days and 3 fence movements throughout the day (6.00, 10.00 and 15.00 h). Cows with similar milk production and lactating days were randomly assigned to 1 of the 3 supplementation rates (1 kg of the supplement per 2, 3 or 4 kg of milk produced). Measurements were taken per cow per paddock for each combination of milk production and supplementation rate.

Forage management, supplement composition and chemical analysis

Pre-grazing and post-grazing forage mass were measured in each paddock during the last 5 days of each experimental period. Pre-grazing forage mass was measured using the plate-meter (EC-10, Jenquip®), while quantification of post-grazing forage mass was done using a metric ruler following the methodology of Avellaneda et al. (2020) because the resting cows crushed the grass, affecting the measurement with the forage plate-meter. Pre-grazing forage samples for each paddock were collected, dried and conserved for subsequent analysis. Supplements were manufactured for each experiment to supply the animal requirements (NRC 2001) and offered individually at the milking parlor. A sample of each supplement was retained for subsequent analysis. During the measurement period, orts of each supplement were weighed to calculate the supplement intake. Forages and supplements were analyzed using the near-infrared spectroscopy (NIRS) methodology (Ariza-Nieto et al. 2017). The agronomic and chemical composition of Kikuyu grass, and the chemical composition of supplements of each experiment are presented in tables 1 and 2, respectively.

Table 1. Agronomic characteristics and chemical composition of Kikuyu grass.

Agronomic characteristics	Trial					
	1	2			3	4
Regrowth period (d)	43	28	42	56	43	43
Plant height (cm)	17.0	10.2	18.3	25.0	19.1	16.9
Pre-grazing mass (kg DM/m^2)	0.17	0.11	0.18	0.21	0.12	0.11
Chemical composition	% DM					
Dry matter[1]	24.6	25.8	23.4	19.6	15.0	16.6
Crude protein	12.8	16.6	14.2	13.8	19.5	18.1
Neutral detergent fiber	61.4	56.6	59.4	60.0	55.8	56.4
Acid detergent fiber	34.4	32.7	33.0	33.4	32.4	33.2
Calcium	0.30	0.27	0.32	0.27	0.20	0.24
Phosphorus	0.34	0.32	0.36	0.34	0.40	0.39
NE^L (Mcal/kgDM)	1.20	1.28	1.24	1.22	1.33	1.30

[1] (% as fed)

Table 2. Ingredients and chemical composition of supplements.

Item	Trial			
	1	2	3	4
Corn grain meal	26.1	23.0	23.0	23.0
Bakery residues	15.0	10.0	12.0	12.0
Glycerin	20.0	25.0	25.0	25.0
Cottonseed, whole	20.0	23.0	23.0	23.0
Distiller's dried grains with solubles (%)	18.9	19.0	17.0	17.0
Chemical composition	% DM			
Dry matter	91.0	91.8	92.6	89.9
Crude protein	12.2	13.2	11.0	12.8
Neutral detergent fiber	20.7	25.5	22.2	21.9
Acid detergent fiber	12.0	17.2	11.5	15.0
Ether extract	5.55	5.62	5.31	5.26
Calcium	0.11	0.11	0.10	0.11
Phosphorus	0.51	0.48	0.50	0.48
NE_L (Mcal/kgDM)	1.87	1.74	1.88	1.79

Variables evaluated

Individual DMI was estimated using different methodologies.

a. Forage mass difference: Forage intake was calculated individually as the difference between pre- and post-grazing forage mass. Total DMI was defined as forage intake plus supplement intake.

Equation 1: Intake (kg/d)=(Pre-grazing biomass – post-grazing biomass) + supplement

b. Ingestive behavior: Forage intake was estimated as the product between grazing time, bite rate, and bite mass. Total DMI was defined as the addition of forage and supplement intake.

Equation 2: Intake (kg/d)=(grazing time × bite rate × bite mass) + supplement

Animal behavior was classified as grazing, ruminating and resting. The grazing time was defined following the animal behavior during each experimental period. Each animal was observed every 10 min for 24 h during the measurement period of each trial. Grazing time was calculated as the time that animals spent in grazing activity. Bite rate was calculated as the number of bites during 5 min, observed every 15 min during the grazing period. Mouth movements during rumination (rumination rate) were calculated for 5 min observed every 15 min during the rumination period. Bite mass was defined through 2 different approaches. Initially, a hand-picked sample was determined considering the width and depth of the bites of each cow, mimicking the ingestive behavior. Also, bite mass was estimated using the relation between bite mass and liveweight (equation 3, Boval and Sauvant 2019). The methodologies to estimate bite mass were applied each day during the measurement period of each trial.

Equation 3: Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight

c. Markers: Internal and external markers were used to estimate forage intake (Correa et al. 2009). Cows received 10 g of chromium oxide (Cr_2O_3), divided into 2 doses daily, to estimate fecal production, assuming 79 % of chromium-marker recovery rate (Lippke 2002; Correa et al. 2009). Feces were collected twice a day during the measurement period of each trial. Feces were dried and mixed by cow per period. Undegradable acid detergent fibre (uADF) at 144 h of incubation and chromium concentration were calculated for forage samples, supplements and feces. The recovery of uADF was assumed as 0.8 (Sunvold and Cochran 1991).

Equation 4: Intake=((Feces × concentration of uADF in feces/0.8) - (supplement intake × concentration of uADF in supplement))/ concentration of uADF in forage) + supplement

Equation 5: Feces=(Chromium supplied + concentration of chromium in the supplement × supplement intake) / (Concentration of chromium in feces/0.79)

d. Model estimation: Intake was estimated using equations described in NRC (2001), CSIRO (2007) and AFRC (Alderman and Cottrill 1993) for dairy cows, respectively.

Equation 6 (NRC): Intake=(fat-corrected milk × 0.372 + 0.0968 × body weight$^{0.75}$) × (1-e $^{(-0.192*(\text{Days in milk}) +3.67)}$)

Fat-corrected milk is milk adjusted on a 4 % fat basis ((0.4+(0.15*milk fat(%)))*milk production).

Equation 7 (CSIRO): Intake=Potential intake × intake level

Equation 8 (AFRC): Intake=0.076 + 0.404 × concentrate intake + 0.013 × Body weight + 4.12 × $\log_{10}$ (days in milk) – 0.129*n + 0.14 × milk production

Milk production was measured for each cow at 1, 3 and 5 days of each measurement period. A milk sub-sample of each animal per measurement day was analyzed for protein and fat (MilkoScan™ FT120, AOAC 2016).

Statistical analysis

Data on feeding behavior of different trials were evaluated with regression analysis. The independent variables were days in milk, liveweight, metabolic liveweight, pre-grazing forage mass, milk production, corrected milk production, milk fat concentration, milk protein concentration and supplement intake. The REG procedure was used for linear regression. The stepwise selection method, assessing contributions of effects as they were added to or removed from the model, was used to select the explicative variables (P<0.05, SAS 2017). Cow behavior of experiments 2 and 3 were analyzed as a completely randomized design using a GLM procedure (SAS 2017), where the fixed effect was the regrowth period or the movements of the electric fence, respectively, and the error was the variation of each cow between measurements. Differences were considered with an alpha value lower than 5 %. The linear and quadratic responses of fixed effects were determined.

The individual DMI using forage mass difference, cow behavior and markers were calculated through regression analysis. The independent variables were pre-grazing forage mass, forage height, animal activity, bite rate, rumination rate, bite mass, supplement intake, liveweight, metabolic liveweight, milk production, corrected milk production, milk fat and protein concentration. The REG procedure and the stepwise option were used to select the explicative variables (P<0.1, SAS 2017). Similarly, the DMI of experiments 2 and 3 were analyzed as a completely randomized design using a GLM procedure (SAS 2017). DMI using different approaches was evaluated through the Pearson correlation. The percentage and absolute mean bias error were defined to evaluate the relationship between different methodologies.

Results

Behavior and intake characteristics in dairy cows

Dairy cows spent 18, 30 and 39 % of time resting, grazing and ruminating throughout the day, respectively (Table 3). The average bite and rumination rate were 0.55 bite/sec and 1.03 bite/sec, respectively (Table 3). Regardless of the methodology, the average bite mass was 0.71g DM/bite or 0.72 g DM/bite (Table 3).

Table 3. Description of animal behavior and intake traits of dairy cows in grassland systems.

Variable	Unit	Mean	Range
Grazing	h/d	7.2	5.1 - 8.8
Ruminating	h/d	9.3	6.4 - 11.4
Resting	h/d	4.2	0.7 - 6.8
Bite rate	times/s	0.55	0.41 - 0.70
Rumination rate	times/s	1.03	0.94 - 1.12
Bite mass[1]	g DM/bite	0.71	0.55 - 0.99
Bite mass[2]	g DM/bite	0.72	0.30 - 1.13

[1]Hand-picked sample simulating animal bite.
[2]Log10 Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Grazing time showed a positive relationship with cow liveweight (Table 4). While rumination was negatively related to pre-grazing forage mass and supplement intake, it positively correlated with corrected milk production. Inversely, time resting showed a positive relationship with pre-grazing forage mass and supplement intake and a negative relationship with the fat-corrected milk production (Table 4). The bite rate was negatively related to pre-grazing forage mass, while the rumination rate had a positive relationship with supplement intake (Table 4). Only the bite mass, using the hand-picked methodology, was positively related to the animal's liveweight (Table 4).

Regrowth period of the Kikuyu grass affected the proportion of time spent in grazing (P<0.05) but not the duration of resting or rumination (P>0.05). Regrowth period did not affect the bite rate, rumination rate, or bite mass (P>0.05). Conversely, more fence movements increased resting and decreased rumination times (P<0.05) but did not affect grazing time (P>0.05). Fence movement did not change the bite rate, rumination rate or bite mass (P>0.05) (Table 5).

Estimation of DMI using different methodologies

The average DMI in dairy cows was estimated between 13.7 and 14.2 kg/d using the different methodologies (Table 6). The linear regression of DMI, calculated by different methodologies according to the variables of forage, ingestive behavior, and animal performance, is presented in Table 7. Pre-grazing forage mass, supplement intake and metabolic body weight variables proved suitable for estimating DMI, calculated as the difference between pre- and post-grazing forage mass.

Table 4. Regression analysis of cow behavior or intake traits with forage mass, supplement intake and animal characteristics in dairy cows (mean from all experiments).

Variable	Intercept	FM	IS	CMP	BW	R^2	P
Circadian behavior (h/d)							
Grazing	5.27***				0.0035*	0.12	*
Rumination	9.94***	-0.98+	-0.34*	0.16*		0.20	+
Rest	3.51*	1.23+	0.37+	-0.18+		0.15	ns
Intake behavior (times/s)							
Bite rate	0.61***	-0.06*				0.11	*
Rumination rate	0.95***		0.014*			0.17	*
Bite mass (g DM/bite)							
Hand-picked	0.32+				0.00074*	0.15	*

FM = pre-grazing forage mass (t/ha); IS = intake of supplement (kg/d); CMP = fat-corrected milk production; BW = liveweight; ns = non-significant; + = P< 0.1; * = P< 0.05; *** = P< 0.001.

Table 5. Behavior of lactating cows under different grass-regrowth periods and electric fence movement schemes in Kikuyu pastures.

Variable	Regrowth period (d)			MSE	Effect	Fence movement			MSE[3]	Effect[4]
	28	42	56			2	4	6		
Circadian behavior (h/d)										
Grazing	7.3[a]	5.9[b]	7.8[a]	0.34	C	7.1	7.6	6.4	0.61	ns
Rumination	9.3	9.2	9.0	0.74	ns	10.8[a]	9.4[ab]	7.9[b]	0.60	L
Rest	3.9	5.4	3.7	0.67	ns	2.6[b]	3.5[b]	6.1[a]	0.87	L
Intake behavior (times/s)										
Bite rate	0.55	0.50	0.49	0.04	ns	0.5	0.5	0.5	0.02	ns
Rumination rate	1.03	1.03	1.09	0.04	ns	1.1	1.0	1.0	0.03	ns
Bite mass (g DM/bite)										
Hand-picked[1]	0.63	0.94	0.87	0.21	ns	0.7	0.6	0.7	0.09	ns
Estimated[2]	0.67	0.66	0.74	0.08	ns	0.7	0.7	0.6	0.05	ns

[1]Hand-picked sample simulating animal bite; [2]Log_{10} Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019); [3]MSE = mean square error; [4]L = lineal effect; ns = not significant. Different letters in the same row mean significant differences (P<0.05).

Table 6. DMI (kg/d) of dairy cows estimated using different methodologies in Kikuyu grassland systems.

Variable	Mean	Range
Forage mass difference	13.7	8.7 – 19.1
Ingestive behavior[1]	13.8	8.7 – 19.9
Ingestive behavior[2]	14.2	7.7 – 21.0
Markers	13.8	10.2 – 18.9

[1]Hand-picked sample simulating animal bite; [2]Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Table 7. Regression analysis of DMI (kg/d) estimation using different methodologies in dairy cattle.

Variable	Intercept	FM	FH	GT	BR	BS	IS	MP	BW	MBW	R
Forage mass difference	-1.18[ns]	3.72***					0.55**			0.078***	0.81
Ingestive behavior[1]	-18.51*			1.54*	16.98*	11.30*	0.02*				0.84
Markers	8.94**		-0.11*					0.27*	0.01*		0.38

FM = pre-grazing forage mass (t/ha); FH = forage height (cm); GT = grazing time (h/d); BR = bite rate (times/sec); IS = intake of supplement (kg/d); MP = milk production (kg/d); BW = liveweight (kg); MBW = metabolic live weight (kg); [1]Hand-picked sample simulating animal bite; *** = P<0.0001; * = P<0.05; ns = not significant.

The estimation of DMI using ingestive behavior had a positive relationship with grazing time, bite rate, bite mass, and supplement intake. The estimation of DMI using markers showed a positive relationship between milk production and body weight and a negative relationship with forage height. The coefficients of determination to estimate DMI through forage mass difference or ingestive behavior were greater than internal and external markers (Table 7).

The regrowth period did not affect DMI, regardless of the methodology used to determine intake (P>0.05). Greater fence movements increased the DMI only when calculated as the difference between pre- and post-grazing. DMI increased 36 % when the electric fence was moved 6 vs 2 times throughout the day. However, the number of fence movements did not affect the estimation of DMI using other methodologies (Table 8).

DMI estimated as the difference between pre- and post-grazing showed a positive correlation (0.62) with the NRC model. Estimation of DMI using ingestive behavior and calculating the bite mass (Boval and Sauvant 2019) had a positive correlation (0.64 and 0.70) with the AFRC and NRC models, respectively. The estimation of DMI using the 2 methodologies of ingestive behavior showed a positive correlation (0.68) between them. DMI estimated with the AFRC model had a positive correlation (0.68 and 0.78) with NRC and CSIRO models, respectively. Estimation of DMI with internal and external markers did not show a significant relationship with other estimation methodologies or the CSIRO model (Table 9).

Table 8. DMI (kg/d) of lactating cows under different grass-regrowth periods and electric fence movements schemes in Kikuyu pastures.

Variable	Regrowth period (d)			MSE[3]	Effect[4]	Fence movement (times/day)			MSE	Effect
	28	42	56			2	4	6		
Forage mass difference	11.9	12.6	14.7	1.31	ns	12.0[b]	12.3[b]	16.5[a]	1.0	L
Ingestive Behavior[1]	13.4	13.0	15.5	2.64	ns	13.0	13.0	12.0	1.4	ns
Ingestive Behavior[2]	13.6	10.7	13.8	1.56	ns	12.4	13.3	11.3	0.9	ns
Markers	15.4	12.7	14.8	3.94	ns	12.1	12.7	12.5	1.1	ns

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019); [3]MSE = mean square error; [4]L = lineal effect; ns = not significant. Different letters in the same row mean significant differences (P<0.05).

Table 9. Pearson correlations between methodologies and models to estimate DMI in dairy cows.

Methodology	Behavior[1]	Behavior[2]	Markers	NRC	CSIRO	AFRC
Mass difference	0.50**	0.51**	0.18[ns]	0.62***	0.11[ns]	0.41*
Ingestive Behavior[1]		0.68***	0.16[ns]	0.46**	0.32[+]	0.47**
Ingestive Behavior[2]			0.25[ns]	0.70***	0.49**	0.64***
Markers				0.47**	0.21ns	0.31[+]
NRC					0.44**	0.68***
CSIRO						0.78***

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Table 10. Percentage difference between estimates of DMI in dairy cows (absolute means above the diagonal while percentage of change under the diagonal) using different methodologies.

Methodology	Forage mass difference	Behavior[1]	Behavior[2]	Markers	NRC	CSIRO	AFRC
Forage mass difference		2.28	1.85	2.21	2.11	2.30	1.89
Ingestive Behavior[1]	3.33 %		1.92	2.79	2.50	2.45	2.56
Ingestive Behavior[2]	0.63 %	2.73 %		2.56	2.20	2.28	1.92
Markers	-0.07 %	3.26 %	0.55 %		2.24	2.31	2.18
NRC	-12.14 %	-8.39 %	-11.44%	-12.05 %		1.67	2.86
CSIRO	-6.84 %	-3.27 %	-6.18 %	-6.76 %	4.71 %		2.11
AFRC	8.41 %	11.46 %	8.98 %	8.47 %	18.31 %	14.27 %	

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

The NRC and CSIRO models overestimated (i.e. negative percentage bias), while the AFRC model underestimated (i.e. positive percentage bias) DMI calculated through different forage mass approaches. Also, the AFRC model showed closer estimations of DMI (i.e. lower absolute bias) with respect to the other models. Ultimately, the estimation of DMI through forage mass difference had the lowest absolute bias relative to other methodologies (Table 10).

Discussion

Grazing behavior is affected by internal and external factors that modify the animal response, resulting in different levels of intake and production. Dairy cows on ryegrass and clover pastures spent 38 % of their time grazing (Rombach et al. 2019). The shorter grazing time on Kikuyu grass in this experiment may be explained by a greater concentration of neutral detergent fiber relative to ryegrass (Vargas et al. 2014; Aguilar et al. 2009), constraining the total daily intake due to a lower passage rate and physical restriction (Allen 2000; NASEM 2016). Ruminants can increase DMI in diets with a lower concentration of structural carbohydrates (Mertens 1987). However, a similar concentration of structural carbohydrates in Kikuyu grass across regrowth periods precluded reaching any conclusions on their effect on DMI in lactating cows in the current study.

Forage traits may explain animal grazing behavior. Rombach et al. (2019) reported that bite rate and bite mass were 1.21 bite/s and 0.47 g DM/bite, respectively, in dairy cows grazing ryegrass and clover pastures. Those values suggested lower DMI per bite relative to the current experiments, requiring more grazing time to supply nutrient requirements. It is recognized that cattle can modulate grazing time, bite mass, or bite rate according to the forage characteristics (Boval and Sauvant 2019). However, the biological ranges across which ruminants can modify these responses under grazing are not well defined (Sollenberger et al. 2020b). Younger forages have greater nutritive value but less mass than older ones, requiring more grazing time to acquire the nutrient requirements due to the small bite mass. Mature forages show greater forage mass but lesser forage quality, increasing the grass selection and requiring more grazing time to supply the energy requirements (Galyean and Gunter 2016).

The bite mass is associated with the capability of the animal to access forage and is associated with the animal's liveweight and forage characteristics (Gordon et al. 1996; Boval and Sauvant 2019; Sollenberger et al. 2020a). Bite mass increases in taller forages (Gregorini et al. 2008). However, long stems reduce bite mass, especially in pastures with low bulk density (Galyean and Gunter 2016). In the current experiment, there were no bite mass differences among the regrowth periods. However, there was a positive correlation between the grazing time, bite mass, and bite rate with DMI, suggesting that the animal response to forage characteristics may modify forage intake (Holecheck et al. 1995; Sollenberger et al. 2020a).

Determining DMI in grazing conditions presents challenges due to the difficulty of accurately defining the animal response for forage selection, especially in diverse pastures or rangeland conditions (Boval and Sauvant 2019). DMI showed different relationships with forage traits and animal characteristics according to the methodology used to estimate intake with greater cow liveweight, grazing time, bite mass, bite rate, supplementation intake and forage mass positively associated with greater DMI.

Forage management may promote or reduce DMI and modify animal behavior and performance (Holecheck et al. 1995). Abrahamse et al. (2008) reported that cows grazing in a small paddock with frequent rotation showed greater intake than those grazing in bigger ones with less rotation. In this experiment, increasing the frequency at which new grass was offered increased the DMI in dairy cows as calculated using the forage mass difference methodology. However, there were no differences in the DMI using other methodologies when increasing the frequency of fence movements. The forage mass difference methodology may have an implicit methodological bias that limits the accuracy of DMI estimation.

There was a positive but not strong relationship between measurements of DMI and those calculated using nutritional models. Correa et al. (2009) suggested a strong relationship between the DMI estimate with external and internal markers and the NRC (2001) or CNCPS (Fox et al.1992) models. However, NRC (2001) and CSIRO (2007) models tended to overestimate, while Alderman and Cottrill (1993) tended to underestimate DMI relative to the measurement methodologies evaluated. Therefore, it is necessary to recognize the main factors that influence DMI to determine the most appropriate methodology to define DMI in grazing conditions of Kikuyu pastures.

Conclusions

DMI is a cornerstone variable, and it is necessary to identify methodologies that provide more accurate estimations under grazing conditions. Cow behavior was related to forage mass, supplement intake and animal traits. Frequency of fence movements affected cow behavior, while grazing Kikuyu pastures at an intermediate regrowth period of 42 d reduced the grazing time. Conversely, average DMI was related to forage traits, cow behavior and milk production. There was a positive but weak relationship between methodologies used to measure intake and the different models used to predict intake. Ultimately, NRC (2001) and CSIRO (2007) models overestimated DMI, while Alderman and Cottrill (1993) underestimated DMI using the measurement methodologies in the study. Based on these data, we conclude that measurement of forage mass, nutritional quality and cow liveweight are relatively easy to measure and can be used to estimate DMI in field conditions. The measurement of the DMI through the other methodologies tested was laborious and required high investment with no consistent results.

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