UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

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SWARD HEIGHTS FOR MAXIMIZING HERBAGE AND NUTRIENT INTAKE RATE OF DAIRY HEIFERS GRAZING KIKUYU GRASS AND REDUCE *IN VITRO* METHANE PRODUCTION

Porto Alegre (RS), Brasil
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(TESE EM COTUTELA)

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Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.

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(TESIS EN COTUTELA)

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A Omar,

La esencia misma de la vida y del amor sincero

y

A Gloria,

Mi mayor inspiración y amor eterno

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ALTURA ÓTIMA DE PASTO QUICUIO PARA MAXIMIZAR A TAXA DE INGESTÃO DE FORRAGEM E NUTRIENTES DE NOVILHAS LEITEIRAS [1]

Autora: Alejandra Marín
Orientadores: Paulo César de Faccio Carvalho e Jérôme Bindelle

RESUMO

A definição de uma altura do pasto que permite maximizar a taxa de ingestão de forragem e de nutrientes tem sido implementada como uma estratégia de manejo sustentável de pastoreio em sistemas de produção de leite a pasto. O objetivo desta tese foi determinar a altura do pasto quicuiu (*Cenchrus clandestinus - Hochst. ex Chiov*) que permitam às novilhas leiteiras maximizar sua taxa de ingestão de forragem e de nutrientes digestíveis. Além disso, avaliar o efeito da altura do pasto a partir de amostras da metade do estrato superior (estrato potencialmente pastado pelo gado) sobre composição química e a digestibilidade *in vitro* da matéria orgânica (DIVMO) e os principais parâmetros da fermentação ruminal *in vitro*, incluindo a produção de CH_4. Foi realizado um experimento de pastejo, cujos tratamentos consistiram em cinco alturas de capim quicuiu (10, 15, 20, 25 e 30 cm). A taxa de ingestão de curto prazo (STIR, por sigla em inglês) de forragem foi medida com a técnica da dupla pesagem e para a determinação da massa do bocado (MB), da taxa de bocado (TB) e tempo de alimentação foi usado o registrador de movimentos mandibulares IGER Behaviour. Verificou-se que a altura do pasto quicuiu que maximiza o consumo de forragem e de nutrientes por unidade de tempo de alimentação foi de aproximadamente 20 cm. Definir alturas de pasto quicuiu muito baixas (10 cm) ou muito altas (30 cm) como meta de manejo de pastagem restringiria a MB e, portanto, a STIR. A composição química e a DIVMO das amostras de forragem do estrato superior não diferiram dentro de uma faixa de alturas entre 10 a 25 cm; além disso, essas alturas do pasto apresentaram maior DIVMO do que a altura do pasto de 30 cm. A produção de gás *in vitro* e a DIVMS mostraram um ajuste negativo e linear ao aumento da altura do pasto. O CH_4 (ml / g DIVMS) apresentou uma resposta broken line sendo semelhante entre as alturas de 10 a 20 cm e depois de 21 cm aumentou com o aumento da altura do pasto. A razão molar de acetato (mol / 100 mol) teve uma resposta semelhante ao CH_4 (mL /g de DIVMS). A razão molar do propionato (mol / 100 mol) também apresentou uma resposta broken line, mas oposta ao CH_4 (ml / g DIVMS), aumentando até alturas de pasto próximas a 20 cm e depois diminuindo. Em conclusão, para otimizar a STIR e TDN-STIR de novilhas leiteiras, o capim quicuiu deve ser manejado a 20 cm de altura do pasto. Além disso, nessa altura do pasto, a produção de CH_4 *in vitro* (mL / g de DIVMS) também seria reduzida.

Palavras chave: altura do pasto; comportamento ingestivo; metas de manejo de pastoreio; mitigação do CH_4;

[1]Tese de Doutorado em Zootecnia - Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (155 p.) dezembro, 2019.

OPTIMAL SWARD HEIGHT OF KIKUYU GRASS FOR MAXIMIZING HERBAGE AND NUTRIENT INTAKE RATE OF DAIRY HEIFERS [1]

Author: Alejandra Marín
Advisors: Paulo César de Faccio Carvalho and Jérôme Bindelle

ABSTRACT

The definition of a sward height that allows maximizing the herbage and nutrient intake rate has been implemented as a sustainable grazing management strategy in forage-based dairy production systems. The aim of this thesis was to determine the sward height of the kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) that allows dairy heifers to maximize the herbage and total digestible nutrients intake rate. Additionally, assess the effect of sward height from herbage samples of top stratum (stratum potentially grazed by cattle) on the chemical composition, *in vitro* organic matter digestibility (IVOMD), and the main *in vitro* ruminal fermentation parameters, including CH_4 production. A grazing experiment was carried out whose treatments consisted of five sward heights of kikuyu grass (10, 15, 20, 25 and 30 cm). Short-term intake rate (STIR) of herbage was measured with the double weighing technique and for the determination of bite mass (BM), bite rate (BR) and the eating time the IGER Behaviour recorder was used. It was found that the sward height of kikuyu grass that maximizes the herbage and nutrient intake per unit of eating time was approximately 20 cm. Define very low (10 cm) or very tall (30 cm) sward heights of kikuyu grass as a grazing management target would constraint the BM, and thus, the STIR. The chemical composition and IVOMD of the herbage samples from the top stratum did not differ in sward heights ranged between 10 to 25 cm, in addition, these sward heights tended to have a higher IVOMD than 30 cm. The *in vitro* gas production and the IVDMD displayed a negative and linear fit to increasing sward height. The CH_4 (ml /g IVDMD) showed a broken line response being similar between 10 to 20 cm sward height, and then, increased with increases in sward height above to 21 cm. The acetate molar proportion (mol/100 mol) had a similar response to CH_4 (mL/g IVDMD). The propionate molar proportion (mol/100 mol) also displayed a broken line response but opposite to CH_4 (ml /g IVDMD), increasing until sward heights close to 20 cm and then decreasing. In conclusion, to optimize the STIR and TDN-STIR of dairy heifers, kikuyu grass should be managed at 20 cm of sward height. Additionally, at this sward height the *in vitro* CH_4 production (mL/g IVDMD) would be also reduced.

Keywords: dairy heifers; CH_4 mitigation; grazing management target; sward height; grazing behavior

[1]Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (155 p) December, 2019.

ALTURA ÓPTIMA DEL PASTO KIKUYU PARA MAXIMIZAR LA TASA DE CONSUMO DE FORRAJE Y NUTRIENTES DE NOVILLAS LECHERAS [1]

Autora: Alejandra Marín
Orientadores: Paulo César de Faccio Carvalho y Jérôme Bindelle

RESUMEN

La definición de una altura de la pastura que permita maximizar la tasa de consumo de forraje y de nutrientes se ha implementado como una estrategia de manejo sostenible del pastoreo en los sistemas de producción de leche basados en pasturas. El objetivo de esta tesis fue determinar la altura del pasto kikuyo (*Cenchrus clandestinus - Hochst. ex Chiov*) que permite a las novillas lecheras maximizar la tasa de consumo de forraje y de total de nutrientes digestibles. Adicionalmente, evaluar el efecto de la altura de la pastura de las muestras de forraje del estrato superior (estrato potencialmente pastado por el ganado) sobre la composición química, la digestibilidad *in vitro* de la materia orgánica (DIVMO) y los principales parámetros de fermentación ruminal *in vitro*, incluida la producción de CH_4. Se llevó a cabo un experimento de pastoreo cuyos tratamientos consistieron en cinco alturas del pasto kikuyo (10, 15, 20, 25 y 30 cm). La tasa de consumo en el corto plazo (STIR, por su sigla en inglés) de forraje se midió con la técnica de doble pesaje y para la determinación de la masa de bocado (MB), tasa de bocado (TB) y tiempo efectivo de alimentación se usó el registrador de movimientos mandibulares IGER behaviour recorder. Se encontró que la altura del pasto kikuyo que maximiza el consumo de forraje y de nutrientes por unidad de tiempo de alimentación fue aproximadamente en 20 cm. Definir alturas del pasto kikuyu muy bajas (10 cm) o muy altas (30 cm) como meta de manejo de pastoreo restringiría la MB y, por lo tanto, la STIR. La composición química y la DIVMO de las muestras de forraje del estrato superior no difirieron en las alturas de la pastura que osciló entre 10 y 25 cm, además, estas alturas de la pastura mostraron una DIVMO más alta que en la altura de 30 cm. La producción de gas *in vitro* y la DIVMS mostraron un ajuste negativo y lineal con el incremento de la altura. El CH_4 (ml/g DIVMS) mostró una respuesta doble lineal similar entre 10 y 20 cm de altura de la pastura, y luego, incrementó con los incrementos de la altura de la pastura superiores a los 21 cm. La proporción molar de acetato (mol / 100 mol) tuvo una respuesta similar a la del CH_4 (ml/g DIVMS). La proporción molar del propionato también presentó una respuesta doble lineal pero opuesta a la del CH_4 (ml / g DIVMS), aumentando hasta alturas de pastura cercanas a 20 cm y luego disminuyendo. En conclusión, para optimizar la STIR y el TDN-STIR de las novillas lecheras, el pasto kikuyo debe manejarse a 20 cm de altura de la pastura. Adicionalmente, a esta altura de la pastura, la producción *in vitro* de CH_4 (ml / g DIVMS) también se reduciría

Palabras clave altura de la pastura; comportamiento ingestivo; novillas lecheras; metas de manejo del pastoreo; mitigación de CH_4

[1]Tesis de Doctorado en Zootecnia, Producción Animal, Facultad de Agronomía, Universidad Federal Rio Grande del Sul, Porto Alegre, RS, Brasil. (155 p) diciembre de 2019.

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ABBREVIATIONS LIST

Symbol	Description
ADF	acid detergent fiber
BM	Bite mass
BR	Bite rate
C3	temperate grasses
C4	tropical grasses
CH_4	methane
CO_2	carbon dioxide
CP	crude protein
DM	dry matter
DMi	dry matter incubated
DMI	dry matter intake
ET	eating time
GEI	gases de efecto invernadero
GP	gas production
IVDMD	*in vitro* dry matter digestibility
IVOMD	*in vitro* organic matter digestibility
NDF	neutral detergent fiber
OM	organic matter
OMI	organic matter intake
SH	sward height
STIR	short-term intake rate
TDN-STIR	short-term total digestible nutrients intake rate
VFA	volatile fatty acid

1. CHAPTER I.

1.1. GENERAL INTRODUCTION

Herbage and nutrient intake is the most determining factor of productivity of grazing animals (BOVAL; DIXON, 2012) as well as the most significant lever to comply with climate change mitigation goals of pasture-based livestock systems due to the influence of the quantity and quality of the herbage ingested by animals on their enteric methane emissions (HRISTOV *et al*., 2013b). Experts agree that among different management practices, those with greatest potential to mitigate the intensity of methane emissions (i.e. g CH_4/g DMI, g CH_4/kg digestible OMI, g CH_4/Kg product) are those associated with a higher intake of digestible forages (BEAUCHEMIN; MCGINN; GRAINGER, 2008; GERBER *et al*., 2013; GERSSEN-GONDELACH *et al*., 2017; HERRERO *et al*., 2016; HRISTOV *et al*., 2013a). In that sense, and due to restrictions in time for cows to graze because of the share of other activities such as ruminating, milking in the daily time budget (BEGGS *et al*., 2018; CHILIBROSTE *et al*., 2015; GIBB; HUCKLE; NUTHALL, 1998), Carvalho (2013) stressed the importance for dairy cattle in pasture-based systems to achieve high levels of herbage and nutrient intake in the shortest possible time to meet their nutritional requirements. Therefore, grazing management actions that provide adequate sward structures to achieve those goals are called for (FONSECA *et al*., 2012).

Several studies have been highlighting the influence of the sward structure on short-term herbage intake rate (STIR) and animal performances (CARVALHO *et al*., 2001; FORBES, 1988; LACA *et al*., 1992). Among the different characteristics of sward structure, the sward height (SH) is influencing bite mass (BM) the most and consequently STIR as well (FORBES, 1988; HODGSON, 1990; LACA *et al*., 1992). Therefore, adequate SH should be sought as grazing management targets to allow grazing cattle to achieve high levels of herbage intake and diet quality (CARVALHO, 2013). Based on the understanding of grazing processes and ingestive behavior, an innovative grazing management concept oriented to maximizing herbage and nutrient intake per unit of eating time of grazing ruminants was proposed (CARVALHO, 2013). According with this concept, there is an optimal sward structure (i.e height) for each grass species in which animals maximize the herbage and nutrient intake rate (AMARAL *et al*., 2013; BREMM *et* *al.*, 2012; FONSECA *et al*., 2012; GONÇALVES *et al*., 2009; MEZZALIRA *et al*., 2014). Recent studies have demonstrated the potential of this grazing management concept to increase the quantity and quality of the forage consumed by sheep grazing Italian ryegrass and to mitigate CH_4 emissions intensity and suggested that the best diet quality with a sward height that optimizes herbage intake rate contributes to reduce the daily CH_4 emissions (SAVIAN *et al*., 2018).

The kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) is a highly productive pasture species that is well adapted to the forage-based dairy systems and widely used in some countries of Latin America, Australia, and Africa (GARCIA *et al*., 2014). Although the information on grazing management targets of kikuyu to improve quantity and quality of the pasture consumed by dairy cattle has been increasing in recent years (DOBOS *et al.*, 2009; SBRISSIA *et al*., 2018; SCHMITT *et al*., 2019), there is still a lack of information about the sward height that leads to maximize STIR and BM as well as its effect on the nutritive characteristics and the *in vitro* ruminal fermentation parameters, including CH_4 production.

In this context, this thesis presents a conceptual model developed on the relationships between sward structure, ingestive behavior, nutritive characteristics and *in vitro* digestibility of the kikuyu grass, and its implications on ruminal fermentation assessed through an *in vitro* model. It is guided by the hypothesis that there exists an optimal sward height of the kikuyu grass that allows animals maximize the herbage intake per unit of eating time, and that sward height of kikuyu grass that lead to better chemical composition and higher IVOMD also reduce *in vitro* CH_4 production per unit of dry matter digested.

1.2. LITERATURE REVIEW

1.2.1 Metas de manejo del pastoreo basadas en comportamiento ingestivo y sus implicaciones en la mitigación de las emisiones de metano- cotexto

En las últimas décadas muchas investigaciones enfocadas en la relación planta animal han sido conducidas por ecólogos, agrónomos y zootecnistas. La unión interdisciplinar de estas ciencias ha permitido avanzar en el entendimiento de cómo las plantas y los animales se relacionan en un ambiente pastoril y ha contribuido a una mejor comprensión del proceso de pastoreo y de los mecanismos involucrados en la adquisición del forraje (CARVALHO, 2013). El enfoque original de los estudios, en la interfaz planta animal, ha demostrado la importancia de incluir la “perspectiva” animal en la definición de metas de manejo del pastoreo (CARVALHO, 2013).

El pastoreo es un proceso basado en interacciones complejas la cuales se han investigando a partir de las variaciones en el comportamiento de los animales en el tiempo y el espacio (BAILEY Y PROVENZA, 2008). La forma en que un animal reacciona ante las variaciones de la pastura y despliega, en consecuencia, mecanismos de pastoreo, es conocida como comportamiento ingestivo (CARVALHO *et al*., 2001; CARVALHO Y MORAES, 2005). El consumo de forraje diario, desde este enfoque, puede ser definido como el producto de los componentes del comportamiento ingestivo: la masa del bocado, la tasa de bocado y el tiempo de pastoreo (ALLDEN Y WHITTAKER, 1970). La masa de bocado es fundamental y determinante de la tasa de consumo y, consecuentemente, del consumo diario y está altamente influenciada por la estructura de la pastura (CHACON Y STOBBS, 1976; HODGSON, 1989). La estructura de pastura, por su parte, ha sido reconocida como factor determinante de la productividad de los pastos y como el principal vínculo entre la composición de la planta y el comportamiento de los animales en pastoreo (HODGSON, 1985).

De acuerdo con Carvalho et al. (2001), la estructura de la pastura juega un rol muy importante en las variaciones del consumo en pastoreo, pues es la forma en que el forraje esta disponible para el animal y, en última instancia, la que define la cantidad de nutrientes ingeridos por los herbívoros en pastoreo. Los herbívoros seleccionan las plantas y sus componentes morfológicos con el propósito de optimizar la ingestión de nutrientes, así como también, de minimizar los costos energéticos (CARVALHO, 2013). Desde esa perspectiva, Carvalho et al. (2001) propusieron que en el manejo del pastoreo se tiene por desafío crear estructuras de pastura que permitan al animal maximizar el consumo en la unidad de tiempo, en otras palabras, los autores sugirieron que la velocidad de ingestión de forraje (tasa de consumo), pautada o definida por los animales, podría ser una herramienta clave para definir metas de manejo del pastoreo.

El entendimiento alcanzado en las últimas décadas sobre las relaciones entre la estructura de la pastura, la tasa de consumo y el comportamiento de los animales en pastoreo, ha resultado clave para la definición de metas innovadoras de manejo del pastoreo orientadas a optimizar el consumo de forraje en la unidad de tiempo y mejorar así la productividad animal (CARVALHO, 2013). Con base en el comportamiento ingestivo de los animales en pastoreo y con el propósito de definir metas de manejo pre y pos-pastoreo se ha adelantado diversos estudios bajo métodos de pastoreo continuo y rotativo y con pastos de clima templado, tropicales e incluso pasturas nativas (AMARAL *et al*., 2013; BREMM *et al*., 2012; FONSECA *et al*., 2012, 2013; GONÇALVES *et al*., 2009; MEZZALIRA *et al*., 2014). De acuerdo con Fonseca et al. (2013) y Mezzalira et al. (2014), cada especie de forraje tiene una estructura óptima para ser pastada (e.g, altura del pasto) y una estructura óptima en la que los animales deberían abandonarla la cual correspondería aproximadamente al 40% de la altura de entrada.

El concepto de manejo de pasturas basado en el comportamiento ingestivo de los rumiantes fue introducido con el nombre de “Rotatinuo” (CARVALHO, 2013). En estudios recientes realizados en una escala espacio-temporal mayor (potrero-días a meses) se han demostrado mejoras en la productividad animal como producto de un mayor consumo diario de materia seca e indirectamente, dado que los animales sólo consumen hojas del estrato superior, de nutrientes también (SAVIAN *et al*., 2018). En la actualidad, estos avances han cobrado mayor importancia debido a las mayores demandas de intensificación sostenible de los sistemas de producción ganaderos basados en pasturas y al compromiso perentorio de mitigar el impacto ambiental (e.g emisiones de metano entéricas) asumido por este sector productivo (GERSSEN-GONDELACH *et al*., 2017; RAO *et al*., 2015). Estudios más recientes realizados en función de evaluar en campo los efectos de la implementación del concepto de manejo Rotatinuo, corroboran su gran potencial dual de mitigar las emisiones de metano entérico e incrementar la productividad animal (SOUZA FILHO *et al*., 2019; SAVIAN *et al*., 2018).

En esta revisión se presentan los avances cientificos y los conceptos más relevantes sobre la relación planta animal, entre ellos los conceptos de comportamiento ingestivo que respaldan los principios del concepto de manejo del pastoreo, Rotatinuo, y además se presentan algunas implicaciones de este concepto de manejo del pastoreo sobre las emisiones de metano enterico en estudios *in vivo* e *in vitro.*

1.2.2 Comportamiento ingestivo: comprendiendo el consumo de forraje de rumiantes en pastoreo

El pastoreo es una combinación compleja de movimientos y actividades realizados de forma jerárquica en escalas espacio-temporales que difiere según los patrones, los niveles de alimentación y los objetivos de los animales (BAILEY *et al.*, 1996; LACA Y ORTEGA, 1996; BAILEY Y PROVENZA, 2008). En un ejercicio de síntesis sobre las principales actividades y definiciones en cada escala espacio-temporal realizado por Carvalho y Moraes (2005), el bocado (segundos) es reconocido como la menor escala de decisión de los animales y el componente fundamental del pastoreo. En segunda instancia está la estación de alimentación, la cual se define como un semicírculo hipotético disponible frente del animal al alcance de este sin mover las patas delanteras (segundos). Le sigue la sesión de pastoreo o *patch* (minutos), que es un agregado de estaciones de alimentación separadas unas de otras por una parada en la secuencia de pastoreo hecha por el animal para reorientarse y elegir un nuevo *patch* al cual dirigirse. Un *patch* también se entiende como el área donde se observa una agregación espacial de bocados. En esta escala la respuesta funcional (relación entre el consumo de forraje y la abundancia de forraje) es denominada tasa de consumo instantánea, velocidad de ingestión o tasa de consumo a corto plazo (expresada, por ejemplo, g MS / min). Una comida o evento de pastoreo, por su parte, es un agregado (*cluster*) de sesiones de pastoreo que ocurre cada día (horas). Finalmente, el potrero es un área de mayores dimensiones, usualmente cubre 1 ha o más, que puede ser ocupada desde algunos días hasta varios meses (ANDRIAMANDROSO *et al*., 2016) y en algunos casos el potrero simplemente puede estar constituido por un único campo de pastoreo (BAILEY *et al*., 1996) (Figura 1).

En cada nivel jerárquico diferentes factores afectan y determinan los patrones de forrajeo de los herbívoros (GREGORINI *et al*., 2008; GREGORINI; TAMMINGA; GUNTER, 2006). En una escala espacio-temporal corta, tal como una sesión de pastoreo, el consumo de forraje es el resultado de la estructura y la accesibilidad al pasto (CARVALHO *et al*., 2005). Desde esta perspectiva, la estructura del pasto es considerada la principal limitante de la tasa de consumo en la medida en que esta determina las dimensiones del bocado (área y profundidad), el consumo por comida, el consumo diario y, en ultima instancia, el desempeño animal (CARVALHO, 2013). De acuerdo con lo anterior, el bocado, considerado hipotéticamente en forma cilíndrica y definido como el "átomo del pastoreo" (LACA Y ORTEGA, 1996) es un factor determinante en el desempeño animal pues en pastoreo los animales toman el forraje de bocado en bocado, en comidas discretas acumulativas, que lo largo de un día definen el consumo diario (FORBES, 1995; GIBB; HUCKLE; NUTHALL, 1998). Todas las escalas espaciales que ascienden en dirección de la definición del desempeño animal tienen su unidad de medida en una escala temporal, por lo tanto, puede decirse que el proceso de pastoreo es un proceso tiempo-dependiente (CARVALHO, 2013) (Figura 1).

En este contexto, el consumo de forraje en condiciones de pastoreo puede definirse como el producto de los componentes de comportamiento ingestivo, masa de bocado (MB, g), tasa de bocado (TB, nº bocado.min-^{1}) y tiempo total de pastoreo (TP, min) (Allden y Whittaker, 1970). La tasa de consumo (TC, g MS.min^{-1}), por su parte, es el producto de la masa de bocado y la tasa de bocados. La masa de bocado es, a su vez, el producto entre la densidad aparente del forraje y el volumen del bocado (área y profundidad del bocado). En términos generales, la masa de bocado, la variable más importante y determinante del consumo de forraje de los animales en pastoreo (HODGSON, 1985), está altamente influenciada por la profundidad del bocado y está, a su vez, por la altura del pasto (CARVALHO, 1997) y menos influenciada por el área del bocado. De acuerdo con este modelo mecanicista, el consumo diario de forraje también puede ser visto como el producto entre la tasa de consumo (TC) y el tiempo total de pastoreo (TP) (Figura 1). El tiempo de pastoreo, por su parte, se considera el vínculo entre la tasa de consumo en el corto plazo y el consumo diario (HODGSON; CLARK; MITCHELL, 1994).

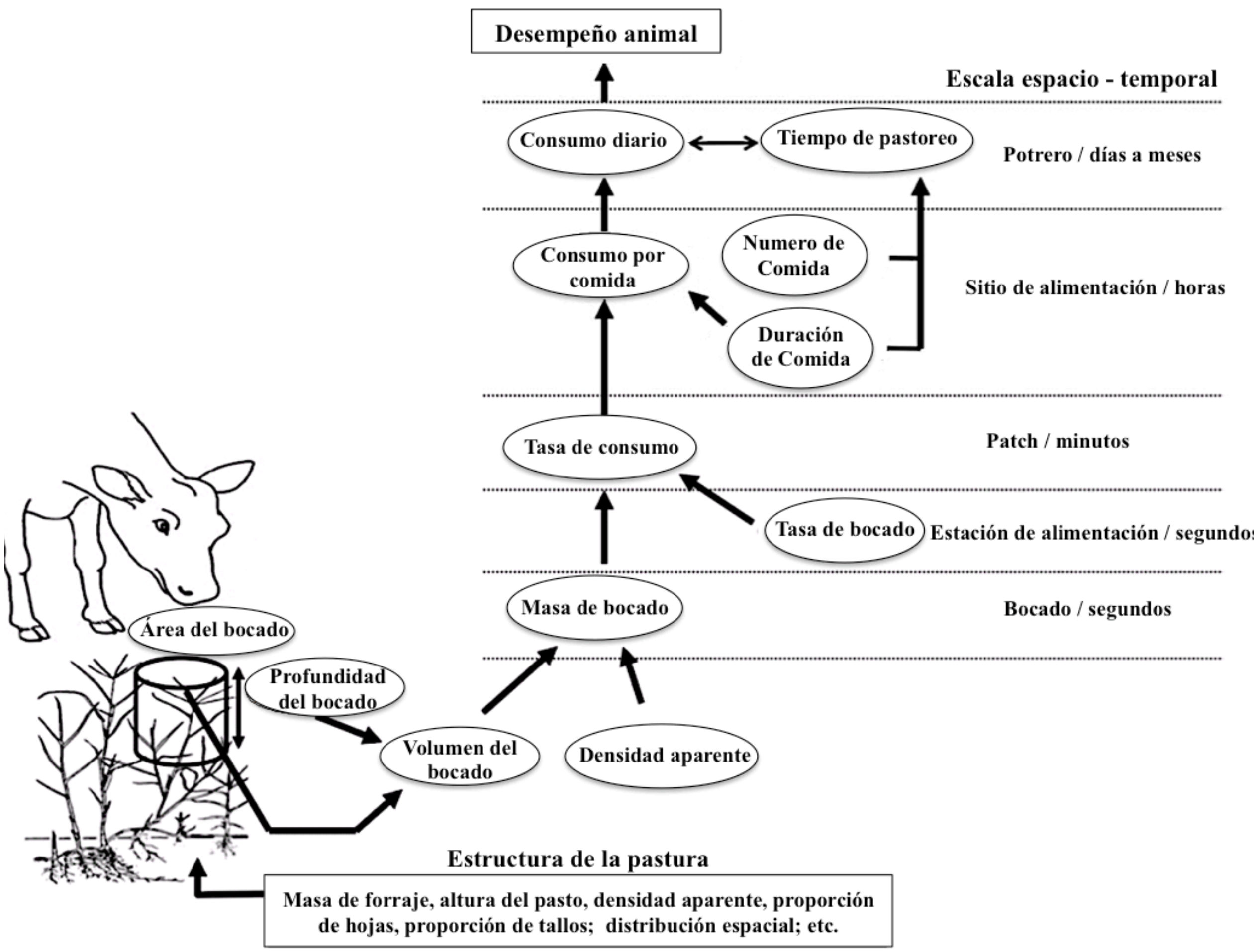

Figura 1. Escalas espacio temporales del pastoreo. Componentes de comportamiento ingestivo de un animal en pastoreo (Adaptado, CARVALHO, 2013).

No obstante, el comportamiento en pastoreo no es uniforme durante la duración de una comida (GIBB; HUCKLE; NUTHALL, 1998; GIBB, 1996). De acuerdo con las definiciones y terminología presentada por Gibb (1996) comer es una actividad que implica la adquisición y manipulación de forraje con la lengua (en el caso de los bovinos), su masticación y su posterior deglución; por su parte, el pastoreo es una actividad que incluye períodos cortos de no-comida, aunque directamente asociados a ella, como los de búsqueda y movilización entre *patchs*. En ese orden de ideas, y de acuerdo con lo sugerido por Gibb (1996) el tiempo de pastoreo y el tiempo de alimentación no deben tratarse como sinónimos en los estudios de pastoreo.

En un estudio sobre la incidencia y la duración de tales períodos de inactividad en vacas lecheras en pastoreo (ROOK; HUCKLE; PENNING, 1994) concluyeron que los períodos de inactividad mayores de 5 minutos podrían considerarse como intervalos entre-comidas, mientras que los períodos de inactividad mayores de 3 segundos y menores de 5 minutos podrían considerarse como intervalos intra-comidas. De acuerdo con esto, una estimación más precisa del consumo diario se puede lograr con el producto de la tasa de consumo y el tiempo de alimentación (TA), que sería el tiempo total de pastoreo excluyendo todos los intervalos de inactividad mandibular mayores de 3 segundos (GIBB; HUCKLE; NUTHALL, 1998; GIBB, 1996). Por lo tanto, el consumo podría ser calculado así:

$$\text{Consumo} = \text{MB} \times \text{TB} \times \text{TA}$$

Sin embargo, la estimación del tiempo de alimentación es casi imposible de registrar manualmente, pues algunas intra-comidas son muy pequeñas, por lo cual se han desarrollado varias herramientas automatizadas y/o software para la caracterización de los movimientos mandibulares del ganado y su comportamiento en pastoreo. El IGER behavior y el software GRAZE se han implementado ampliamente para registrar y discriminar movimientos mandibulares (bocados, masticación, bocados de masticación) y detectar comportamientos como alimentación, rumia y ocio (RUTTER; CHAMPION; PENNING, 1997; RUTTER, 2000). Más recientemente otras tecnologías se han venido desarrollando (ANDRIAMANDROSO *et al*., 2016).

En términos generales puede decirse que el modelo de consumo (basado en los componentes de comportamiento ingestivo) propuesto por (ALLDEN Y WHITTAKER, 1970) en los años 70 ha servido de base para muchas investigaciones sobre la relación planta animal y la comprensión de los mecanismos del proceso de pastoreo además de haber rescatado la importancia de la estructura de la pastura sobre consumo de forraje de los animales herbívoros bajo pastoreo (CARVALHO, 2013; CHILIBROSTE *et al*., 2015).

1.2.3 Influencia de la estructura de la pastura sobre el consumo

La estructura de un pasto se define como la disposición espacial de las partes aéreas de las plantas individuales en una comunidad dada y se puede describir a través de los parámetros tales como la masa de forraje, la altura del pasto, la densidad aparente, la proporción de hojas, la proporción de tallos, la relación hoja/tallo o distribución espacial (LACA Y LEMAIRE, 2000). Desde otra perspectiva, la estructura del pasto es la forma en que el forraje está disponible para un animal jugando así un rol importante en la cantidad de forraje y nutrientes consumido por los animales bajo pastoreo (CARVALHO *et al*., 2001; STOBBS, 1973a) así como también en la proporción de la biomasa que es removida de la pastura (RHODES Y COLLINS, 1993). De acuerdo con Carvalho et al. (2001), la estructura de la pastura ha sido considerada causa y consecuencia del proceso de pastoreo, por un lado, porque la defoliación provoca cambios en los tejidos de las plantas alterando la competencia de la vegetación y sus patrones de crecimiento y, por otro lado, porque la estructura del pasto determina los patrones de defoliación de los animales y el consumo de forraje que, en última instancia, definen el desempeño animal.

Según Carvalho et al. (2001), la estructura de la pastura es el resultado de la dinámica de crecimiento (expansión y aparición) de sus partes en el espacio cuyas variables morfogénicas (tasa de aparecimiento de las hojas, tasa de expansión de las hojas y duración de vida de la hoja) dan lugar a variables estructurales, tales como tamaño de la hoja, densidad de puntos de crecimiento o macollos y número de hojas por macollo (LEMAIRE Y CHAPMAN, 1996). Estas variables, en última instancia, caracterizan la presentación espacial de la materia verde al animal en pastoreo y con la cual un animal debe interactuar. Desde el enfoque del animal, la estructura de la pastura es importante por constituir la base de los componentes morfológicos del pasto y su accesibilidad (CARVALHO *et al*., 2005). De acuerdo con Stobbs (1973a), las características estructurales de la pastura determinan el grado de selectividad de los animales en pastoreo y la eficiencia de colecta influyendo, finalmente, en la cantidad total de los nutrientes ingeridos.

El efecto de varias características estructurales de la pastura, como la masa total de forraje, la masa de las hojas verdes (MS, kg / ha) y la altura del dosel (cm), han sido evaluados sobre el comportamiento ingestivo y el consumo de forraje, tanto en condiciones de campo (ORR *et al*., 1997; PENNING *et al*., 1994), como con pasturas construidas artificialmente (BLACK Y KENNEY, 1984; LACA *et al*., 1992). Penning et al. (1994) mostraron que la masa de las hojas verdes y el índice de área foliar, en lugar de la altura de la pastura, fueron las variables que reflejaron mejor el consumo y el estado de la pastura. Otros autores, por su parte, demostraron que tanto la masa de hojas verdes como la altura de la pastura se correlacionan alta y positivamente con la masa de bocado (ORR *et al*., 1997). Hodgson et al (1994), al respecto, indicaron que la variación en la densidad aparente del forraje puede afectar la masa de del bocado y esta, a su vez, el consumo de forraje. Los trabajos pioneros con pastos tropicales cultivados de (STOBBS, 1973b, 1973a) y Chacon y Stobbs (1976), indicaron que la masa de bocado es el factor principal que influye en el consumo de forraje de animales en pastoreo. Sin embargo, a diferencia de los pastos templados, donde la variación en la altura influye fuertemente en la masa de bocado (HODGSON, 1985), con pastos tropicales, además de la altura, la masa de hojas, la relación hoja:tallo y la densidad aparente son las características estructurales que afectan significativamente la masa de bocado y la tasa de consumo a corto plazo (STOBBS, 1973a). Otros autores señalan que el máximo consumo en pasturas tropicales ocurre cuando los animales pastan en estructuras con alta densidad de hojas accesibles al animal (DEMMENT; PEYRAUD; LACA, 1995; FLORES *et al*., 1993). En este sentido, diversos estudios con pastos tropicales coinciden en señalar que la densidad aparente es una de las características estructurales más determinantes en el consumo de forraje en animales en pastoreo (HODGSON, 1985; LACA *et al*., 1992; STOBBS, 1973a). Stobbs (1973a) observó que, a diferencia de los pastos templados, los pastos tropicales varían mucho en su composición del estrato superior hasta el estrato inferior de la pastura. Silva y Carvalho, (2005) señalaron que la elongación de los tallos durante el desarrollo vegetativo en pastos tropicales contribuye en gran medida con la densidad aparente del forraje y esta es una diferencia importante para resaltar, con relación a los pastos de clima templado. En otros estudios se ha demostrado que la presencia de tallos actúa como una barrera física vertical para las dimensiones del bocado (profundidad y área) (BENVENUTTI; GORDON; POPPI, 2006) limitando, por lo tanto, la tasa de consumo (DRESCHER, 2003; FLORES *et al*., 1993). De acuerdo con esto, los animales prefieren hojas a tallos, lo que quedó demostrado al evaluar el efecto de barrera vertical y horizontal que ejercen los tallos de *Panicum maximum* sobre la masa de bocado y la tasa de consumo; donde se halló que además de la limitación física por la presencia de tallos, la densidad de los tallos también afecta la formación del bocado (BENVENUTTI; GORDON; POPPI, 2006). Benvenutti et al. (2008) observaron de manera similar que el aumento en la proporción de tallos en el estrato más bajo de la pastura no permitió una alta tasa de ingestión de forraje en el corto plazo. Los animales, a fin de optimizar su tasa de consumo, prefieren pastar *patches* altos que bajos, a menos que los primeros reflejen mayor madurez (alto contenido de tallos y/o mayor esfuerzo para tomar un bocado), en cuyo caso prefieren a los segundos (cortos e inmaduros) lo que enfatiza sobre la importancia de distinguir entre estructuras donde predominen hojas y tallos, y sugiere que considerados sobre una misma base, las especies de pastos templados y tropicales pueden influir de manera similar sobre la masa de bocado y la tasa de consumo de forraje de animales de pastoreo (revisado en Silva y Carvalho, (2005). Estudios recientes han reforzado este argumento, indicando que a pesar de los hábitos de crecimiento contrastantes de las especies tropicales y de clima templado, los patrones de respuesta de la masa de bocado y la tasa de consumo a la altura del pasto, en general, son similares (MEZZALIRA, 2012).

La importancia de la altura del pasto en la definición del consumo y su relación con las dimensiones del bocado, especialmente con la profundidad del bocado, ha sido ratificada por varios autores (CANGIANO, 1999; CARVALHO, 1997; HODGSON; CLARK; MITCHELL, 1994; LACA *et al*., 1992; WADE, 1991). De acuerdo con Carvalho (1997), y a partir de diferentes estudios en diversas condiciones se concluyó que la profundidad del bocado esta relacionada lineal y positivamente con la altura de la pastura y lineal y negativamente con la densidad aparente del forraje, aunque existen algunas excepciones que describen una forma asintótica (LACA *et al*., 1992; PARSONS *et al*., 1994). Demment y Laca, (1993) con una técnica donde variaban la altura y la densidad de forma independiente, demostraron la importancia de la altura del pasto para maximizar la masa de bocado. De acuerdo con Carvalho et al. (2001) la altura, para los animales, representa la cantidad de biomasa disponible y su preferencia significa la oportunidad de alta ingestión en la medida en que la altura potencializa la profundidad del bocado. Según

Hodgson (1990), la altura del dosel de la pastura es la característica estructural que más influye en las dimensiones del bocado y, consecuentemente, en la tasa de consumo en el corto plazo (CARVALHO *et al*., 2001; FORBES, 1988). En una escala menor de pastoreo (comida), la altura y la densidad volumétrica de los pastos son las variables estructurales que más influyen en la tasa de consumo (CARVALHO *et al*., 2017). La altura, además de ser un descriptor importante del estado de la pastura, ha recibido una atención considerable en los últimos años por ser una herramienta útil y de fácil implementación por lo que se ha sugerido como un parámetro viable para el establecimiento de metas de manejo del pasto (HODGSON, 1985, 1990).

En este contexto y dada la influencia de la estructura de la pastura en la tasa de consumo de forraje, y consecuentemente en la producción animal, Carvalho et al. (2001) sugirieron que el manejo de pasturas debe ser visto como la construcción de estructuras que permitan a los animales en pastoreo mantener altas tasas de consumo de forraje.

1.2.4 Relaciones entre el valor nutritivo y la estructura de la pastura y sus implicaciones sobre el consumo de forraje

El aumento de la producción de leche o carne requiere un mayor consumo de energía metabolizable y, por lo tanto, un aumento del consumo voluntario y/o dietas digestibles seleccionadas por los animales bajo pastoreo (BOVAL Y DIXON, 2012). Los mecanismos clásicos de control del consumo voluntario de forrajes por parte de los rumiantes han sido principalmente explicados en el marco de las teorías convencionales de los controles metabólicos y físicos del apetito (ALLISON, 1985; FORBES Y BARRIO, 1992).

De acuerdo con Poppi et al. (1987), el consumo de forraje de los animales en pastoreo representa un comportamiento asintótico que está regido por factores no nutricionales y nutricionales. Los factores no nutricionales son aquellos relacionados con la estructura y el comportamiento ingestivo de los animales en pastoreo mientras que los factores nutricionales están relacionados con los aspectos inherentes a la digestibilidad, composición química del forraje y factores metabólicos (POPPI; HUGHES; L'HUILLIER, 1987) (figura 2).

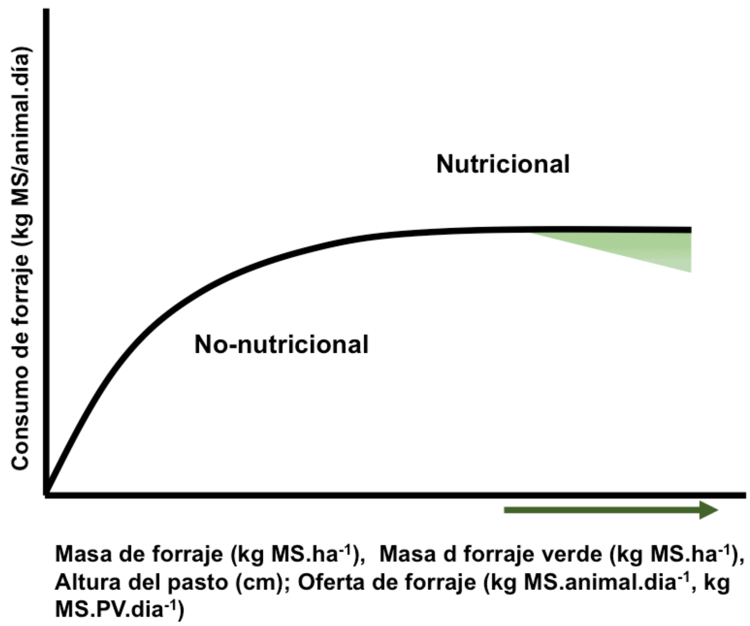

Figura 2. Controles de consumo de forraje en condiciones de pastoreo (Reis y Silva, 2011) adaptado de (Poppi et al., 1987).

Durante una sesión de pastoreo, el consumo describe una curva asintótica. La capacidad del animal para cosechar forraje en la fase inicial ascendente de la curva de consumo, parece ser el factor más importante que limita el consumo (factores no nutricionales). En este caso, esos factores son influenciados por el comportamiento ingestivo de los animales en pastoreo (selección de la dieta, tiempo de pastoreo, masa de bocado y tasa de bocado), muy sensibles a los cambios en la estructura de la pastura la cual, a su vez, está fuertemente influenciada por las prácticas de manejo del pastoreo (SILVA Y CARVALHO, 2005). Ya en la fase asintótica de la curva, los factores nutricionales como la digestibilidad, tiempo de retención del alimento en el rumen y concentración de productos metabólicos parecen ser más importantes en el control del consumo de forraje (POPPI; HUGHES; L'HUILLIER, 1987), aunque hay pruebas crecientes de que también pueden operar los factores no nutricionales a este nivel (CARVALHO, 1997; SILVA Y CARVALHO, 2005) (Figura 2).

La importancia de la composición química y la digestibilidad ha sido ampliamente resaltada en la literatura (CARVALHO *et al.*, 2017). La estrecha relación entre la digestibilidad y el consumo de materia seca fue señalada por Van Soest. (1994). La fibra en detergente neutro (FDN) es también considerada un indicador confiable del consumo en la medida en que aumentos en la FDN se traducen en reducciones en el consumo de materia seca, lo cual se ha atribuido, principalmente, a una limitación de tipo físico, el llenado ruminal (MERTENS, 1994). Recientemente, Harper y McNeill, (2015) señalaron que el consumo es dependiente también del potencial de digestibilidad de la FDN, sugiriendo la necesidad de incluir su fracción indigerible (FDNi) en los modelos de predicción de consumo. La cantidad y tipo de proteína, por su parte, también pueden afectar el consumo (CARVALHO et al., 2017). De acuerdo con un estudio de Boval et al. (2007) existe una correlación positiva entre el contenido de proteína y el consumo diario de forraje. Van Soest (1994) ya había señalado que los bajos contenidos de proteína cruda (e.g. PC, < 7%) reducen la disponibilidad de N para los microorganismos del rumen, por cuanto limitan su crecimiento y eficiencia y, consecuentemente, la degradación de la fibra (LENG, 1990). No obstante, esos mecanismos de relación entre consumo, digestibilidad y composición química son válidos cuando el alimento, en este caso el forraje, ya se encuentra en el tracto digestivo, principalmente a nivel del rumen, o en aquellos casos donde los animales no requieren cosechar el forraje, como por ejemplo con animales estabulados, a diferencia de lo que ocurre con animales en pastoreo (ILLIUS Y GORDON, 1987; REIS Y SILVA, 2011; CARVALHO *et al*., 2017). Como se señaló anteriormente, en pastoreo los animales se enfrentan a un ambiente complejo que varía en el espacio-tiempo; bajo esas condiciones los animales deben buscar y manipular el forraje a fin de alcanzar a llenar sus demandas nutricionales lo que implica retos adicionales (CARVALHO Y MORAES, 2005). En este contexto, la estructura tiene una importancia relativamente mayor frente al valor nutritivo como limitante del consumo de forraje de los animales en pastoreo (CARVALHO Y MORAES, 2005).

La estructura de la pastura, desde esa perspectiva, juega un rol determinante en la calidad de la dieta de los herbívoros en pastoreo tanto con pastos tropicales como templados (HARDY; MEISSNER; O’REAGAIN, 1997). Dentro de las diferentes variables estructurales de la pastura, las hojas verdes son el componente de mayor digestibilidad y el de mayor efecto sobre la calidad de la dieta (CHACON Y STOBBS, 1976; O’REAGAIN Y MENTIS, 1988; HARDY et al., 1997). De acuerdo con Wade y Carvalho (2000), la masa de hojas es determinante tanto en la cantidad como en la calidad del forraje consumido. La disponibilidad y accesibilidad de las hojas, por lo tanto, están estrechamente relacionadas con la calidad del forraje consumido en cada bocado (O'REAGAIN Y MENTIS, 1988; BAILEY, 1995; DRESCHER, 2003). En términos generales, los animales tienden a seleccionar plantas ricas en hojas por su terneza y alto contenido de nutrientes y evitan los tallos por su baja calidad, rigidez y mayor resistencia a la tracción (O'REAGAIN Y MENTIS, 1989; O'REAGAIN, 1993). De acuerdo con Prache et al. (1998), los animales interactúan con la estructura de la pastura ajustando las dimensiones del bocado a fin de seleccionar las partes más nutritivas y maximizar la calidad de lo consumido. Los pastos tropicales varían significativamente en la cantidad y calidad de las hojas y consecuentemente en su valor nutritivo desde el estrato superior hasta el estrato inferior (distribución vertical) (CHACON Y STOBBS, 1976). El valor nutritivo del estrato superior, en general, se asocia con mayor proteína cruda, mayor digestibilidad aparente de la materia seca y menor concentración de FDN (SOLLENBERGER Y BURNS, 2001). En ese sentido, la calidad y la cantidad de la dieta seleccionada por los animales en pastoreo va a depender de la intensidad de defoliación (CHACON Y STOBBS, 1976).

En un pastoreo rotativo tradicional, el ganado pasta por horizontes o capas aprehendiendo primero los bocados potenciales ubicados en el horizonte más expuesto para asegurar una mayor proporción de hojas en la dieta (CARVALHO, 2013; CARVALHO et al., 2016). Independientemente de la altura del pasto y del peso vivo de los animales, estos pastan en capas sucesivas removiendo aproximadamente el 50% de la altura inicial de la pastura (proporcionalidad constante de remoción de forraje) (LACA Y DEMMENT, 1991; GALLI et al., 1996; BAUMONT et al., 2004). Los cambios horizontales (distribución horizontal) en la disponibilidad y accesibilidad de las hojas verdes condicionan la concentración de nutrientes a nivel de bocado (CARVALHO, 2013). En la medida que los animales van accediendo a los horizontes más bajos durante la defoliación progresiva de la pastura, menores son los volúmenes de los bocados potenciales y, paralelamente, la calidad del forraje ingerido en la medida en que esta representa el promedio de los bocados de los horizontes pastados (CARVALHO et al., 2017). De acuerdo con Gregorini et al. (2011), si los animales prefieren las hojas verdes, el componente más digerible del forraje, es claro que una reducción en la masa del forraje conducirá a reducciones en la digestibilidad total del forraje. Estudios recientes han demostrado que la mayor tasa de consumo de forraje se logra cuando el animal come principalmente las hojas predominantes del estrato superior de la pastura. Fonseca et al. (2012); Mezzalira et al. (2014) y Benvenutti et al. (2016) hallaron que la calidad de la dieta es mayor cuando los animales tienen acceso al estrato frondoso superior de la pastura y disminuye en la medida en que los animales acceden a los estratos inferiores, donde la proporción de tallos es mayor concluyendo que la masa de bocados y el consumo de nutrientes es mayor cuando hay un consumo predominante de las hojas del estrato superior.

En la práctica, el ganado, sobre todo el lechero especializado, al momento del pastoreo podría estarse comportando simultáneamente como un minimizador de tiempo y un maximizador de energía, es decir, durante el restringido lapso de tiempo que dispone para pastar, éste buscaría ganar la mayor energía en el menor tiempo posible de tal manera que puedan, por un lado, asegurar su potencial éxito reproductivo (PIANKA, 1976; PYKE; PULLIAM; CHARNOV, 1977) y por el otro, disponer del tiempo suficiente para sus otras actividades (BERGMAN et al., 2001; GIBB; HUCKLE; NUTHALL, 1998).

Para Schoener (1971), un minimizador de tiempo es un animal cuya *fitness* (adaptación y éxito reproductivo) se maximiza cuando se minimiza el tiempo dedicado a la alimentación para reunir un requisito de energía determinado. Un maximizador de energía, por su parte, es un animal cuya *fitness* se maximiza cuando la energía neta se maximiza durante un tiempo determinado de alimentación.

Si se tiene en cuenta que la única distinción real que se puede establecer entre estas dos categorías de forrajeros propuesta por Schoener (1971) es que, durante un período de tiempo determinado disponible para el pastoreo (y se enfatiza "disponible"), un minimizador de tiempo deja de pastar después de obtener un requerimiento de energía neta, mientras que un maximizador de energía continua haciéndolo, se llega a la conclusión entonces que la proporción relativa de tiempo disponible gastada en pastar representa el único criterio de distinción para clasificarlos. Así las cosas, cuando el tiempo disponible es restringido, se imposibilita su distinción, si es que la hay (HIXON, 1982). Solo cuando se disponga del tiempo suficiente, podrían observarse los maximizadores de energía, cuando los haya.

En concepto de Hixon (1982), si se aplica rigurosamente la base empírica que sustenta los criterios de clasificación en el sentido original de Schoener, un maximizador de energía que se sacia y deja de pastar durante el período en cuestión podría ser etiquetado como un minimizador de tiempo mientras que, a la inversa, un minimizador de tiempo que de otra manera no alcanza a llenar sus requerimientos de energía neta y, por lo tanto, se ve obligado a seguir alimentándose continuamente durante el mismo período, se etiquetaría como maximizador de energía (HIXON, 1980). En ese sentido sugiere cambiar el nombre asignado a estos forrajeros por el de "minimizadores del tiempo de alimentación" y "maximizadores del tiempo de alimentación" teniendo al período de tiempo dado como el factor determinante más importante (HIXON, 1982).

Mientras que, en los animales silvestres, sobre los cuales se construyen estos modelos empíricos, los tiempos de forrajeo y no-forrajeo están determinados por la relación animal-ambiente, principalmente, en las vacas de leche bajo pastoreo, ese no es el caso pues esta relación es relegada a un segundo plano y sometida al servicio de los fines productivos de los animales y a los horarios que estos imponen. En ese orden de ideas, y dadas las restricciones de tiempo a que se ve sujeto el ganado de leche en producción bajo pastoreo durante una jornada (GIBB MJ; HUCKLE CA; NUTHALL R; PENNING PD., 1996; GIBB *et al*., 1998; DOHME-MEIER *et al*., 2014; CHILIBROSTE *et al*., 2015; DELAGARDE Y LAMBERTON, 2015), se hace difícil, si no imposible, ubicarlo inequívocamente en una de estas dos estrategias de alimentación. En conclusión, a las vacas de leche de alta producción en pastoreo, para llenar sus requerimientos, no les quede otra opción que ser minimizadoras de tiempo de alimentación.

Lo relacionado concretamente con los condicionantes, la eficiencia y los resultados del proceso como tal, se estudian y se explican desde el concepto de respuesta funcional en el cual el estado de la pastura (cantidad y calidad) juega un papel principal (revisado en Searle y Shipley, 2008) mientras que otros factores, como condición corporal del animal, se le otorgan papeles secundarios (VISSCHER Y MERRILL, 2018).

El estado de la pastura por afectar la selectividad al momento de la cosecha del forraje se convierte en una característica clave para tener en cuenta al momento de diseñar estrategias de alimentación animal conducentes a promover una mayor ganancia energética en el menor tiempo posible (VISSCHER Y MERRILL, 2018). Los animales pueden intercambiar (*trade-off*) calidad por cantidad, aunque menos preferida, más conveniente en términos energéticos (BERGMAN *et al*., 2001; PRACHE, S.; GORDON, I. J.; ROOK, A. J., 1998). En otras palabras, los animales en procura de una mayor eficiencia buscan los bocados potenciales más rentables en términos de costos de energía y tiempo asociado a la manipulación y búsqueda (GREGORINI; GUNTER; BECK, 2008). Los rumiantes en pastoreo, por lo tanto, prefieren el forraje que se puede consumir con mayor rapidez, a una alta tasa de ingestión en la unidad de tiempo (BLACK Y KENNEY, 1984; ILLIUS et al., 1992; PRACHE, 1997; PRACHE, S.; GORDON, I. J.; ROOK, A. J., 1998; BERGMAN et al., 2001; UTSUMI et al., 2009; CARVALHO, 2013). Carvalho et al. (2001) sugirieron que en situaciones de elevadas ofertas de forraje (e.g. pastoreo rotativo tradicional) las restricciones del consumo de forraje pueden estar asociadas con el aumento del tiempo de manipulación. Por lo tanto, parece que el consumo diario de materia seca de los animales que pastan en pastos tropicales no esta en función exclusiva de la mala calidad del forraje, como lo sugirieron Silva y Carvalho (2005).

En este contexto, la adquisición del forraje bajo pastoreo es un limitante del consumo previo y predominante a los mecanismos clásicos, físicos y metabólicos de control de consumo; es decir, la captura de forraje (ingestión), y no la composición química del mismo (digestión), es el principal limitante del consumo y de la producción animal, de lo cual se puede concluir que la ingestión estaría fundamentalmente limitada por el tiempo de adquisición de forraje (e.g. tiempo pastoreo) (CARVALHO *et al*., 2017).

1.2.5 Metas de manejo de pastoreo basados en el comportamiento ingestivo.

1.2.5.1 El tiempo como un factor limitante de la optimización del consumo en pastoreo

El pastoreo es un proceso tiempo-dependiente cuyas actividades relacionadas con la adquisición de forraje compiten entre sí (figura 1) (CARVALHO et al., 2001). De acuerdo con Carvalho (2013), los herbívoros seleccionan las plantas y sus componentes morfológicos con el propósito de optimizar la ingestión de nutrientes, así como también, minimizar los costos energéticos. Otros autores por su parte han argumentado que los rumiantes prefieren el forraje que se puede consumir a una alta tasa de consumo (mayor rapidez) incluso si esa decisión significa consumir una dieta de menor calidad (BLACK Y KENNEY, 1984; PRACHE, S.; GORDON, I. J.; ROOK, A. J., 1998; BERGMAN et al., 2001). En este sentido y teniendo en cuenta que los animales en pastoreo toman el forraje de bocado en bocado, en comidas discretas acumulativas que lo largo de un día definen en consumo diario (CARVALHO, 2013; FORBES, 1995; GIBB; HUCKLE; NUTHALL, 1998) y que, además, en un día los animales pueden aprehender entre 25 mil y 30 mil bocados, en una frecuencia que puede variar entre 45 y 72 bocados por minuto (ANDRIAMANDROSO *et al*., 2016). Carvalho et al. (2017), sugirieron que, si cada uno de esos bocados fuese colocado en una estructura óptima que les permitiera aumentar la ingestión de forraje en la unidad de tiempo de alimentación, al final de día los animales habrían consumido la máxima cantidad de forraje de la mejor calidad.

A través de un ejemplo hipotético, Carvalho et al. (2017) mostraron como pequeñas variaciones en la masa de bocado (escala inferior de pastoreo), multiplicada por miles de veces en que cada bocado ocurre en el día, podrían tener grandes influencias en el consumo diario (escala superior) de los animales y, consecuentemente, en su productividad. En otras palabras, una pequeña variación en el tiempo debida, por ejemplo, a una estructura de la pastura (e.g. altura) que implique mayor tiempo de manipulación y/o procesamiento, y dado que mientras un bocado es procesado otro no puede ser realizado, implicará mayor tiempo total de pastoreo (CARVALHO et al., 2001).

No obstante, a fin de mantener el consumo diario de forraje, los animales pueden compensar una baja tasa de consumo instantánea, a través de la reasignación movimientos mandibulares (LACA *et al*., 1994, GIBB *et al*., 1999), o el aumento de la tasa de bocados (CHILIBROSTE, 1999, MEZZALIRA *et al*., 2014) o el tiempo de pastoreo (DIFANTE *et al*., 2009; GIBB *et al.*, 1997), respuestas compensatorias que no siempre son eficientes (CHILIBROSTE *et al*., 2015; GIBB; HUCKLE; NUTHALL, 1998). Gibb *et al*. (1999) evaluando el efecto de la estructura de la pastura sobre la masa de bocados y la tasa de consumo, encontraron que cuando la masa de bocado se vio limitada por las condiciones de la estructura de la pastura, las vacas lecheras aumentaron la tasa de bocados (frecuencia de bocados) y el tiempo total dedicado al pastoreo cada día, sin embargo, no pudieron compensar completamente la reducción en la tasa de consumo ni el consumo diario de forraje. Por otro lado, el tiempo en pastoreo es finito, normalmente se restringe a menos de 10 horas / día (HODGSON, 1990) y compite con otras actividades que son normales del comportamiento en pastoreo como la rumia, el descanso, la socialización y el ocio, entre otras jornadas (GIBB ET AL., 1998; DOHME-MEIER *et al*., 2014; CHILIBROSTE *et al*., 2015; DELAGARDE Y LAMBERTON, 2015). Esto es particularmente importante en los sistemas de producción de leche donde además de estas actividades aparecen las actividades asociadas al ordeño (desplazamientos, espera, ordeño), como una actividad adicional que disminuye de manera significativa el tiempo de que dispone el animal para recolectar forraje mediante el pastoreo (CARVALHO, 2013; GIBB *et al*., 1996; ISLAM *et al*., 2015; THOMPSON; WEARY; VON KEYSERLINGK, 2017).

Partiendo del argumento que el tiempo es el principal factor limitante para la adquisición de forraje y de nutrientes en pastoreo y de la necesidad de identificar y ofrecer las estructuras del pasto que permitan a los animales optimizar la tasa de consumo de forraje, un concepto de manejo de pasturas denominado Rotatinuo, revisado a continuación, ha sido propuesto (CARVALHO, 2013).

1.2.5.2 *Rotatinuo*: un concepto innovador de manejo de pasturas

Rotatinuo es un concepto de manejo del pastoreo basado en comportamiento ingestivo en el que se establecen metas de manejo pre y pos-pastoreo que permitan a los animales optimizar y mantener constante la tasa de consumo de forraje en la unidad de tiempo de alimentación (CARVALHO, 2013). Este concepto, fue denominada Rotatinuo (ROTAtional y conTINUO) por presentar al animal, gracias a su estrategia de manejo, un pasto con una estructura (altura) óptima continuamente.

Bajo este concepto, el comportamiento ingestivo, y no los atributos de la producción de biomasa, rigen la definición de metas de manejo del pasto (CARVALHO, 2013). La importancia del Rotatinuo radica en el tiempo como principal factor limitante de la producción de los animales en pastoreo (CARVALHO *et al*., 2017).

Carvalho et al. (2001) sugirió que con pastos tropicales el tiempo para la acción del bocado (movimientos de la cabeza y lengua, el tiempo para la aprehensión y formación del bocado) podría ser un factor limitante en el consumo de los animales principalmente en situaciones de alta oferta y/o con alto acúmulo de material senescente debido, probablemente, a que la dispersión espacial de las hojas podría limitar la ingestión de forraje, no por efecto simplemente de la densidad aparente del forraje, si no por el aumento del tiempo (función lineal) requerido en el proceso de captura de hojas por parte del animal (Figura 3).

En dicho trabajo se halló que en situaciones de elevadas ofertas de forraje (e.g. pastoreo rotativo) las restricciones del consumo de forraje pueden estar asociadas con el aumento del tiempo de manipulación (CARVALHO et al., 2001).

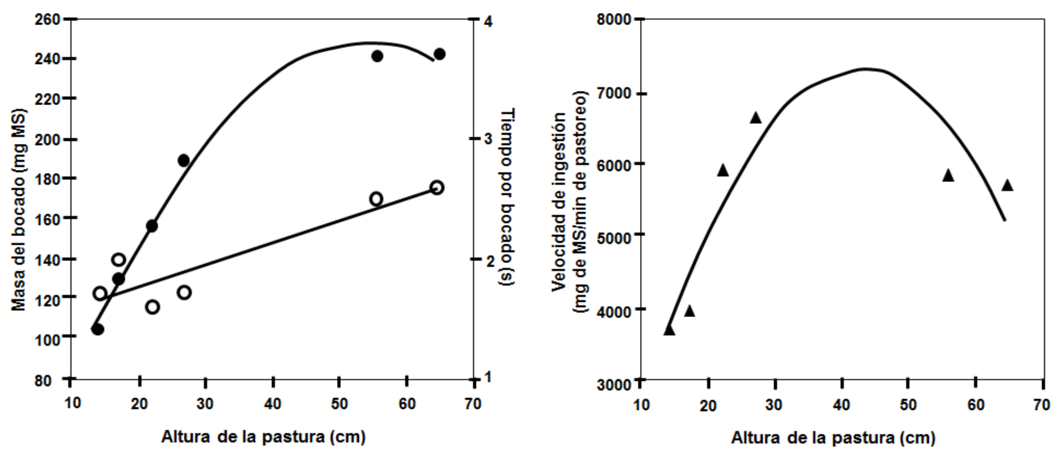

Figura 3. Comportamiento ingestivo de borregas en pasturas de pasto Tanzania (*Megathyrsus maximus*) con diferentes alturas pre-pastoreo (masa de bocado (●), tiempo por bocado (o) y velocidad de ingestión (▲), tomado (CARVALHO et al., 2001).

En una serie secuencial de experimentos y protocolos realizados posteriormente, se aportaron nuevas evidencias de cómo la estructura de los pastos tropicales influye sobre el consumo de forraje (CARVALHO, 2013). Dichos estudios sugirieron que los animales en pastoreo se toman más tiempo para adquirir una determinada masa de bocado con pastos tropicales que con los templados (AMARAL *et al*., 2013; BREMM *et al*., 2012; FONSECA *et al*., 2012; MEZZALIRA *et al*., 2014). Mezzalira (2012) evaluó en dos especies de hábitos de crecimiento contrastante (*Avena strigosa* y *Cynodon* sp Tifton 85) y bajo dos métodos de pastoreo (continuo y rotacional), los mecanismos mediante los cuales la altura de la pastura influencia la tasa de consumo en el corto plazo. Los resultados de ese trabajo mostraron que tanto en alturas bajas como en las altas, la masa de bocado y la tasa de consumo disminuyeron para ambas especies, aunque por mecanismos diferentes; mientras que en las alturas bajas esa disminución estuvo relacionada con la limitación en la profundidad del bocado, en las alturas de pasto más altas estuvo asociada con el mayor tiempo por bocado debido al decrecimiento de la densidad aparente del forraje en el estrato superior (MEZZALIRA, 2012; MEZZALIRA *et al*., 2014). Resultados similares fueron encontrados en otros pastos tropicales como *Panicum maximum* cv. Mombaza (PALHANO *et al*., 2007) y *Sorghum bicolor* (FONSECA *et al*., 2012).

A partir de estos hallazgos fue sugerido que los componentes de comportamiento ingestivo y concretamente la masa de bocado (que influye en la tasa de consumo y en última instancia en el desempeño animal), es un indicador clave para identificar la estructura óptima en la que los animales maximizan la ingestión de forraje en la unidad de tiempo, y por lo tanto, metas de manejo pre-pastoreo (e.g. momento de entrada a un potrero en un pastoreo rotativo) (CARVALHO, 2013). En los trabajos de Mezzalira (2012) y Fonseca et al. (2012), también se evaluaron diferentes niveles de defoliación de forraje como una proporción de la altura de entrada donde los animales optimizaban la masa de bocado con el propósito de definir la estructura óptima de salida. Los resultados mostraron que la tasa de consumo inicialmente se mantuvo constante y luego decreció linealmente en la medida que el forraje era defoliado (Figura 4).

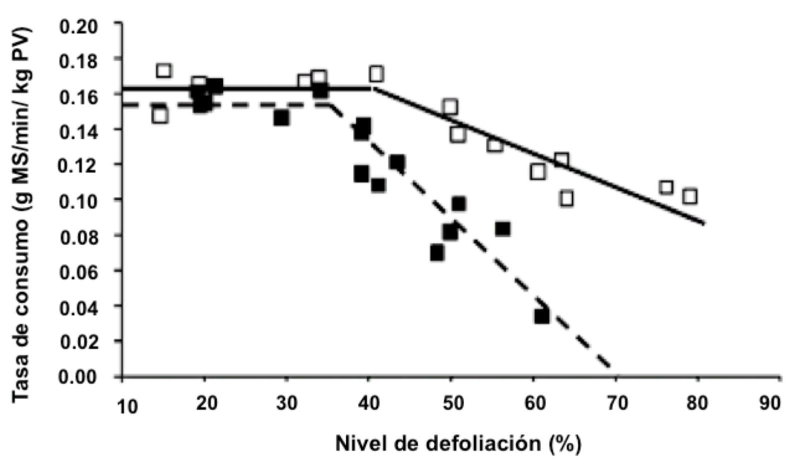

Figura 4. Tasa de consumo en función de diferentes niveles de defoliación (% de reducción de la altura inicial de pasto) en pastos de *Cynodon* sp (■) *Sorghum bicolor* (□) (Adaptado de Carvalho (2013).

A pesar que estos trabajos fueron realizados con estructuras contrastantes de diferentes pastos y que la velocidad de disminución de la tasa de consumo fue más rápida para *Cynodon* sp (MEZZALIRA, 2012) que para *Sorghum bicolor* (FONSECA *et al*., 2012, 2013), en ambos estudios la tasa de consumo comenzó a caer en un nivel de defoliación semejante, correspondiente al 40% de la altura del pasto de entrada; además, los resultados permitieron mostrar que la eficiencia de colecta de nutrientes (proporción de hojas pos-pastoreo) por unidad de tiempo también disminuía después de una defoliación media del dosel forrajero del 40%.

Las investigaciones sobre el comportamiento ingestivo no solo han contribuido a un mayor entendimiento sobre el proceso del pastoreo y de la interface planta animal, sino que también han permitido proponer metas de manejo de la altura pre pastoreo óptima, que permite a los animales maximizar la ingestión de forraje y de nutrientes en la unidad de tiempo de alimentación, y de la altura pos-pastoreo, en la cual la velocidad de ingestión se mantendría constante (altura pos-pastoreo sería la correspondiente al 40% de la altura media pre-pastoreo óptima). El concepto de manejo del pastoreo Rotatinuo puede ser implementado en pastoreo rotativo, en el cual las metas de manejo se implementarían, por ejemplo, en la entrada y salida de una franja en un potrero. Por lo tanto, también se puede aplicar o en pastoreo continuo, desde una perspectiva de desplazamiento entre estaciones de alimentación (CARVALHO et al., 2016).

Tras la implementación de este concepto de manejo de pasturas se espera que los animales (e.g. vacas lecheras), siempre puedan ingerir el forraje de la mejor calidad (principalmente hojas), a las más altas tasas de consumo lo que permitiría un uso más eficiente del tiempo que los animales pueden dedicar al pastoreo (CARVALHO, 2013) lo cual cobra mayor relevancia en los sistemas de producción actuales que están siendo rediseñados para conciliar la producción animal con la gestión ambiental (BOVAL Y DIXON, 2012; AMARAL *et al*., 2013).

En general, las prácticas de manejo del pastoreo que permitan mejorar la eficiencia de utilización de las pasturas y aumentar la productividad animal, han recibido una atención importante en los últimos años debido a los beneficios asociados con la mitigación de los gases con efecto invernadero –GEI, particularmente las emisiones de metano por unidad de producto animal o por área (HERRERO *et al*., 2016; MONTES *et* *al*., 2013; SAVIAN *et al*., 2014, 2018) y a sus efectos sobre la sostenibilidad de los sistemas ganaderos basados en pasturas (RAO *et al*., 2015).

1.2.6 Implicaciones del concepto de manejo Rotatinuo en la mitigación de las emisiones de metano

La ganadería está bajo la mirada de los críticos por sus altos requisitos en el uso de la tierra y por su significativa participación en las emisiones globales de gases de efecto invernadero (GEI) de origen antropogénico, de alrededor del 14,5%, de las cuales aproximadamente el 44% son en forma de metano (CH_4) producto de la fermentación entérica, el estiércol y el pienso de arroz (GERBER *et al*., 2013; GERSSEN-GONDELACH *et al*., 2017). No obstante, el CH_4 al igual que el CO_2 son subproductos naturales del proceso de fermentación microbiana de los carbohidratos en el rumen (HRISTOV *et al*., 2013a). Los microorganismos ruminales fermentan los carbohidratos y aminoácidos en ácidos grasos volátiles (AGV) de cadena corta (principal fuente energética para el rumiante), hidrógeno (H_2), dióxido de carbono (CO_2), metano CH_4 y otros productos finales de la fermentación (HOOK; WRIGHT; MCBRIDE, 2010; JANSSEN, 2010; MOSS; JOUANY; NEWBOLD, 2000). En ese proceso, las arqueas metanogénicas reducen el CO_2 a metano CH_4 evitando la acumulación de H_2 en el rumen (MCALLISTER *et al*., 1996). Las moléculas de H_2, aunque son utilizadas como fuente de energía por las arqueas metanogénicas (JANSSEN, 2010) deben ser removidas para mantener la eficiencia energética de la fermentación (BEAUCHEMIN *et al*., 2008).

Entre la variedad de sistemas de producción pecuaria, los rumiantes en pasturas son los más controversiales en la literatura actual (TEAGUE *et al*., 2016) por su reconocida contribución al cambio climático y al mismo tiempo por su potencial significativo para reducir sus impactos ambientales (GERSSEN-GONDELACH *et al*., 2017) y contribuir con la sostenibilidad de las lecherías por medio de menores costos de alimentación (FRENCH; O'BRIEN; SHALLOO, 2015), mayor bienestar animal, menor incidencia de cojeras y mastitis y aumento de la calidad de la leche (ELGERSMA, 2015). Los pastos, además, pueden desempeñar un papel importante en la captura del CO_2 atmosférico a través del secuestro del suelo ofreciendo servicios ecosistémicos

(HERRERO *et al*., 2016; MOTTET *et al*., 2017) además de generar servicios sociales y ambientales (RAO *et al*., 2015; WERLING *et al*., 2014). Por otro lado, los rumiantes son capaces de transformar el forraje (un recurso que los humanos no podrían utilizar de otra manera), en alimentos comestibles de alto valor biológico y, por lo tanto, son el recurso alimenticio más importante en producción de alimentos de origen animal (MCGINN *et al*., 2014).

En general, los pastizales proporcionan numerosos alimentos, bienes y servicios y son fundamentales para los medios de vida y las economías de muchas personas (Boval y Dixon, 2012). En este contexto, optimizar el manejo de las pasturas no sólo es necesario sino también deseable, por lo cual, los sistemas de pastoreo actuales se están rediseñando para vincular la producción animal con la gestión ambiental (BOVAL Y DIXON, 2012; CARVALHO, 2013; GARNETT *et al*., 2017) a la luz de las demandas actuales de producción agraria sostenible en todo el mundo (FAO, 2017; RAO *et al*., 2015).

La intensificación de los sistemas de producción ganadera y, consecuentemente, la mejora en la eficiencia de producción es considerada una estrategia de gran importancia para mitigar las emisiones antropogénicas de GEI (GARNETT *et al*., 2017; GERBER *et al*., 2013; GERSSEN-GONDELACH *et al*., 2017). De acuerdo con Gerssen-Gondelach et al. (2017), en los países en desarrollo, especialmente, existe un mayor potencial de reducción de emisiones a través de la intensificación dentro del sistema basado en pasturas la cual puede hacerse de diversas formas (GERSSEN-GONDELACH *et al*., 2017), siendo el manejo del pastoreo una de ellas, considerada una estrategia clave para mitigar las emisiones de metano entérico e incrementar la productividad animal, toda vez que las metas de manejo de pasturas definidas permitan aumentar la cantidad y calidad (digestibilidad) del forraje consumido por los animales (ULYATT Y LASSEY, 2001; DERAMUS *et al*., 2003; SAVIAN *et al*., 2018; SOUZA FILHO *et al*., 2019). Sin embargo, si bien el aumento del consumo de materia seca y la productividad individual de los animales, conducen a una reducción de la intensidad de emisión de metano por materia seca consumida, o por unidad de producto animal, carne o leche (en términos relativos), las emisiones totales diarias se incrementan en términos absolutos (SOUZA FILHO *et al*., 2019; HAMMOND *et al*., 2013; KURIHARA *et al*., 1999; SAVIAN *et al*., 2014, 2018). En ese sentido, cuando la emisión de metano es expresada por unidad de producto animal (intensidad de emisión) se refleja con mayor exactitud del impacto de las estrategias de mitigación implementada (HRISTOV et al., 2013b), por lo que se ha sugerido que las estimaciones de las emisiones de CH_4 de los rumiantes en pastoreo sea expresada sobre la base del consumo o producto animal (BEAUCHEMIN et al., 2008; KURIHARA *et al*., 1999).

El aumento de la productividad animal (carne o leche) requiere de un aumento del consumo de dietas digestibles que conduzcan a un incremento del consumo de energía metabolizable por los animales en pastoreo (BOVAL Y DIXON, 2012). Cuando la alimentación de rumiantes se basa en forrajes de alta digestibilidad se aumenta el consumo y se reduce la intensidad de las emisiones de metano (HERRERO *et al*., 2016; HRISTOV *et al.*, 2013b; SAVIAN *et al*., 2014).

De acuerdo con Carvalho (2013), bajo el concepto Rotatinuo el manejo del pastoreo se modifica para mejorar el consumo de forraje y de nutrientes digestibles de los animales en la unidad de tiempo de alimentación. Estudios posteriores indicaron que la estructura óptima del pasto y la mejor composición química bajo el concepto de manejo Rotatinuo implementado en un pastoreo rotativo, comparativamente con un manejo del pastoreo rotativo tradicional, dieron como resultado una mayor digestibilidad del forraje, un mayor consumo de materia orgánica y de energía metabolizable de ovejas pastando ryegras anual (*Lolium multiflorium*) y, consecuentemente, una menor intensidad de las emisiones de metano tanto por área como por unidad de producto animal. Si bien la emisión diaria de CH_4 por animal fue mayor, la emisión diaria de CH_4 no difirió cuando fue expresada por kg de peso vivo (SAVIAN *et al*., 2018).

Los resultados de Savian et al. (2018) demuestran que la estructura pre y pos-pastoreo definida para que los animales maximicen la tasa de consumo en la unidad de tiempo, de acuerdo con los principios del concepto Rotatinuo (CARVALHO, 2013) permite aumentar el consumo de forraje y de nutrientes digestibles totales y disminuir la intensidad de las emisiones de CH_4. Al definir como criterio de salida (e.g. franja en el pastoreo rotativo) el 40% de la altura media pre-pastoreo óptima, los animales incrementan el consumo de hojas, el componente más digestible de la planta y no tienen la necesidad de explorar partes inferiores del dosel forrajero donde predominan los tallos, menos digestibles y menos preferidos por los animales (AMARAL *et al*., 2013; EUCLIDES *et al*., 2018; SCHMITT *et al*., 2013; ZANINI *et al*., 2012). Varios estudios han indicado que el consumo predominante de hojas está asociado con el nivel de consumo y la digestibilidad de la materia seca ingerida, lo cual se correlaciona positivamente con la emisión diaria pero negativamente con la intensidad de las emisiones de metano (JOHNSON Y JOHNSON, 1995; ELLIS *et al*., 2007; HEGARTY, 2009). Cuando los animales consumen una dieta de mejor calidad (e.g mayor consumo de hojas más digestibles), como en el sistema Rotatinuo, es de esperarse que los parámetros de fermentación ruminal se modifiquen para disminuir la relación acetato/propínato y, consecuentemente, influyan en la menor emisión de metano individual (Johnson y Johnson, 1995). En el estudio de Savian et al. (2018), no se detectaron diferencias en los principales parámetros de la fermentación (pH, NH_3-N, AGV y relación A:P) entre los líquidos ruminales de las dos estrategias de manejo evaluadas debido, probablemente, al menor poder estadístico por el número de ovejas a las que se les colectó liquido ruminal, una oveja por tratamiento. No obstante, estos autores sugieren que la mejor calidad de la dieta del tratamiento Rotatinuo contribuyó a minimizar las diferencias en las emisiones diarias entre los tratamientos.

Las implicaciones del concepto de manejo del pastoreo Rotatinuo sobre la cantidad y calidad del forraje consumido por los animales en pastoreo demuestran que tiene un alto potencial para mitigar las emisiones de metano de animales en pastoreo (SAVIAN *et al*., 2018).

1.3. CONCEPTUAL MODEL

The conceptual model presented on Figure 5 shows the main interactions between sward structure, chemical composition and *in vitro* organic matter digestibility (nutritive characteristics), and ingestive behavior components of the grazing animals defined as plant-animal (A), and their implications on the main *in vitro* ruminal fermentation parameters defined as *in vitro* rumen (B).

In this model, sward height is the central structural characteristic given its influence on the bite mass (bite area, bite depth). The bite is considered as the basic unit of acquisition of forage and nutrients under grazing conditions (LACA Y ORTEGA, 1996) and the most determining factor of the STIR and, ultimately, of animal performance (CARVALHO, 2013). This relationship is studied through the basic components of ingestive behavior (bite mass, bite rate) (LLDEN; WHITTAKER, 1970). Additionally, the sward structure characteristics like bulk density and morphological components (green leaf mass, stems + sheath mass) are analyzed, due to their influence on bite mass. The chemical composition and IVOMD, also influenced by the sward structure, are included to explore how sward structure ultimately impact the ingestion of digestible nutrients (TDN-STIR, TDN as a percentage of digestible organic matter) and the end products of *in vitro* ruminal fermentation such as methane (CH_4), volatile fatty acids (AGV) and *in vitro* dry matter digestibility (IVDMD) included in the model *in vitro* rumen (B).

The plant-animal model (A) is defined on the spatial-temporal scale of the grazing process from bite to the patch level, and excludes digestive processes that occur on a larger and longer scale, while in the model *in vitro* rumen (B) the extent of forage digestion (after 24 and 48 h) is analyzed through the *in vitro* gas production technique. In the whole model it is assumed that there is a sward structure, defined through the sward height, that allows animals maximize the herbage and nutrient intake per unit of eating time (FONSECA *et al*., 2013; MEZZALIRA *et al*., 2014), and at the same time it has high potential to mitigate the *in vitro* methane production.

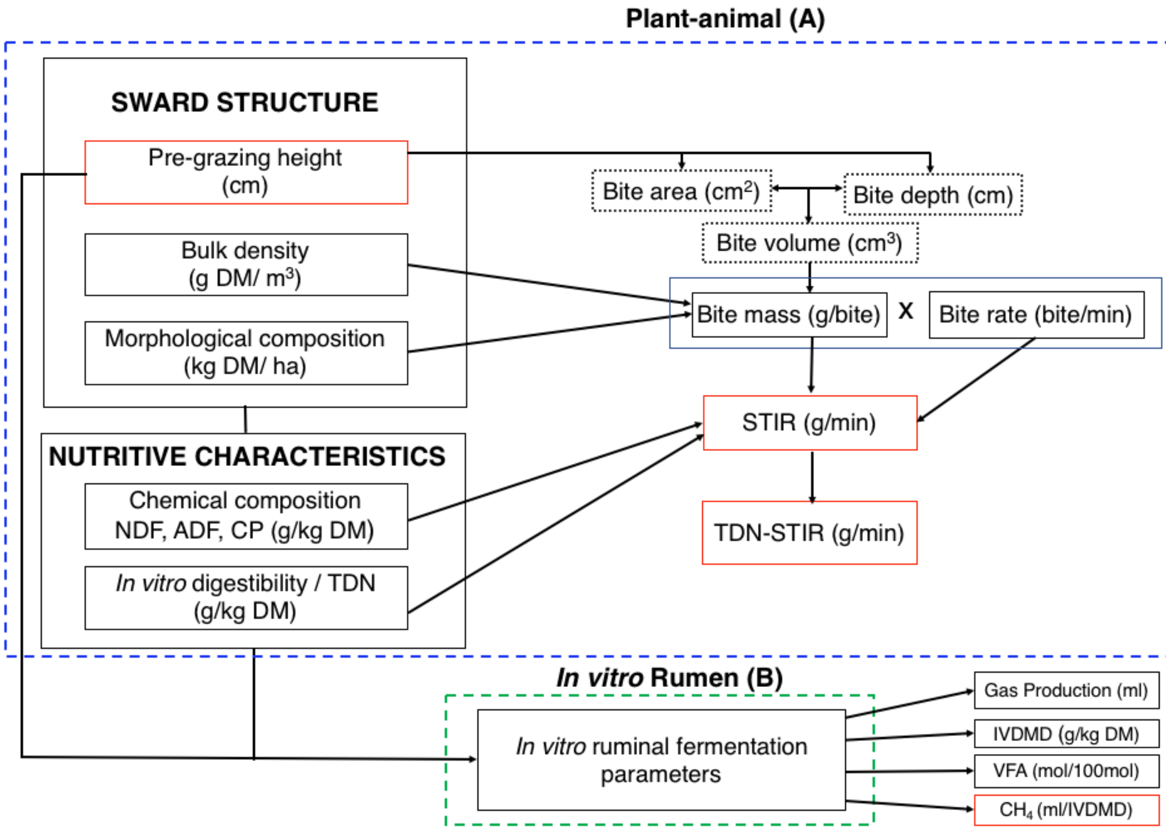

Figure 5. Conceptual model of defining sward height targets of kikuyu grass. Blue dashed boxes: Plant-animal (A); green dashed boxes: *in vitro* rumen (B); black solid boxes: measured variables; dotted boxes: estimated variables, and red solid boxes: response variables (Adapted from BAUMONT et al., 2004 AND CARVALHO, 2013).

1.4. HYPOTHESES

This thesis hypothesized that an optimal sward height of the kikuyu grass that allows dairy heifers maximize the herbage intake in the unit of eating time does exist, and considering only the potentially grazed stratum by animals, it would allow maximize the digestible nutrients intake rate. Additionally, it was postulated that sward height of the kikuyu grass that lead to better chemical composition and a higher IVOMD also reduce the *in vitro* CH_4 production per unit degraded forage mass.

1.5. OBJECTIVES

1) Determine the sward height of the kikuyu grass that allows dairy heifers to maximize the herbage and total digestible nutrients intake per unit eating time.

2) Assess the effect of kikuyu sward height from herbage samples of the top stratum on the chemical composition and the main *in vitro* ruminal fermentation parameters and identify the sward heights with the greatest potential for mitigating methane emissions.

2. CHAPTER II

[1]Article prepared in accordance with the Journal of Dairy Science (appendix 1).

Optimal sward height of kikuyu grass for maximizing herbage and nutrient intake rate of dairy heifers

Abstract

In order to maximize dairy cattle herbage and nutrient intake rate from tropical forage-based systems an optimal sward structure should be defined. The objective of this study was to determine the optimal sward height of kikuyu grass that maximizes herbage and total digestible nutrients intake per unit eating time of dairy heifers. The treatments consisted in 5 swards heights of kikuyu grass (10, 15, 20, 25 and 30 cm) which were evaluated under a randomized complete block design with two spatial (paddocks) replicates, and two temporal (morning and afternoon) replicates. This study evaluated the short-term herbage and total digestible nutrients intake rate (STIR and TDN-STIR, respectively), the bite mass (BM), the bite rate (BR), some sward structural characteristics and nutritive characteristics. It was found that the sward height of kikuyu grass that maximized the dry matter intake per unit eating time was approximately 20 cm. This sward height also optimized the total digestible nutrients intake rate. Herbage mass did not vary between treatments and it was stabilized from 20 cm. The bulk density of forage decreased as management sward height increased. Leaf mass predominated in swards heights equal to and greater than 20 cm. The chemical composition and *in vitro* organic matter digestibility (IVOMD) of the simulated grazing herbage samples were similar between treatments. These findings indicate that managing the sward height of kikuyu grass at 20 cm is the best strategy to maintain high and continuous intake rate of dry matter and digestible nutrients of heifers at tropical pasture-based dairy systems.

Keywords: grazing management target, sward structure, grazing behavior, dairy heifer.

2.1 Introduction

Intake of herbage mass and nutrients is the most determining factor of productivity of grazing animals (Boval and Dixon, 2012). Due to restrictions in time for cows to graze because of the share of other activities such as ruminating, milking in the daily time budget (Gibb et al., 1997; Chilibroste et al., 2015; Beggs et al., 2018), Carvalho (2013) stressed the importance for dairy cattle to achieve high levels of herbage and nutrient intake in the shortest possible time to meet their nutritional requirements in pasture-based systems (Carvalho, 2013). Recent studies, based on the understanding of grazing processes and ingestive behavior, proposed an innovative grazing management concept oriented to maximizing herbage and nutrient intake per unit of eating time of dairy cattle (Carvalho, 2013).

Several studies have been highlighted the influence of the sward structure on short-term herbage intake rate (STIR) and animal performance (Forbes, 1988; Laca et al., 1992; Carvalho et al., 2001). Among the different sward structural characteristics, the sward height (SH) influences bite mass (BM) and consequently STIR the most (Forbes, 1988; Hodgson, 1990; Laca et al., 1992). Therefore, adequate SH should be sought as grazing management targets to allow grazing cattle to achieve high levels of herbage intake and highest diet quality (Carvalho, 2013). According with this grazing concept, there is an optimal sward structure (i.e sward height) for each grass species in which animals maximize the herbage and nutrient intake rate (Gonçalves et al., 2009; Bremm et al., 2012; Fonseca et al., 2012; Amaral et al., 2013; Mezzalira et al., 2014). In rotational stocking pasture, target refers to pre-grazing sward height (Carvalho, 2013). Moreover, grazing animals can maintain such highest levels of intake until the average sward height reaches 40% of its pre-grazing value, which can thus be used as target post-grazing value for an optimal sward management (Fonseca et al., 2012; Mezzalira et al., 2014).

High STIR combined with high quality of the consumed forage is key in forage-based dairy production systems in the tropics whose low levels of performance are, among others, ascribed to the intrinsic lower quality of C4 grasses compared to C3 grasses (Sollenberger and Burns, 2001). Many dairy systems of Latin America and Australia are characterized by Holstein cattle fed with kikuyu grass (*Cenchrus clandestinum - Hochst. ex Chiov*) and supplement, in different proportions (Garcia et al., 2014; Sbrissia et al., 2018). However, many of them vary substantially in terms of level of intensification and grazing management and therefore they face several challenges to achieving high levels of productivity (Garcia et al., 2014). The ideal sward structure, which is translated in sward height, that allows the animals the maximization of STIR is different according to the grass species. Hence, the use of innovative grazing management based on this concept with this species still lacks such an information. The aim of this study was to determine the sward height of the kikuyu grass that allows dairy heifers to maximize their herbage and total digestible nutrients intake rate.

2.2 Material and Methods

2.2.1 Animals and Procedures

All procedures were conducted in accordance to the legal requirements of Brazilian animal, ethical, labor and environmental legislation.

2.2.2 Experimental site

The present study was conducted at the Agricultural Research and Rural Extension (EPAGRI) Lages, SC, Brazil (27°47’10.5”S, 50°18’20.5”W, 937m alt.) from December 2016 to April 2017. According to Köppen’s climate classification the region is humid subtropical under oceanic influences and has average annual temperature of 17°C and an average annual precipitation of 1460 mm. Weather data recorded by a local meteorological station during the experimental period are shown in Table 1.

Table 1. Monthly weather data during the experimental period in Lages, SC, Brazil

	Dec.	**Jan.**	**Feb.**	**Mar.**	**Apr.**
Average temperature (°C)	18,91	20,16	18,39	18,99	17,22
Min temperature (°C)	14,85	16,39	14,15	11,56	13,78
Max temperature (°C)	23,00	23,92	22,98	22,26	20,64
Rainfall (mm)	124,55	223,72	86,60	106,40	18,54

The experiment was carried out in an area of 5000 m^2 of kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) spread vegetatively in the early 1990s and grazed by dairy and beef cattle since then. The total area was divided into 10 paddocks of 500 m^2 each and mowed homogeneously until 5 cm height (the cuttings were removed). The pasture received one application of 250 kg/ha of fertilizer (N-P-K, 9-33-12). Nitrogen was applied in the form of urea, 135 kg/ha in December 2016 (first period of evaluation) and 67.5 kg/ha in March 2017 (second period of evaluation).

2.2.3 Treatments and experimental design

The treatments consisted of five kikuyu sward heights (10, 15, 20, 25, and 30 cm) which were obtained through a homogeneous mechanical cutting at 5 cm followed by a regrowth until reaching the target sward height for a given plot and the initiation of a grazing session for the measurement of STIR. The experimental design was a complete randomized block with two spatial replicates (paddocks), and two temporal replicates corresponding to time of day in which the grazing sessions were performed (beginning of the main daily meals, morning around 6:30 and afternoon around 16:30). The temporal replications were performed in different two periods of evaluation and the time of the day (morning or afternoon) was defined as blocking criterion. A total of 20 grazing sessions were evaluated. The duration of each grazing session was 45 ± 5 min to minimized digestive constraints. The size of the paddocks (500 m^2) was scaled so that average sward height reduction during a grazing session was less than 10%, ensuring that the same sward structure, at the bite level, was available to the animals over the course of each grazing sessions.

2.2.4 Grazing sessions and STIR

Short-term intake rate (STIR) was determined using three Holstein heifers (22 ± 2 months old; 440 ± 42 kg of body weight). Heifers were familiarized to the experimental protocol and grazed in an adjacent area with kikuyu grass for approximately one month before the start of the experiment. They were not fasted at any time to avoid any alteration of their ingestive behavior (Greenwood and Demment, 1988; Gregorini et al., 2009) and diet selection (Newman et al., 1994). During the grazing sessions, heifers were fitted with IGER Behavior Recorders (Rutter et al., 1997) to record the effective eating time and the number of grazing jaw movements (biting and non-biting). The data were analyzed with the Graze software (Rutter, 2000). To determine the STIR, the double weighing technique described by (Penning and Hooper, 1985) was used. Each heifer was fitted with a fecal and urine collecting bag and weighed before and after each grazing session. Following the second weighing, immediately after the grazing session, heifers were taken to an adjacent non-vegetated area deprived of feed and water for 45 ± 5 min to estimate insensible weight losses (water evaporation and carbonic dioxide and methane production). All weights were taken on a 10 g precision scale. The STIR of fresh matter was subsequently calculated using following equation:

$$\boldsymbol{STIR} = \left[\frac{W2 - W1}{t2 - t1} + \frac{W3 - W4}{t4 - t3}\right] x \; \frac{t2 - t1}{ET}$$

where STIR is short-term herbage intake rate (g/min) corrected by forage DM content, which was estimated based on the simulated grazing herbage sample (as described below), W1 and W2 are pre- and post-grazing weight (kg); t1 and t2 pre- and post-grazing time (min); W3 and W4 animal are pre- and post- insensible weight losses (kg); t3 and t4 pre- and post-insensible weight losses time (min); and ET is eating time (min). ET was calculated as the total session time excluding intervals of jaw inactivity greater than 3 s (Gibb, 1998). Bite rate (BR, bites per min) was determined for each animal and each grazing session dividing the total number of bites by effective eating time. Time per bite was calculated as the inverse of BR. Bite mass (BM, g DM) was calculated by dividing herbage dry matter intake (herbage fresh matter intake corrected by forage DM content) by the total number of bites.

Bite area (BA, cm^2) was estimated using the equation five (5) published by Carvalho et al (2015) as follows:

$$\mathbf{BA} = 2 \; x \; DA^2 \; (1 \; + \; 50 \; /\mathrm{SH})^{-1} x \; e^{-0.3x(HBd-1))}$$

Where DA = the animal's dental arcade breadth, SH = sward height (cm), and HBd= herbage bulk density of the grazed stratum (top stratum) of the sward height. The dental arcade (DA) was calculated using the allometric equation published by Illius and Gordon (1987). Short-term total digestible nutrients intake rate (TDN-STIR, g/min), was calculated as a product of STIR and total digestible nutrients concentration (g/kg) of simulated grazing herbage sample analyzed.

2.2.5 Sward measurements

Sward height was measured at 150 haphazardly selected points per paddock before and after each grazing session using a sward stick (Barthram, 1985). Pre-grazing herbage mass was assessed through three herbage mass samples clipped at ground level in two strata, top and bottom, using a quadrat of 0.25 m^2 and the sward stick. Each sample was later separated into morphological components (leaf lamina, stem + sheath, and dead material) and dried in a forced air oven at 55°C for 72 hours. The dry weights of the morphological components were used to calculate herbage mass (kg of DM/ha), as the sum of the mass of each component. The herbage bulk density (g of DM/m^3) was calculated by the ratio between the total herbage mass by the total volume of sample. The bulk density of the morphological components (leaf, stem + sheath, and dead material) was calculated as the ratio between the mass of each component by the volume of each component both in the top and bottom stratum (product of quadrat area (0.5 x 0.5 m) and average sward height). Four representative samples of the herbage potentially consumed by the animals (simulated grazing) were collected at the beginning and final of each grazing sessions by hand plucking method (De Vries, 1995) for dry matter content estimation, chemical composition and *in vitro* digestibility analysis.

2.2.6 Chemical composition and *in vitro* digestibility

The chemical composition and the *in vitro* organic matter digestibility of the simulated grazing samples were analyzed in duplicate for dry matter (DM; 930.04, AOAC, 2016), ash (930.05, AOAC, 2016). Neutral detergent fiber (NDF) without heat-stable alpha-amylase and acid detergent fiber (ADF) (Van Soest et al., 1991) using an Ankom200 Fiber Analyzer. The NDF and ADF were expressed including residual ash. Crude protein calculated as a N x 6.25 (N; 984.13, AOAC, 2016). The *in vitro* organic matter digestibility (IVOMD) were analyzed according to (Tilley and Terry, 1963) adapted by (Van Soest et al., 1991). The total digestible nutrients (TDN) concentration of the simulated grazing samples was estimated as a percentage of digestible organic matter (Moore et al., 1999).

2.2.7 Statistical analysis

All statistical analyses were done using R 3.5.1 (R Core Team, 2018). For sward characteristics, chemical composition and *in vitro* digestibility the differences among treatments were performed with Tukey's honestly significant difference test including the sward height and the time of day (turn) as a fixed effect. A period of evaluation (period) was included as a random effect. The experimental unit for sward variables and nutritive characteristics of the herbage was the grazing paddock.

Before assessing treatment effect, animal effect was subtracted from all the ingestive behavior analyses as follows: Y corrected= y (original data) – y (animal media) + y (general media). STIR, TDN-STIR, BM, BR, and time per bite were analyzed as a quadratic function of observed sward heights (SH) as fixed effects with the time of day (turn) and period of evaluation (period) as a random effect, according to the following model: y= SH + (SH^2) + turn + period. Those variables were also fitted using a compound function of sward height (SH), consisting in a double linear model or broken line, as described below: $y = f\{p + a1 \times (SH - v), p + a2 \times (SH - v)\}$, where y is STIR, TDN-STIR, BM, BR or time per bite, f is the min (for STIR, TDN-STIR, and BM) or max (for BR or time per bite) function, v and p are the coordinates of crossing point of sward height for STIR and BM at the maximum value and for the BR corresponds to the minimum value, SH are the observed values of sward height, and a1 and a2 are the slopes of the component lines (Mezzalira et al., 2017). The bite area (BA) was fitted as a linear function of SH (fixed effects with turn) as follows: BA = SH + turn.

All mixed linear models were fitted with the lme function from the nlme package version 3.1-137 (Pinheiro et al., 2018) in R 3.5.3. The broken line models were fitted by deviance minimization with the OPTIM function of the STATS package in R 3.5.1 (R Core Team, 2018). The different models for each variable were compared using Akaike's information criterion (AIC), which was calculated according to the formula: *-2*log-likelihood + 2*npar*, where npar represents the number of parameters in the fitted model. The best model was selected by the smaller value of AIC.

2.3 Results

2.3.1 Sward characteristics and chemical composition

Sward height values during the grazing sessions were similar to the target treatments established, and significantly different between them ($P < 0.05$). The difference between pre- and post-grazing sward heights was smaller than 10%, according to the proposed assumption (Table 2). Herbage mass, steam + sheath mass, and steam + sheath bulk density did not differ between treatments ($P > 0.05$). Green leaf mass was smaller at lower sward height (10 cm) than taller ones (P < 0.05). But it did not differ among the other heights evaluated (P > 0.05). Herbage bulk density decreased as management sward height increased, being lower in the 30 cm and 25 cm treatments than in 10 cm treatment (P < 0.05). Leaf bulk density was lower in the 30 cm (P < 0.05) than the 10 cm treatment, but there was no difference among the other sward heights (p >0.05). Steam + sheath bulk density did not differ between treatments (P < 0.05) (Table 2).

Table 2. Pre and post-grazing sward height, herbage mass, green leaf mass, stem + sheath mass, herbage bulk density, leaf bulk density, and steam + sheath bulk density of the kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) under different sward height managements

Variable	**Kikuyu**					**P-value**	**SEM**
	10	**15**	**20**	**25**	**30**		
Pre-grazing sward height (cm)	9.8 e	15.1 d	20.1 c	24.3 b	31.3 a	<.0001	0.51
Post-grazing sward height (cm)	9.3 e	14.4 d	18.5 c	22.6 b	29.3 a	<.0001	0.60
Herbage mass (kg of DM / ha)	3301.0	4056.3	4484.6	4401.3	4338.9	0.325	425.95
Green leaf mass (kg of DM / ha)	1003.0 b	1269.4 ab	1869.0 a	1721.8 a	1827.9 a	0.004	152.21
Stem + sheath mass (kg of DM / ha)	972.2	1062.7	1508.9	1531.4	1495.5	0.146	190.72
Herbage bulk density (g of DM / m^3)	6406.4 a	5145.8 ab	4156.3 abc	3579.8 bc	2762.3 c	0.002	523.58
Leaf bulk density	2004.2 a	1646.5 ab	1749.7 ab	1437.9 ab	1168.7 b	0.033	165.69
Steam + sheath bulk density	1856.6	1323.2	1385.4	1226.1	942.9	0.155	234.84

Means followed by the same letter within lines are not statistically different, as determined by Tukey's test (P < 0.05). S.E.M., standard error of the mean; p-value, significance level.

In general, no differences in the chemical composition nor *in vitro* organic matter digestibility (IVOMD) of the simulated grazing herbage samples were found (P > 0.05, Table 3). However, the CP, IVOMD, and thus, the TDN shown a tendency to decrease with taller sward heights (P < 0.10, Table 3).

Table 3. Chemical composition and *in vitro* organic matter digestibility (IVOMD) of simulated grazing herbage samples of kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) under different sward height managements

Item (g/kgDM)	kikuyu					P-value	SEM
	10	15	20	25	30		
OM	908.1	914.7	907.4	909.5	906.4	0.376	3.0
NDF	507.1	522.1	503.9	518.9	510.3	0.780	11.8
ADF	189.3	205.7	195.6	197.7	196.4	0.626	7.2
CP	316.0	282.9	299.7	292.3	290.8	0.074	7.6
IVOMD	712.7	690.2	651.0	682.2	643.9	0.060	16.4
TDN	647.5	631.8	591.6	620.6	583.9	0.060	15.6

Means followed by the same letter within lines are not statistically different, as determined by Tukey's test (P < 0.05). NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein, VOMD, *in vitro* organic matter digestibility, and TDN, total digestible nutrients. P-value, significance level. S.E.M., standard error of the mean.

2.3.2 Short-term herbage intake rate (STIR).

The STIR of kikuyu grass was reduced at both lower and taller swards heights, following a double linear response (Figure 1, a). However, the decreasing slope (a2= -0.44, P < 0.04) for STIR did not decline as much with increasing sward heights (past 20 cm) as the increasing slope did (a1=1.36, P < 0.001). The maximum STIR (44.02 g DM /min) was reached at sward height of 19.3 cm. The BM had a similar response as STIR between 10 and 20 cm sward heights but declined much more than the STIR after 20 cm with increasing (a1= 0.023, P < 0.001) and decreasing slope (a2= -0.016, P < 0.001) statistically significant (Figure 1, b). Conversely, the BR showed an opposite relationship to BM with sward height with a minimum value at 20.8 cm. The slope of the decreasing (a1= -0.44, P=0.016) and increasing (a2= 0.53, P=0.003) line for BR were significant (Figure 1, c). Having found no differences in the concentration of TDN between treatments, the TDN-STIR had a similar response that the dry matter intake rate (STIR) with a maximum in 20.3 cm, (increasing slope, a1= 0.62, P < 0.001, and decreasing slope, a2= -0.32, P= 0.04) (Figure 1, d).

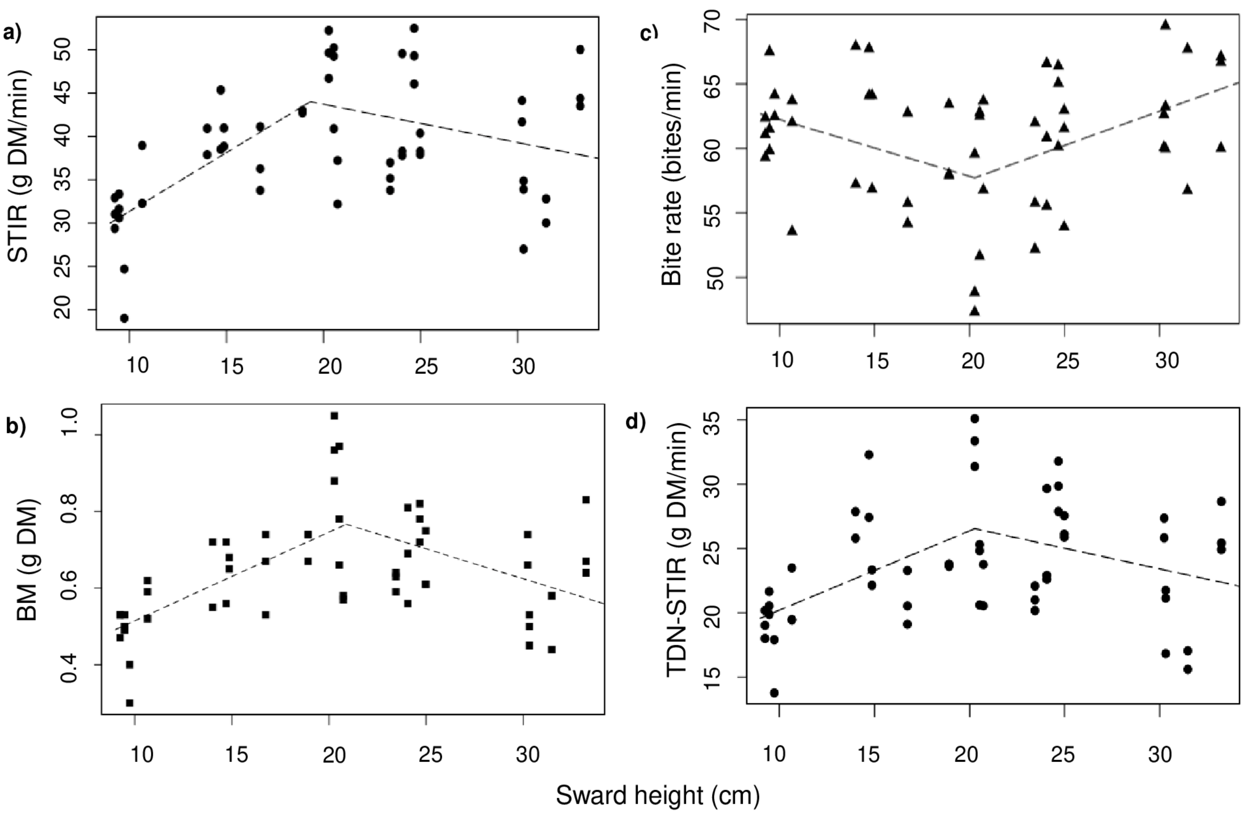

Figure 1. Relationships between short-term intake rate (STIR, a), bite mass (BM, b), bite rate (BR, c), and short-term total digestible nutrients intake rate (TDN-STIR, d) of dairy heifers as a function of sward height (SH) in monoculture of kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*). Equation for STIR=min(44.02 + 1.36 (SH– 19.3), (44.02 – 0.44 (SH-19.3)), P <0 .001, R^2=0.35; BM=min(0.77 + 0.023 (SH – 20.88), (0.77 – 0.016 (SH – 20.88), P < 0.001, R^2=0.36; BR=min(57.71 - 0.44 (SH – 20.27); (57.71 + 0.53 (SH – 20.27), P < 0.009, R^2=0.14; TDN-STIR=min((26.53 + 0.62 * (SH – 20.27); (26.53 – 0.32 * (SH – 20.27)), P < 0.002, R^2=0.20.

Time per bite was 1.03 seconds (s) when BM was 0.88 g DM/bite (slope, a1= 0.18, P=0.024), and continue increasing with the BM (g DM/bite) in a rate of 1.62 seconds (s) per bite (slope, a2= 1.62, P < 0.001), (Figure 2).

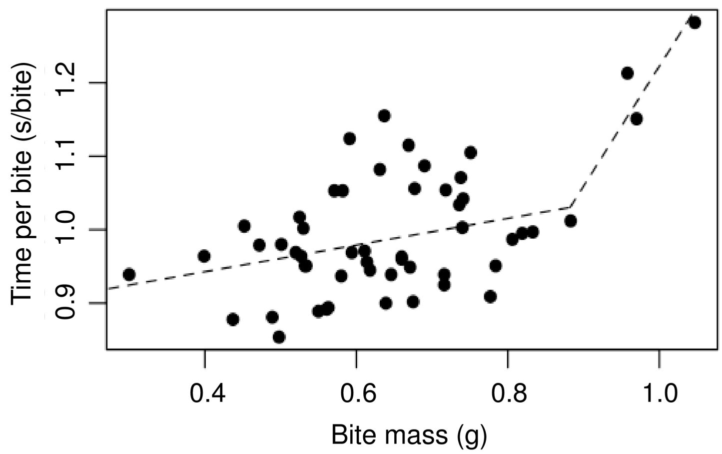

Figure 2. Relationships between time per bite (s) and bite mass (BM, g DM/bite) of dairy heifers grazing on kikuyu grass sward heights. Equation for Time/bite= min(1.03 + 0.18 * (BM – 0.88); (1.03 – 1.62 * (BM – 0.88)), P<0.001, R^2=0.42.

The bite area (BA) increased linearly (1.42 cm^2) as a function of sward height (P < 0.001, R2= 0.94) (Figure 3).

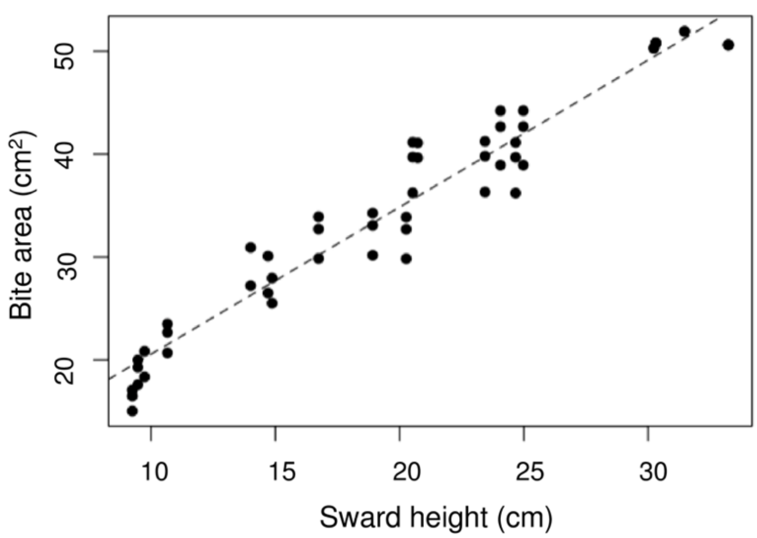

Figure 3. Relationship between bite area (BA) and sward height (SH) of dairy heifers grazing on kikuyu grass sward heights. (BA = 6.27 + 1.42SH, P < 0.001, R^2= 0.94).

2.4 Discussion

The key finding of this experiment was that 20 cm sward height of kikuyu grass that leads to maximizing the dry matter intake rate for dairy heifers, simultaneously optimizes the total digestible nutrient intake rate. The chemical composition and *in vitro* digestibility of the grazed stratum did not limit the STIR, highlighting the primacy of sward structure as a determinant factor of herbage intake by grazing animals, over those components of the nutritive value, at least on a short-term basis.

2.4.1 Components of ingestive behavior and STIR

Our results showed that the STIR can be reduced more than 30% in very low swards heights, and also it can be reduced on taller heights although the decreasing slope is more subtle than at lower ones. It indicated, that sward heights managed lower than 20 cm would restrict more the STIR of dairy heifers grazing on kikuyu grass pastures than taller ones until 30 cm. The BM, bite volume and bulk density (Burlison et al., 1991; Laca et al., 1992), was the most influential factor for these changes in STIR. To better understanding of the mechanisms for which the BM may have decreased we focus on the conceptual model initially presented (Figure 5, chapter I) and discuss each in turn.

At sward heights lower than 20 cm, the decrease in the BM was due to the constraints in bite formation caused by the lower bite volume (product of bite area and bite depth). The restriction in bite formation at low sward heights (Laca et al., 1992; Flores et al., 1993; Gregorini et al., 2011; Silva et al., 2013), as well as the amount of herbage that can be harvested by the animal (Illius and Gordon, 1987a; Benvenutti et al., 2008), has been widely reported. Considering that, the bite area decreased linearly with declining sward height (Figure 3), and besides, the bite depth is positively correlated with sward height (Laca et al., 1992) and maintains an almost constant proportion, close to 50% of sward height (Wade, 1991; Laca et al., 1992; Flores et al., 1993; Cangiano et al., 2002), it is reasonable to stress that the kikuyu structure at 10 and 15 cm would reduce the BM, and consequently, the STIR. Although, at these sward heights it was found that the BR increased (Figure 1.c), it was not sufficient to compensate for a low BM, resulting in a STIR significantly lower than that found at tall pastures.

On the other hand, at tall sward heights (>20 cm), the STIR (Figure 1.a) did not decline at the same proportion as the BM did (Figure 1.b). In this sense, the increase in the BR (Figure 1.c) in the tall pastures appears to have almost completely compensated the BM reduction, and therefore, the only way that STIR could have declined is due to the BM declined proportionally more than the amount that BR increased. In concordance with these results, the key question is why BM decreased in sward heights managed after 20 cm?

The BM reduction not compensated for BR must have been related to the components that define the BM, such as bite volume (bite area and bite depth) and bulk density of the grazed stratum. Taking into account that, the relationship between the BA estimated and the sward height was linear and positive (Figure, 3), and assuming that, bite depth increases linearly with sward height as previously reported in cattle (Laca et al., 1992; Cangiano et al., 2002) the decrease in BM must have been a result of the decrease in bulk density which ultimately affected the amount of dry matter gathered in the same bite volume. It is widely accepted that BM is predominantly constrained by the sward height and herbage and leaf bulk density (Black and Kenney, 1984; Laca et al., 1994; Searle and Shipley, 2008). We thought that the fact of the herbage mass kept constant in sward heights from 20 to 30 cm had an important influence on the bulk density of the grazed stratum. The high and negative correlation between herbage and leaf bulk density in the total with the sward height (-0.82 and -0.68 respectively), also support these results.

Although, it was not found statistical differences among treatment, It was noted that the herbage mass increased from 10 to 15 cm, but then, it was stabilized between 20 and 30 cm of sward height which probably suggests a tiller size/number compensation mechanism (Sbrissia and Silva, 2008; Sbrissia et al., 2018). We hypothesized therefore that from 20 cm probably there was a reduction in the tiller population density and a concomitant increase in the average tiller weight which did not allow that the total herbage mass increased as a function of sward height. The inverse relationship between the number of tillers and tiller weight in sward canopy has been well described (Matthew et al., 1995). A recent study showed that the same type of homeostatic mechanism occurred with sward heights of kikuyu grass between 15 and 25 cm subjected to moderate defoliation of 50% (Sbrissia et al., 2018). The authors showed that the taller pre-grazing sward heights of kikuyu grass had less tillers number, but they were bigger (heavier tillers weight), and consequently in that range of sward heights not differences in forage accumulation were found (Sbrissia et al., 2018).

The increase of the sward height combining with a constant herbage mass had an important influence in the bulk density decrease, which ultimately appears to be the most decisive variable in the BM decrease at tall pastures. Stobbs (1973a; b) highlighted the influence of bulk density in the context of tropical pastures and showed the negative relationship between BM and sward height, which was attributed to the low density of leaves in the upper stratum of the sward. The importance of the herbage and leaf bulk density as a determinant of BM in higher canopy heights has been reported for different tropical grasses (Palhano et al., 2007; Benvenutti et al., 2008; Fonseca et al., 2012).

On the other hand, it is widely accepted that grazing ruminants are capable to increase their BR in response to lower BM (Hodgson, 1985; Forbes, 1988). The inverse response between BR and BM suggest a compensatory response as having been argued by some authors (Gibb et al., 1997; Chilibroste, 1999) but it may also indicate a reallocation of grazing jaw movements (GJM) due to changes in the ratio of biting to non-biting GJM (Laca et al., 1994; Gibb et al., 1999). Contrary to expectations, in this study, the time per bite did not increase as a sward height increase suggesting that the reduction of BM in tall sward heights was not directly related with the increase in time to locate and manipulate bites (Figure 2). The reduction of BM at higher pastures heights could be related to the increasing time per bite due to an increase in herbage manipulation caused by the decreasing bulk density in the upper stratum of the pasture (Carvalho, 2013). Grazing animals also could increase time per bite due to the presence of the undesirable components plant (e.g. the presence of stem + pseudostem) (Benvenutti et al., 2008; Guzatti et al., 2017), however, neither differences in the morphological components were found through sward heights studied.

Given the conceptual model, the bite area was explored to better understand of the phenomenon by which BM decreased after 20 cm of sward height. As was showed in Figure 3, the BA increased as a function of sward height which is in accordance with (Laca et al., 1992; Flores et al., 1993; Cangiano et al., 2002). In general, BA is directly related to mouth dimensions in herbivores (Illius and Gordon, 1987b) and although, it is a variable more difficult to determine experimentally it has been also pointed out that it is the primary parameter that animals adjust to increase bite mass (Wade and Carvalho, 2000). In this study, however, the BA seems to have been little sensible to sward height variations as other studies (Burlison et al., 1991; Laca et al., 1992).

The sward height that maximized the STIR of the kikuyu grass was achieved in approximately 20 cm which is in accordance with other studies conducted with *Cynodon sp* (Mezzarilla et al 2014) as well as within the pre-grazing sward height range suggested by other authors with other approaches of grazing management for the kikuyu grass (Sbrissia et al., 2013, 2018; Schmitt et al., 2019b). Concerning the model selection, which was based on the AIC as a metric of model performance (the smaller value represents the best model), it was found that the broken line model for STIR (AIC =344.52) was fitted better to the data than the quadratic model (AIC = 347.94). Mezzalira et al (2017) also described a dome-type model for STIR and BM with *Cynodon sp* which is similar to our results. Previous studies comparing adaptative variations of Type IV functional response models suggested a double quadratic and the double quadratic with plateau broken line models for estimating short-term intake rate by ruminants grazing *Cynodon dactylon* and *Avena strigosa* respectively (Mezzalira et al 2012). In this study, we examined which model could provide a reasonable statistical fit to the data, however, it worth recognizing that the best model for estimating the maximum point of STIR with kikuyu grass remains an open research area given the complexity of the ingestive behavior of grazing ruminants.

Mezzalira et al (2017) also suggested that this dome-shaped relationship seems to prevail in large herbivores even in conditions with little variation in the chemical quality of the swards. Under the experimental conditions of this study, the IVOMD of the potentially grazed stratum (simulated grazing samples) was similar among sward heights evaluated then the TDN (estimated as a percentage of digestible organic matter) also did not vary between treatments, therefore, the total digestible nutrient intake rate (TDN-STIR) (Figure 1, d) of the kikuyu grass had a similar response to the intake rate of dry matter (STIR).

Other authors described this similar response for instantaneous intake rate of digestible energy by herbivores (Wilmshurst and Fryxell, 1995; Wilmshurst et al., 1999). Nevertheless, additional work is required to fully understand the differences between STIR and STIR of nutrients with the kikuyu grass.

2.4.2 Morphological components, nutritive characteristics, and STIR

The reduction in the BM was not well explained by the morphological components which is in accordance with (Boval et al., 2007). Although at sward height managed very low (10 cm) the lowest proportion of leaf mass could have influenced the decrease in the BM, and thus, the STIR, at sward heights higher or equal than 15 cm the green leaf mass was not statistically different. Besides, the stem + sheath mass as well the herbage mass remained constant from 20 cm sward heights. These results allow us to infer that the stems, at least in the top stratum, did not act as a barrier of bite formation at tall pastures as other authors suggested (Benvenutti et al., 2008; Fonseca et al., 2012; Mezzalira et al., 2017). We suggest the arrangement of leaves and stems of the kikuyu grass at sward height of 20 cm allowed animals to apprehend more leaves in each bite which substantially increases the STIR. Contrary, the spatial arrangement of those plant parts must have changed in sward heights past 20 cm reducing the amount of herbage gathered by heifers.

In general, the morphological characteristics of the sward heights studied were similar to the reported in the literature for kikuyu grass (Sbrissia et al., 2013, 2018; Schmitt et al., 2019). According to some authors, the similarities of the morphological and chemical composition between the sward heights of 15, 20, and 25 cm along with a moderate defoliation (e. g. 50% of the sward height) suggest a flexible grazing management for kikuyu grass (Sbrissia et al., 2013). Sbrissia et al. (2018), studying similar sward structures to those in this study, found that with the exception of the sward height grazed at 10 cm, the forage accumulation and the stem elongation rate were the same for sward heights between 15 and 25 cm. Although, in the present study, some sward structure characteristics and the chemical composition of the kikuyu grass were not affected over a range of sward heights evaluated, the components of the ingestive behavior were constrained at both lower and taller swards heights of 20 cm.

On that sense, it was found that the chemical composition and the digestibility of the potentially grazed stratum (simulated grazing samples) remained similar through all sward heights (Table 3) and, consequently, did not constraint the STIR. It seems the leaf spatial arrangement in 30 cm sward height was the main cause of the decrease in BM, and thus, STIR. In tropical pastures, the limited availability and accessibility of green leaves at bite level in the grazed horizon, along with the increase in the proportion of stems, negatively influences the BM, decreasing it and consequently limiting the STIR (Benvenutti et al., 2008, 2016; Fonseca et al., 2012; Mezzalira et al., 2014). The acquisition of the forage under grazing is the first to constrain the intake rate (ingestion) previous and predominant to the chemical composition (digestion) (Silva and Carvalho, 2005). The tendency to decrease in the IVOMD and TDN, however, could have an effect on post-ingestive processes such as changes in the end products of fermentation which could influence on intake in the long term (Chilibroste, 1999). Moreover, the impact of the nutritive value on intake is expressed mainly when top grazing stratum become highly depleted (Benvenutti et al., 2016), which was not the case in our experiment.

Sbrissia et al. (2013) defining grazing management targets of kikuyu grass suggested that, despite higher concentration of CP and a lower concentration of fiber (NDF and ADF), grazing at 10 cm would not result in higher animal performance, since that sward structure could have a negative effect on BM, STIR, and therefore, on herbage and total nutrient intake. Other authors, also argued about the relatively less importance of the nutritive value over the sward structure in determining herbage intake rate (Silva et al., 2013; Silva et al., 2018). Recent studies evaluating contrasting structures of kikuyu grass, have suggested that when defining a level of defoliation between 40 or 50% of the initial sward height, the morphological and chemical characteristics of the pasture in the stratum harvested by the animals remain constant (Schmitt et al., 2019a; b).

In general, in our study, the chemical composition of simulated grazing sample exhibited lower fiber concentration (NDF and ADF) and higher CP and IVOMD concentration than those reported by other authors working with kikuyu grass (Garcia et al., 2014; Marín et al., 2014; Clark et al., 2018). While these studies have presented values of chemical composition and digestibility of the kikuyu grass of samples cut at ground level or between 5 and 10 cm above ground level, it was expected that in our simulated grazing samples the high proportion of leaves and the low proportion of stems + sheath influenced in these results.

2.4.3 Implications of this study

The results of this study besides allowing a better understanding of the grazing processes by dairy heifers and their reactions to the constraints imposed by the sward height of kikuyu grass have important and practical implications for the definition of grazing management targets oriented for the maximization of dry matter and nutrients intake in the short term. The sward height of the kikuyu grass suggested here could be used under intermittent stocking, as a pre-grazing sward height.

It is worth mention that, the sward height represents a useful and easy-to-implementation tool which could improve current management practices, for example in a country like Colombia, where farmers are mainly smallholders who are usually more conservationists regard to adopt new technologies. Finally, the definition of the optimal sward height of kikuyu grass also supports the innovative grazing management concept, *Rotatinuo*.

2.5 Conclusions

To maximize the STIR of dairy heifers, kikuyu grass should be managed at 20 cm of sward height. Define very low (10 cm) or very tall (30 cm) sward heights of kikuyu grass as a grazing management target would constraint the BM, and thus, the STIR. Another interesting finding is that considering only potentially grazed stratum by animals the total digestible nutrient intake rate (TDN-STIR) of the kikuyu grass would be also optimized around 20 cm. We also concluded, the BM, and thus, the STIR of the kikuyu grass, were predominantly constrained by the sward height and herbage bulk density.

2.6 References

Amaral, M.F., J.C. Mezzalira, C. Bremm, J.K. Da Trindade, M.J. Gibb, R.W.M. Suñe, and P.C. de F. Carvalho. 2013. Sward structure management for a maximum short-term intake rate in annual ryegrass. Grass Forage Sci. 68:271–277. doi:10.1111/j.1365-2494.2012.00898.x.

Barthram, G.T. 1985. Experimental techniques: the HFRO sward stick. M.M. (Ed. . Alcock, ed. Midlothian.

Beggs, D.S., E.C. Jongman, P.E. Hemsworth, and A.D. Fisher. 2018. Implications of prolonged milking time on time budgets and lying behavior of cows in large pasture-based dairy herds. J. Dairy Sci. 101:10391–10397.

Benvenutti, M.A., I.J. Gordon, and D.P. Poppi. 2008. The effects of stem density of tropical swards and age of grazing cattle on their foraging behaviour. Grass forage Sci. 63:1–8.

Benvenutti, M.A., D.R. Pavetti, D.P. Poppi, I.J. Gordon, and C.A. Cangiano. 2016. Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. Grass Forage Sci. 71:424–436. doi:10.1111/gfs.12186.

Black, J.L., and P.A. Kenney. 1984. Factors affecting diet selection by sheep. 2. Height and density of pasture. Aust. J. Agric. Res. 35:565–578.

Boval, M., and R.M. Dixon. 2012. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. Animal 6:748–762. doi:10.1017/s1751731112000304.

Boval, M., A. Fanchone, H. Archimède, and M.J. Gibb. 2007. Effect of structure of a tropical pasture on ingestive behaviour, digestibility of diet and daily intake by grazing cattle. Grass Forage Sci. 62:44–54. doi:10.1111/j.1365-2494.2007.00560.x.

Bremm, C., E.A. Laca, L. Fonseca, J.C. Mezzalira, D.A.G. Elejalde, H.L. Gonda, and P.C. de F. Carvalho. 2012. Foraging behaviour of beef heifers and ewes in natural grasslands with distinct proportions of tussocks. Appl. Anim. Behav. Sci. 141:108–116. doi:10.1016/j.applanim.2012.08.008.

Burlison, A.J., J. Hodgson, and A.W. Illius. 1991. Sward canopy structure and the bite dimensions and bite weight of grazing sheep. Grass forage Sci. 46:29–38.

Cangiano, C.A., J.R. Galli, M.A. Pece, L. Dichio, and S.H. Rozsypalek. 2002. Effect of liveweight and pasture height on cattle bite dimensions during progressive defoliation. Aust. J. Agric. Res. 53:541–549.

Carvalho, P.C. de F., H. Ribeiro Filho, C. Poli, A. de Moraes, and R. Delagarde. 2001. Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo. Reun. Anu. da Soc. Bras. Zootec. 38:853–871.

Carvalho, P.C. de F. 2013. Harry Stobbs Memorial Lecture: Can grazing behaviour support innovations in grassland management? Pages 1134–1148 in 22nd International Grasslands, Sidney (Australia).

Carvalho, P.C. de F., C. Bremm, J.C. Mezzalira, L. Fonseca, J.K. da Trindade, O.J.F. Bonnet, M. Tischler, T.C.M. Genro, C. Nabinger, and E.A. Laca. 2015. Can animal performance be predicted from short-term grazing processes?. Anim. Prod. Sci. 55:319. doi:10.1071/AN14546.

Chilibroste, P. 1999. Grazing Time: The Missing Link: A Study of the Plant-Animal Interface by Integration of Experimental and Modelling Approaches.

Chilibroste, P., M.J. Gibb, P. Soca, and D.A. Mattiauda. 2015. Behavioural adaptation of grazing dairy cows to changes in feeding management: do they follow a predictable pattern?. Anim. Prod. Sci. 55:328. doi:10.1071/AN14484.

Clark, C.E.F., R. Kaur, L.O. Millapan, H.M. Golder, P.C. Thomson, A. Horadagoda, M.R. Islam, K.L. Kerrisk, and S.C. Garcia. 2018. The effect of temperate or tropical pasture grazing state and grain-based concentrate allocation on dairy cattle production and behavior. J. Dairy Sci. 101:5454–5465. doi:10.3168/jds.2017-13388.

Flores, E.R., E. a. Laca, T.C. Griggs, and M.W. Demment. 1993. Sward Height and Vertical Morphological Differentiation Determine Cattle Bite Dimensions. Agron. J. 85:527. doi:10.2134/agronj1993.00021962008500030001x.

Fonseca, L., J.C. Mezzalira, C. Bremm, R.S.A. Filho, H.L. Gonda, and P.C. d. F. Carvalho. 2012. Management targets for maximising the short-term herbage intake rate of cattle grazing in Sorghum bicolor. Livest. Sci. 145:205–211. doi:10.1016/j.livsci.2012.02.003.

Forbes, T.D. 1988. Researching the plant-animal interface: the investigation of ingestive behavior in grazing animals. J. Anim. Sci. 66:2369–2379. doi:10.2527/jas1988.6692369x.

Garcia, S.C., M.R. Islam, C.E.F. Clark, and P.M. Martin. 2014. Kikuyu-based pasture for dairy production: a review. Crop Pasture Sci. 65:787. doi:10.1071/CP13414.

Gibb, M.J., C.A. Huckle, R. Nuthall, and A.J. Rook. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass Forage Sci. 52:309–321. doi:10.1111/j.1365-2494.1997.tb02361.x.

Gibb, M.J., C.A. Huckle, R. Nuthall, and A.J. Rook. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl. Anim. Behav. Sci. 63:269–287.

Gonçalves, E.N., P.C. de F. Carvalho, T.R. Kunrath, I.J. Carassai, C. Bremm, and V. Fischer. 2009. Relações planta-animal em ambiente pastoril heterogêneo: processo de ingestão de forragem. Rev. Bras. Zootec. Brazilian J. Anim. Sci. Viçosa, MG. Vol. 38, n. 9 (set. 2009), p. 1655-1662.

Greenwood, G.B., and M.W. Demment. 1988. The effect of fasting on short-term cattle grazing behaviour. Grass Forage Sci. 43:377–386.

Gregorini, P., C.E.F. Clark, J.G. Jago, C.B. Glassey, K.L.M. Mcleod, and A.J. Romera. 2009. Restricting time at pasture: Effects on dairy cow herbage intake , foraging behavior , hunger-related hormones , and metabolite concentration during the first grazing session. J. Dairy Sci. 92:4572–4580. doi:10.3168/jds.2009-2322.

Gregorini, P., S.A. Gunter, M.T. Bowman, J.D. Caldwell, C.A. Masino, W.K. Coblentz, and P.A. Beck. 2011. Effect of herbage depletion on short-term foraging dynamics and diet quality of steers grazing wheat pastures1. J. Anim. Sci. 89:3824–3830. doi:10.2527/jas.2010-3725.

Guzatti, G.C., P.G. Duchini, A.F. Sbrissia, J.C. Mezzalira, J.G.R. Almeida, P.C. de F. Carvalho, and H.M.N. Ribeiro-Filho. 2017. Changes in the short-term intake rate of herbage by heifers grazing annual grasses throughout the growing season. Grassl. Sci. 63:255–264. doi:10.1111/grs.12170.

Hodgson, J. 1985. The control of herbage intake in the grazing ruminant. Proc. Nutr. Soc. 44:339–346. doi:10.1079/PNS19850054.

Hodgson, J. 1990. Grazing Management. Science into Practice. Longman Group UK Ltd.

Illius, A.W., and I.J. Gordon. 1987. The Allometry of Food Intake in Grazing Ruminants. J. Anim. Ecol. 56:989. doi:10.2307/4961.

Laca, E.A., E.D. Ungar, and M.W. Demment. 1994. Mechanisms of handling time and intake rate of a large mammalian grazer. Appl. Anim. Behav. Sci. 39:3–19. doi:10.1016/0168-1591(94)90011-6.

Laca, E.A., E.D. Ungar, N. Seligman, and M.W. Demment. 1992. Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. Grass Forage Sci. 47:91–102. doi:10.1111/j.1365-2494.1992.tb02251.x.

Marín, A., L. Giraldo, and G. Correa. 2014. Parámetros de fermentación ruminal *in vitro* del pasto Kikuyo (Pennisetum clandestinum). Liv. Res. Rural Dev. 26.

Matthew, C., G. Lemaire, N.R.S. Hamilton, and A. Hernandez-Garay. 1995. A modified self-thinning equation to describe size/density relationships for defoliated swards. Ann. Bot. 76:579–587.

Mezzalira, J.C., O.J.F. Bonnet, P.C. de F. Carvalho, L. Fonseca, C. Bremm, C.C. Mezzalira, and E.A. Laca. 2017. Mechanisms and implications of a type IV functional response for short-term intake rate of dry matter in large mammalian herbivores. J. Anim. Ecol. 86:1159–1168. doi:10.1111/1365-2656.12698.

Mezzalira, J.C., C. Bremm, L. Fonseca, C.C. Mezzalira, R.A. Oliveira Neto, L.H.S. Correia, and P.C.F. Carvalho. 2012. Adaptations of Type IV functional response for estimating short-term intake rate by grazing herbivores. Page 246-248 in Grassland–a European Resource?

Mezzalira, J.C., P.C. De Faccio Carvalho, L. Fonseca, C. Bremm, C. Cangiano, H.L. Gonda, and E.A. Laca. 2014. Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. Appl. Anim. Behav. Sci. 153:1–9. doi:10.1016/j.applanim.2013.12.014.

Moore, J.E., M.H. Brant, W.E. Kunkle, and D.I. Hopkins. 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. J. Anim. Sci. 77:122–135.

Newman, J.A., P.D. Penning, A.J. Parsons, A. Harvey, and R.J. Orr. 1994. Fasting affects intake behaviour and diet preference of grazing sheep. Anim. Behav. 47:185–193. doi:10.1006/anbe.1994.1021.

Palhano, A.L., P.C.D.F. Carvalho, J.R. Dittrich, A. de Moraes, S.C. Silva, and A.L.G. Monteiro. 2007. Características do processo de ingestão de forragem por novilhas holandesas em pastagens de capim-mombaça. Rev. Bras. Zootec. 36:1014–1021. doi:10.1590/S1516-35982007000500005.

Penning, P.D., and G.E. Hooper. 1985. An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. Grass Forage Sci. 40:79–84. doi:10.1111/j.1365-2494.1985.tb01722.x.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R.C. Team. 2018. nlme: linear and nonlinear mixed effects models. R package version 3.1-137. R Found. Stat. Comput. https//CRAN. R-project. org/package= nlme (accessed 6 Feb. 2019).

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

Rutter, S.M. 2000. Graze: a program to analyze recordings of the jaw movements of ruminants. Behav. Res. Methods, Instruments, Comput. 32:86–92.

Rutter, S.M., R.A. Champion, and P.D. Penning. 1997. An automatic system to record foraging behaviour in free-ranging ruminants. Appl. Anim. Behav. Sci. 54:185–195.

Sbrissia, A.F., P.G. Duchini, G.D. Zanini, G.T. Santos, D.A. Padilha, and D. Schmitt. 2018. Defoliation Strategies in Pastures Submitted to Intermittent Stocking Method: Underlying Mechanisms Buffering Forage Accumulation over a Range of Grazing Heights. Crop Sci. 58:945. doi:10.2135/cropsci2017.07.0447.

Sbrissia, A.F., V.P.B. Euclides, R.A. Barbosa, D.B. Montagner, D.A. Padilha, G.T. Santos, G.D. Zanini, P.G. Duchini, and S.C. Silva. 2013. Grazing management flexibility in pastures subjected to rotational stocking management: herbage production and chemical composition of kikuyu-grass swards. Pages 1038–1040 in Revitalising Grasslands to Sustain our Communities: Proccedings of the 22th International Grassland Congress.

Sbrissia, A.F., and S.C. Silva. 2008. Compensação tamanho/densidade populacional de perfilhos em pastos de capim-marandu. Rev. Bras. Zootec. 37:35–47.

Schmitt, D., D.A. Padilha, K.M. Dias, G.T. Santos, G.R. Rodolfo, G.D. Zanini, and A.F. Sbrissia. 2019a. Chemical composition of two warm-season perennial grasses subjected to proportions of defoliation. Grassl. Sci. 65:171–178. doi:10.1111/grs.12236.

Schmitt, D., D.A. Padilha, C. Medeiros-Neto, H.M.N. Ribeiro Filho, L.E. Sollenberger, and A.F. Sbrissia. 2019b. Herbage intake by cattle in kikuyugrass pastures under intermittent stocking method. Rev. CIÊNCIA AGRONÔMICA 50. doi:10.5935/1806-6690.20190058.

Searle, K.R., and L.A. Shipley. 2008. The comparative feeding bahaviour of large browsing and grazing herbivores. I.J. Gordon and H.H.T. Prins, ed. Springer.

Silva, G.P., C.A. Fialho, L.R. Carvalho, L. Fonseca, P.C.F. Carvalho, C. Bremm, and S.C. Silva. 2018. Sward structure and short-term herbage intake in Arachis pintoi cv. Belmonte subjected to varying intensities of grazing. J. Agric. Sci. 156:92–99. doi:10.1017/S0021859617000855.

Silva, S.C., and P.C. de F. Carvalho. 2005. Foraging behaviour and herbage intake in the favourable tropics/sub-tropics. Grassl. a Glob. Resour. Wageningen Wageningen Acad. Publ. 81–95.

Silva, S.C., F.M.A. Gimenes, D.O.L. Sarmento, A.F. Sbrissia, D.E. Oliveira, A. Hernandez-Garay, and A. V. Pires. 2013. Grazing behaviour, herbage intake and animal performance of beef cattle heifers on marandu palisade grass subjected to intensities of continuous stocking management. J. Agric. Sci. 151:727–739. doi:10.1017/S0021859612000858.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Sollenberger, L.E., and J.C. Burns. 2001. Canopy characteristics, ingestive behaviour and herbage intake in cultivated tropical grasslands. Pages 321–327 in International grassland congress.

Stobbs, T.H. 1973a. The effect of plant structure on the intake of tropical pastures - I. Variation in the bite size of grazing cattle. Aust. J. Agric. Res. 24:809–819.

Stobbs, T.H. 1973b. The effect of plant structure on the intake of tropical pastures - II.* Differences in sward structure, nutritive value, and bite size of animals grazing setarza anceps and chlorzs ga yana at various stages of growth. Aust. J. Agric. Res. 24:821–9.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass forage Sci. 18:104–111.

De Vries, M.F.W. 1995. Estimating forage intake and quality in grazing cattle: a reconsideration of the hand-plucking method. J. Range Manag. 370–375.

Wade, M.H. 1991. Factors affecting the availability of vegetative Lolium perenne to grazing dairy cows with special reference to sward characteristics, stocking rate and grazing method. Rennes.

Wade, M.H., and P.C. de F. Carvalho. 2000. Defoliation patterns and herbage intake on pastures. Grassl. Ecophysiol. grazing Ecol. 1st ed. Wallingsford, UK CABI Publ. 233–248.

Wilmshurst, J.F., and J.M. Fryxell. 1995. Patch selection by red deer in relation to energy and protein intake: a re-evaluation of Langvatn and Hanley's (1993) results. Oecologia 104:297–300. doi:10.1007/BF00328364.

Wilmshurst, J.F., J.M. Fryxell, and P.E. Colucci. 1999. What Constrains Daily Intake in Thomson's Gazelles?. Ecology 80:2338. doi:10.2307/176914.

3 CHAPTER III.

Article prepared according to the norms of the Animal Feed Science and Technology (appendix 2).

Effects of sward heights of kikuyu grass on the main *in vitro* fermentation parameters, including methane production

Abstract

Sward heights that result in greater herbage digestibility have high potential to mitigate methane (CH_4) emissions from forage-based systems. This study aimed to assess the effect of kikuyu grass sward height (10, 15, 20, 25, and 30 cm) from herbage samples of the top stratum (potentially grazed stratum by cattle) on the chemical composition and the main *in vitro* fermentation parameters, including methane (CH_4) production. The *in vitro* incubations were carried out under a randomized block design with four independent runs of each treatment and two ruminal liquid from steers. The chemical composition and the *in vitro* organic matter digestibility (IVOMD), as well as some sward structural characteristics (herbage mass, morphological components), were evaluated. The gas production (GP), *in vitro* dry matter digestibility (IVDMD), CH_4 production, total volatile fatty acid (VFA) concentration, and their acetate, propionate, and butyrate proportions were measured at 24 and 48 hours of *in vitro* fermentation. The results showed that the chemical composition of the top stratum of kikuyu grass sward heights between 10 to 25 cm was similar and tended to have higher IVOMD than 30 cm. The GP and the IVDMD showed a negative and linear fit to the increasing sward height. The CH_4 production (mL/ g IVDMD) showed a broken line response remained similar across treatments below 21 cm height, at which point increased as sward height increased. The acetate molar proportion (mol/100 mol) showed a broken line response similar to CH_4 (mL/g IVDMD) response. The propionate molar proportion (mL/g IVDMD) indicated a broken line

response opposite to CH_4 increasing until sward height near to 20 cm and then decreased. The butyrate molar proportion (mL/g IVDMD) was reduced linearly as the sward heights increased. It was concluded that the less CH_4 (mL/g IVDMD) production between 10 to 25 cm sward heights highlighted their greatest mitigation potential. Sward heights above 25 cm exhibited tended to produce higher ADF contents and lower IVOMD than the other sward heights, and thus, produced less propionate (mol/100 mol) and more CH_4 (mL/g IVDMD) and acetate (mol/100 mol) indicating their greater methanogenic potential. These findings are important to aid decision making on the optimal sward height of the kikuyu grass for being grazing by animals in order to reduce *in vitro* CH_4 production.

Keywords: CH_4 mitigation strategies, forage-based dairy systems, *in vitro* ruminal fermentation

3.1 Introduction

Livestock is under the fire of critics for its major share in the environmental impact of the agricultural sector. Among livestock production systems, grassland-based ruminants are the most controversial in present-day literature (Gerssen-Gondelach et al., 2017; Teague et al., 2016). Grazed pastures are the basis of those systems and they display multiple roles that can benefit the sustainability of dairy production such as lower feeding costs (French et al., 2015), higher animal welfare and lower occurrence of lameness and mastitis, good public image and increased milk quality (Elgersma, 2015; Lobato et al., 2014). Pastures can play a significant role in trapping atmospheric CO_2 through soil carbon sequestration (De la Motte et al., 2016). In addition, grasslands provide many social and environmental services (Werling et al., 2014) such as improving soil health parameters (Horrocks et al., 2019). Grazing ruminants are also able to produce high-quality food from plant material not used by humans (McGinn et al., 2014). However, grazing systems can have a profound impact on environment. For instance, the production of CH_4 from enteric fermentation of feedstuff ingested contributes significantly to climate change (approximately 15% of the global greenhouse gas-GHG (Gerber et al., 2013). Hence, current grazing systems are being re-designed to link animal production with environmental management (Boval and Dixon, 2012; Carvalho, 2013) in the light of current demands for sustainable agricultural production around the world (Herrero et al., 2010; Mottet et al., 2017).

Defining the sward structure (e.g sward height) allowing animals to maximize the short-term herbage and nutrients intake rate has been suggested as a sustainable grazing management concept for the tropical forage-based dairy production systems (Carvalho, 2013). Several studies have defined the optimal sward height, that allows animals reaching the highest levels of herbage intake rate and highest diet quality for different grass species (Amaral et al., 2013; Bremm et al., 2012; Fonseca et al., 2012; Gonçalves et al., 2009; Mezzalira et al., 2014). Savian et al. (2018) demonstrated the potential of this grazing management concept to increase the quantity and quality of the forage consumed by sheep grazing Italian ryegrass and to mitigate CH_4 emissions intensity. Although these authors did not detect differences in the main parameters of *in vitro* ruminal fermentation (pH, NH_3-N, AGV and A: P ratio), it was suggested that the best diet quality with a sward height that optimizes herbage intake rate contributes to reduce the daily CH_4 emissions. In general, grazing management targets improving the quantity and quality of herbage consumed by the animals are key to optimizing productivity and reducing enteric CH_4 emissions per unit of animal product (Hristov et al., 2013a). Increased forage digestibility has been associated with a fermentation profile in the rumen that is unfavorable to CH_4 production (Hristov et al., 2013b; Muñoz et al., 2016). A positive relationship between sward height and diet digestibility have been also found at top leafy stratum (Benvenutti et al., 2016).

Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) is a highly productive pasture species that is well adapted to the forage-based dairy systems and widely used in countries of Africa, Latin America and Australia (Garcia et al., 2014). Although the information on grazing management targets of kikuyu grass to improve quantity and quality of the pasture consumed by dairy cattle has been increasing in recent years (Dobos et al., 2009; Sbrissia et al., 2018; Schmitt et al., 2019b), there is still a lack of information about the chemical composition and the *in vitro* ruminal fermentation parameters including CH_4 production under different sward heights with this species, which are important performance predictors. The aim of this study was to assess the effect of the sward height of kikuyu grass on the chemical composition and the main *in vitro* ruminal fermentation parameters of the potentially grazed stratum by cattle and identify the sward structures with the greatest potential for mitigating CH_4 emissions.

3.2 Material and methods

All methods and protocols involving animals were carried out in accordance with the relevant guidelines, regulations, and requirements of Colombian law No 84/1989 and following protocol approved by the Ethics Committee of the International Center for Tropical Agriculture, assuring the welfare of animals used in the experiment.

3.2.1 Experimental site and laboratory facilities

The *in vitro* incubations were conducted at the Forage Quality and Animal Nutrition Laboratory (certified by the FAO-IAG proficiency test of feed constituents 2017 including *in vitro* gas production) and Greenhouse Gas Laboratory facilities at International Center for Tropical Agriculture (CIAT) located in the Valle del Cauca department, Colombia (3°29'34"N, 76°21'37"W, 965 m alt.) between December 2017 to July 2018. Herbage samples of kikuyu grass were obtained from a grazing trial with dairy heifers (described in detail in chapter III). The region is subtropical humid climate of Southern Brazil (27°47'10.5"S, 50°18'20.5"W, 937m alt.) with an average annual precipitation of 1460 mm and an average annual temperature of 17.7°C according to Köppen's climate classification. A total area of approximately 5000 m^2 with kikuyu grass was mowed homogeneously until 5 cm height. After mowing, the area received 250 kg /ha of fertilizer N-P-K (9-33-12). Nitrogen was applied in the form of urea, 135 kg/ha in December 2016 and 67.5 kg/ha in March 2017. The experimental area was subdivided into ten paddocks of 500 mt^2 each in which five kikuyu grass sward height (10, 15, 20, 25, and 30 cm) with two replicates per paddock were randomized. Once target sward height was achieved the herbage samples from the top stratum were collected for *in vitro* incubations.

3.2.2 Treatments and experimental design

Treatments consisted in herbage samples from the top stratum of five kikuyu grass sward heights (10, 15, 20, 25, and 30 cm). The herbage samples were produced within a randomized complete block design experiment with two spatial (paddocks) replicates, and two temporal (morning and afternoon) replicates. The blocking criterion was the time of day when herbage sample were collected (morning or afternoon). The *in vitro* incubations were carried out through four independent runs of each treatment with two ruminal liquid from steers, and two incubation time (24 and 48 h). Four blanks (no substrate) for each incubation time also were included.

Finally, the experimental scheme resulted in a total of 88 bottles as follows: 5 (herbage sample) × 4 (incubation runs) × 2 (ruminal liquid) × 2 (incubation time) + 8 (Blanks) = 88.

3.2.3 Herbage sampling and sward measurements

Three herbage samples from the top stratum of each kikuyu grass sward height were cut using a 0.25 m^2 metallic quadrat and a graduated sward stick to keep the cutting height as uniform as possible. Each sample was later separated into morphological components (leaf lamina, stem + sheath, and dead material) and dried in a forced air oven at 55°C for 72 hours and then pooled for the *in vitro* incubations. The dry weights of the morphological components were used to calculate herbage mass (kg DM /ha), as the sum of the mass of each component. The sward height, considered as the average height of the undisturbed plant canopy surface of lamina above ground level, was measured in each paddock at 150 points randomly using a sward stick (Barthram, 1985).

3.2.4 Chemical composition and *in vitro* digestibility

The herbage samples from the top stratum of each sward height were analyzed for dry matter (DM; 930.04, AOAC, 2016), ash (930.05, AOAC, 2016), crude protein, calculated as N × 6.25 (N; 984.13, AOAC, 2016), neutral detergent fiber (NDF) without heat-stable alpha-amylase and acid detergent fiber (ADF) (Van Soest et al., 1991) by using an Ankom200 Fiber Analyzer. The ADF and NDF procedures are not ash-free. The *in vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963) adapted by Van Soest et al. (1991).

3.2.5 Rumen fluid and *in vitro* gas production

The *in vitro* gas production was conducted following the procedures detailed by Theodorou (1994). The rumen fluid was collected from two rumen-cannulated Bos indicus Brahman steers grazed on star grass (*Cynodon plectostachyus*) who had free access to water and mineral salts, filtered through 4 layers of cheesecloth, dispensed into 2 thermal flasks pre-warmed to 39 ± 0.5°C, and immediately transferred to the laboratory. Five-hundred milligrams of the herbage samples (DM basis) were incubated in 160 ml glass bottles, pre-warmed in an incubator at 39°C, and with 20 ml filtered rumen fluid mixed with 80 ml rumen medium in 1:4 ratio (Menke and Steingass, 1988), dispensed with continuous flushing with CO_2. The bottles were slightly stirring, sealed with rubber stoppers, and an aluminum caps, and finally incubated in a water bath at 39°C in duplicate and in two different sets corresponding to incubation times 24 and 48 hours. Four blanks of rumen medium (bottles without substrate which contain only inoculum and medium) per each set were also incubated.

The gas production was measured at 3, 6, 9, 12, 24, and 48 h using a pressure transducer (Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan) connected to digital wide-range manometer (Sper Scientific, Arizona, USA) and a 60 mL syringe through three-way valve (Theodorou, 1994). After each measurement, the gas of the bottles was released to avoid partial dissolution of CO_2 (Tagliapietra et al., 2010) and possible disturbance of microbial activity (Theodorou, 1994). Cumulative pressure values were converted into volume (GP, mL) from measured pressure changes at incubations times and after correction for blank pressures values by using the ideal gases law and expressed per unit of incubated dry matter and *in vitro* dry matter digested (IVDMD).

3.2.6 *In vitro* dry matter digestibility, CH_4, and VFA

For *in vitro* methane (CH_4) production analysis, a gas sample in headspace was collected into a 5 ml vacuum vial (Labco Ltd., High Wycombe, England) at 24 and 48 h. The methane concentration was determined by means of a gas chromatograph (Shimadzu GC-2014) equipped with a Hayesep N packed column (0.5m x 1/8" x 2mm ID) and flame ionization detector (FID). The operating temperatures of column, detector, methanizer, and valves were 80, 250, 380, and 80 °C respectively. Ultra-high purity 5.0 grade Nitrogen (N) was used as carrier gas with a linear velocity of 35mL / min. Methane (CH_4) concentration was calculated using a standard of 10 % CH_4 balanced in N (Scott-Marrin Inc., Riverside, CA) and corrected for the CH_4 blank values. The volume of methane (ml) produced at the end of each incubation time (24 and 48 h) was calculated as a product of the total gas produced (ml) with the percentage (%) of methane in the analyzed sample as described by Lopez and Newbold (2007).

After 24 and 48 hours the fermentation was stopped dipping the bottles in cold water with ice, and then processed for determination of volatile fatty acids (VFA), and IVDMD. Ruminal fluid samples (10 ml) were centrifuged at 3000 rpm for 10 min at 4ºC. 1.6 ml of the supernatant was transferred into 2 ml Eppendorf tube and 0.4 ml of metaphosphoric acid (25%; w/v) added for VFA analysis. Samples were then stored frozen at -20°C and later analyze for acetate, propionate and butyrate concentrations by high-performance liquid chromatograph (HPLC) with an SPD-20AV UV-VIS detector (SHIMADZU, Prominence UFLC System) fitted with a BIO-RAD Aminex HPX-87H, 300mm x 7.8mm Ion

Exclusion Column. The VFA of blank samples were analyzed for respective corrections, then the total VFA production was calculated. All contents remaining in the bottle were finally filtered through pre-weighed sintered glass crucibles pore number 1 (Pyrex®), and dried in a forced air oven at 105°C for 24 h to determinate the *in vitro* apparent digestibility of dry matter (IVDMD).

3.2.7 Statistical analysis

All statistical analyses were done using R 3.5.1 (R Core Team, 2018). For sward characteristics, chemical composition and *in vitro* digestibility the differences among treatments means were assessed using the Tukey's honest significant difference test. Treatment differences and trends were declared significant at $P \leq 0.05$ and $P \leq 0.10$, respectively. In all analyses, paddocks were considered as experimental units. The least-squares mean (±SEM) also was reported. The *in vitro* fermentation data were analyzed as a linear and quadratic function of sward height (SH) with treatments and turn (time of day that herbage samples were collected) as fixed effects as follows: y =SH + turn and y= SH + I(SwardH^2) + turn, using the R 3.5.3 nlme package version 3.1-137 for mixed linear models (Pinheiro et al., 2018), as well as a double linear function of sward height according to the following model: $y = f\{p + a1 \times (SH - v), p + a2 \times (SH - v)\}$, where y is *in vitro* CH_4, VFA (acetate, propionate, and butyrate), f is the min or max function, v and p are the coordinates of the crossing point of sward height, SH are the observed values of sward height, and a1 and a2 are the slopes of the component lines adapted of Mezzalira et al. (2017). The double linear models were fitted by deviance minimization with the OPTIM function of the STATS package in R 3.5.1 (R Core Team, 2018). The different models for each variable were compared using Akaike's information criterion (AIC).

Pearson's correlations were used to determine the relationships between some measured variables.

3.3 Results

3.3.1 Sward characteristics

The sward height, herbage mass and morphological composition of the treatments are showed in Table 1. The actual sward heights were as pretended for all treatments and different between them (P < 0.05). The herbage mass was greater in 30 cm with respect to 10 cm (P < 0.05), but it was not statistically different from the other sward heights (P > 0.05). The 25 and 30 cm sward heights resulted in a higher green leaf mass than 10 cm sward height, although, it was similar to 15 and 20 cm (P > 0.05). The stem + sheath mass tended to increase as a function of sward height (P < 0.10).

Table 1. Sward height, herbage mass, green leaf mass, and stem + sheath mass of herbage samples from the top stratum of five kikuyu grass sward heights grazed by dairy heifers.

	Treatments					**P-value**	**SEM**
	10	**15**	**20**	**25**	**30**		
Pre-grazing height (cm)	9.8e	15.1d	20.1c	24.3b	31.3a	<.0001	0.51
Herbage mass (kg DM/ha)	426.0b	502.0ab	796.0ab	870.33ab	950.33a	0.013	107.23
Green leaf mass (kg DM/ha)	363.97b	463.10ab	737.45ab	791.33a	842.84a	0.009	93.63
stem + sheath mass (kg DM/ha)	31.98	24.25	52.94	73.88	91.12	0.097	17.99

Means followed by the same letter within lines are not statistically different, as determined by Tukey's test (p<0.05). S.E.M., standard error of the mean, P-value, significance level.

3.3.2 Chemical composition and *in vitro* organic matter digestibility

The nutritive characteristics of the of the top stratum is shown in the Table 2. No differences were found for OM, NDF, CP, and IVOMD, although, a tendency to decrease IVOMD was detected in 30 cm (P < 0.05). In addition, this sward height resulted in a higher concentration of FDA (g / kg DM) respect to the lower sward height (10 cm), but it was not statistically different with respect to the other heights.

Table 2. Chemical composition and *in vitro* organic matter digestibility (IVOMD) of herbage samples from the top stratum of five kikuyu sward heights.

	Treatments					P-value	SEM
	10	15	20	25	30		
OM (g/kg of DM)	907.15	911.6	905.3	905.8	902.1	0.222	2,58
NDF (g/kg of DM)	535.9	541.9	543.1	541.1	545.6	0.983	10,96
ADF (g/kg of DM)	194.1b	198.7ab	210.9ab	213.1ab	218.8a	0.017	3,80
CP (g/kg of DM)	316.8	301.8	305.0	302.9	281.3	0.222	7,97
IVOMD (g/kg of DM)	686.6	657.5	635.1	610.7	592.3	0.169	30,59

Means values among the sward heights within the lines followed by the same letters are not significantly different, as determined by Tukey's test (P<0.05). OM, organic matter, NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein, and IVOMD, *in vitro* organic matter digestibility. P-value, significance level. S.E.M., standard error of the mean.

3.3.3 The *in vitro* fermentation parameters

The *in vitro* fermentation parameters of the herbage samples from the top stratum of the treatments are showed in Figure 1. The GP, expressed on grams of dry matter incubated (mL / g DMi), was high and positively correlated with IVDMD (g) (r= 0.69 at 24 h and r= 0.74 at 48 h, P < 0.01). The IVDMD (g) showed intermediate and negative correlation with sward height (r= -0.63 and r= -0.59, P < 0.01) at 24 and 48 h, respectively. Conversely, an intermediate and positive correlation (r=0.62, P < 0.01) among the ADF (g / kg) and sward height was observed.

The GP (mL / g DMi) and the IVDMD (g) linearly decreased with sward height at both incubation times (those relationships are shown at 24 hours in Figures 1.a and 1.b). However, when the GP was expressed per unit of dry matter digested (mL / g IVDMD), no differences were found between treatments either at 24 h or 48 h. The GP (mL / g IVDMD) ranged from 179.75 to 182.92 mL at 24 h and from 232.17 to 225.78 at 48h with the treatments from 10 to 30 cm, respectively. There was no general relationship between the total *in vitro* CH_4 production (ml /g DMi) and the sward heights studied at any time of incubation (ranged from 8.07 to 10.31 ml at 24 h and 16.3 to 17.8 ml at 48 h). However, a broken line response was observed between CH_4, expressed per unit of dry matter digested (mL / g IVDMD), and the sward height (Figure 1.c) at 24 hours of fermentation. In this model, the decreasing slope (a1) was slight (-0.22) and not significant (p=0.32), but the increasing slope (a2= 0.61) had the greatest inclination and was significant (p=0.02), showing that the CH_4 (mL / g IVDMD) remained similar from 10 to 20 cm and then, approximately from 21 cm, increased with increasing sward height. At 48 hours, the CH_4 production per unit of dry matter digested (mL / g IVDMD) tended to increase linearly as a function of sward height (p=0.12). Total VFA (mM / L) concentration was similar between treatments at both incubation times. The proportions of acetate, propionate, and butyrate (mol / 100 mol), and the A:P ratio at 24 hours did not show significant relationships with sward height. However, changes in the proportions of VFA were found at 48 hours. The average total volatile fatty acid (VFA, mM / L) for treatments at 48 h was 17.6. The acetate and propionate molar proportions were well described by a broken line model (Figure 1.d and 1.e, respectively). The relationship between acetate (mol/100mol) with sward height first described a straight line slightly inclined (slope a1 = -0.07, P = 0.095) and after 25 cm indicated a steeper line with a higher and significative slope (a2= 1.55, P < 0.0001).

Conversely, the propionate (mol/100mol) response as a function of sward height, first increased (a1= 0.30, P < 0.001) until 21.44 cm and then decreased (a2= -0.34, P < 0.001).

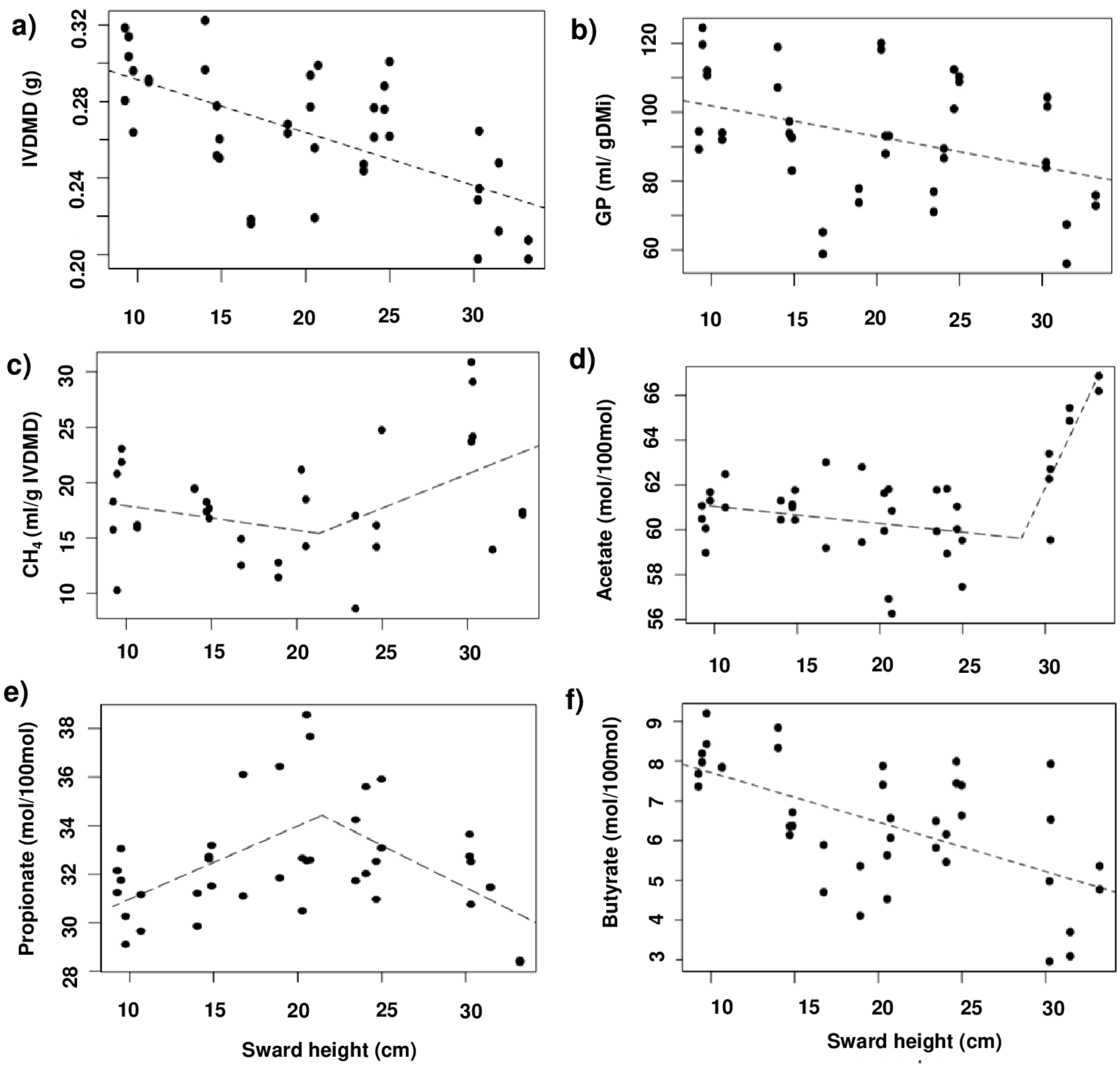

Figure 1. Relationship between *in vitro* fermentation parameters as a function of sward height. IVDMD (g DM, a), GP (ml/g DMi, b), CH_4 (ml/g IVDMD, c) at 24 hours of *in vitro* fermentation. The molar proportion of acetate (mol/100mol, d), propionate (mol/100mol, e), and butyrate (mol/100mol, f) at 48 hours of *in vitro* fermentation. Equation for IVDMD= 0.30 - 0.002SH + 0.023, p<0.0001, R^2=0.49; GP= 96.94 - 0.83SH + 26.21, p<0.0001, R^2=0.68; CH_4=min(15.42 – 0.22 (SH– 21.30), (15.42 + 0.61 (SH-21.30)), p=0.060, R^2=0.11; Acetate= min(59.62 – 0.07 (SH– 28.55), (59.62 + 1.55 (SH-21.30)), p<0.0001, R^2=0.54; Propionate=min(34.42 + 0.30 (SH– 21.44), (34.42 - 0.34 (SH-21.30)), p<0.0001, R^2=0.29; Butyrate= 8.22 – 0.12SH + 1.37, p< 0.0001, R^2=0.53.

The butyrate (mol/100mol) showed a negative and linear fit to as the sward heights increased (Figure 1.f).

3.4 Discussion

This study assessed the effect of the sward height of kikuyu grass on chemical composition, *in vitro* organic matter digestibility and main *in vitro* ruminal fermentation parameters including CH_4 production. The key findings from this study were that the chemical composition as well as the IVOMD of the top stratum of kikuyu grass was similar between 10 to 25 cm sward heights. In this range of sward heights, herbage samples exhibited the highest IVOMD and, consequently, the greatest mitigation potential of CH_4 production per unit of dry matter digested. The observed tendency for a decrease in the IVOMD and the higher ADF concentration at 30 cm sward height regard to the shortest ones contributed both to the increasing of the CH_4 production (mL CH_4 / g IVDMD) and the proportion of acetate, as well as to the decreasing of the proportion of propionate, suggesting a greater methanogenic potential at taller sward heights.

3.4.1 Sward characteristics and chemical composition

The herbage mass and green leaf mass of the top stratum, although similar between the sward heights of 15 to 30 cm seems to increase linearly with the sward height. Even a tendency to increase the stem + sheath mass as a function of height was observed. Schmitt et al. (2019a) reported a linear increase in herbage mass and leaf lamina mass and a quadratic relationship of the stem + dead material mass (grazing stratum) with pre-grazing heights of the kikuyu grass similar to those studied here. Such results are consistent with the variations found in chemical composition (higher FDA) and digestibility (lower IVOMD) of the herbage samples from the top stratum in the swards heights managed at 30 cm and reflects its maturity. In this regard, it was previously suggested in the literature that variations in the chemical composition are closely related to the sward height (Delagarde et al., 2000; Difante et al., 2009).

In agreement with our results, it has been reported that the NDF and ADF content of herbage samples from the upper stratum did not change for kikuyu grazing heights between 10 and 25 cm (Sbrissia et al., 2013; Schmitt et al., 2019a). However, the chemical composition of kikuyu grass was slightly different with what is reported in the literature reviewed by Garcia et al (2014). This probably as a result of different clipping height: top of the canopy in this study *vs* entire plant in others (Correa et al., 2008a; Fulkerson et al., 2006; Marín et al., 2014). Additionally, the effect of the time of day (morning and afternoon) in which the herbage samples were taken may also have influenced on chemical composition (Abrahamse et al., 2009; Delagarde et al., 2000; Mayland et al., 2005). The CP of kikuyu of the pre-grazing heights evaluated ranged from 28.3 to 31.6% of dry matter, which is higher than values usually reported (Correa et al., 2008b; Garcia et al., 2014), even when only the upper stratum of sward structures were evaluated (Sbrissia et al., 2013; Schmitt et al., 2019a). Besides the high CP content of the upper stratum due to green leaves, the higher levels of nitrogen due to fertilization could have influenced the results. According to Correa et al., (2008b), the higher CP content (true protein and non-protein nitrogen (NPN), in highly fertilized kikuyu swards, is closely related with the higher amounts of ruminal ammonia (N-NH_3), and lower efficiency in the use of N.

3.4.2 *In vitro* fermentation parameters

One of the objectives of this study was to assess the effect of kikuyu grass sward height from herbage samples of the top stratum on the main *in vitro* ruminal fermentation parameters, and results showed that the GP, as well as the IVDMD, decreased with increasing sward heights. These results are in agreement with the observed tendency for a decrease in the IVOMD and the higher ADF concentration in sward heights of 30 cm. In general, *in vitro* gas production is a good indicator to predict the carbohydrate degradation of forages (Menke et al., 1979; Theodorou, 1994). It is widely accepted that the higher GP, the higher the IVDMD (Durmic et al., 2010; Meale et al., 2012). Our results, although based on sward height, are consistent with others based on regrowth ages or mature stages of tropical and temperate grasses (Navarro-villa and Brien, 2011; Ribeiro et al., 2014).

In this way, the CH_4 production in an *in vitro* gas system is strongly associated with the fermentation of structural carbohydrates (Johnson and Johnson, 1995). The higher fiber concentration in the forages has been also associated with lower digestibility and higher CH_4 production per unit of dry mater digested (Beauchemin et al., 2008; Boadi et al., 2002; Kurihara et al., 1999; Navarro-Villa et al., 2011). The model obtained for the CH_4 (mL / g IVDMD, Figure 1.c) suggests that there would be a greater mitigation potential with sward heights between 10 up to 21 cm than taller sward heights. Above 25 cm sward height the chemical characteristics and the IVOMD of the top stratum of kikuyu are more liable to produce more CH_4 (mL / g IVDMD). In concordance with our results, other studies assessing the *in vitro* CH_4 production from different maturity stages of kikuyu grass showed a lower CH_4 production per unit of digested organic matter (Vargas et al., 2018) and per gram of digestible dry matter (Ramírez et al., 2015), in the youngest forages compared to the most mature ones. Other authors also observed that the reduction of the digestibility of perennial ryegrass due to maturity increased the CH_4 production per unit of dry matter digested (Purcell et al., 2011). Nevertheless, in this study no differences in the

CH_4 production per unit of dry matter incubated (mL / g DMi) were found probably due to little magnitude of the effect of the sward height on the chemical composition. Conversely, studies reported superior CH_4 production (mL / g DMi) with highly fermentable forages (immatures) (Durmic et al., 2010; Navarro-Villa et al., 2011; Purcell et al., 2011). In regard to chemical composition, although the NDF was similar between all sward heights, the higher concentration of ADF (herbage sample from the top stratum) in 30 cm respect to 10 cm sward height probably contributed to the highest *in vitro* CH_4 production (mL / g IVDMD) found at 24 h and the tendency to increase at 48 hours. The high and positive correlation between ADF (g / kg) and sward height also support these results. It is also widely known that forages with high fiber content are related to greatest CH_4 emissions (Johnson and Johnson, 1995; Moe and Tyrrell, 1979).

On the other hand, the end products of *in vitro* ruminal fermentation like the acetate, propionate, and butyrate proportions are consistent with those published by other authors (Burke et al., 2006; Marín et al., 2014; Ramírez et al., 2015; Vargas et al., 2018) who also evaluated the *in vitro* fermentation of kikuyu. The lack of differences found in the total VFA (mM / L) at both *in vitro* incubation times performed and the molar proportions of the main VFA at 24h, could be due to changes in the fermentation pathways as a possible variation in the microbial fermentation. This phenomenon would seem to be too subtle to elicit a change in the early fermentation or due to a low sensibility of the *in vitro* technique to detect small differences between the same type of substrate (grass) (Meale et al., 2012). Conversely, other responses of the main VFA proportions were observed at 48 hours. Prolonged incubation (48h) in a closed system potentially favor changes in the VFA production as well as its proportions (Ungerfeld and Kohn, 2006). In this study, the higher fiber content at the 30 cm sward height shifted fermentation towards the production of acetate at 48 h. The broken line response of the acetate as a function of sward height (Figure 1.d) matched with the CH_4 response at 24 h, although, the increase in the proportion of acetate was more pronounced after approximately 28 cm of sward height. The molar proportion of propionic acid, for its part, increased up to 21 cm approximately and then decreased as sward height increased (Figure 1.e). The reduction in propionic (mol / 100mL) at tallest sward height was expected due to its ADF contents and the low IVDMD. The higher molar proportion of propionic acid at 20 cm sward height matches with its less methanogenic profile and suggest also a better *in vitro* rumen fermentation efficiency at that sward structure. It is also widely known that forages that increase propionate and decrease acetate are often associated with a reduction in ruminal CH_4 production (Beauchemin et al., 2009; Meale et al., 2012; Moss et al., 2000). Nevertheless, the lower proportion of propionate at smaller heights was unexpected due to the similarities of the chemical composition and IVDMD between the sward heights below 20 cm. A possible explanation of this finding could be related to butyrate response as a function of the sward heights (Figure 1.f) which could have increased at the expense of propionate. In this study the butyrate seems to have acted as an alternative H_2 sinks (Moss et al., 2000; Ungerfeld, 2015) which is also in agreement with the lower CH_4 (mL / g IVDMD) at lower sward heights. It is also suggested that some changes in the fermentation pathways could be associated with superior CP concentrations and, probably, with the higher nitrates contents as a product of the high nitrogen fertilization of the kikuyu (Lovett et al., 2004). It is well known that nitrate is an alternative H_2 sink and an effective inhibitor of methanogenesis (McAllister and Newbold, 2008; Patra et al., 2017; Van Zijderveld et al., 2010; Yang et al., 2016). Other studies have suggested that the inclusion of nitrate on *in vitro* ruminal fermentation could increase the molar proportion of acetic acid and reduce the molar proportion of propionic (Navarro-Villa et al., 2011), as observed in this study at taller sward heights.

3.5 Conclusions

In conclusion, the herbage samples from the top stratum of kikuyu grass between 10 to 25 cm sward heights exhibited a less methanogenic *in vitro* fermentation profile respect to taller sward height (30 cm), which produced a lower propionate proportion and higher acetate proportion highlighting their greater methanogenic potential. The results of this study suggest that in that range of sward heights the grazing management of the kikuyu grass would be more strategic in order to reduce *in vitro* CH_4 production.

3.6 References

Abrahamse, P.A., Tamminga, S., Dijkstra, J., 2009. Effect of daily movement of dairy cattle to fresh grass in morning or afternoon on intake, grazing behaviour, rumen fermentation and milk production. J. Agric. Sci. 147, 721–730. https://doi.org/10.1017/S0021859609990153

Amaral, M.F., Mezzalira, J.C., Bremm, C., Da Trindade, J.K., Gibb, M.J., Suñe, R.W.M., de F. Carvalho, P.C., 2013. Sward structure management for a maximum short-term intake rate in annual ryegrass. Grass Forage Sci. 68, 271–277. https://doi.org/10.1111/j.1365-2494.2012.00898.x

AOAC, 2016. Official methods of analysisof of Association of Official Analytical Chemist., 20th editi. ed. Arlington, VA, USA.

Barthram, G.T., 1985. Experimental techniques: the HFRO sward stick, Biennial Report of the Hill Farming Research Organization. Midlothian.

Beauchemin, K., McAllister, T.A., McGinn, S.M., 2009. Dietary mitigation of enteric methane from cattle. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 4, 1–18. https://doi.org/10.1079/PAVSNNR20094035

Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A., 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Agric. 48, 21–27. https://doi.org/10.1071/EA07199

Benvenutti, M.A., Pavetti, D.R., Poppi, D.P., Gordon, I.J., Cangiano, C.A., 2016. Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. Grass Forage Sci. 71, 424–436. https://doi.org/10.1111/gfs.12186

Boadi, D.A., Wittenberg, K.M., McCaughey, W., 2002. Effects of grain supplementation on methane production of grazing steers using the sulphur (SF6) tracer gas technique. Can. J. Anim. Sci. 82, 151–157. https://doi.org/10.4141/A01-038

Boval, M., Dixon, R.M., 2012. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. Animal 6, 748–762. https://doi.org/10.1017/s1751731112000304

Bremm, C., Laca, E.A., Fonseca, L., Mezzalira, J.C., Elejalde, D.A.G., Gonda, H.L., Carvalho, P.C. de F., 2012. Foraging behaviour of beef heifers and ewes in natural grasslands with distinct proportions of tussocks. Appl. Anim. Behav. Sci. 141, 108–116. https://doi.org/10.1016/j.applanim.2012.08.008

Burke, J.L., Waghor, G.C., Brookes, I.M., Chaves, A.V., Attwood, G.T., 2006. In vitro production of volatile fatty acids from forages, in: Proceedings-New Zealand Society of Animal Production. New Zealand Society of Animal Production; 1999, p. 50.

Carvalho, P.C. de F., 2013. Harry Stobbs Memorial Lecture: Can grazing behaviour support innovations in grassland management?, in: 22nd International Grasslands. Sidney (Australia), pp. 1134–1148.

Correa, H.., Pabón, M.., Carulla, J.., 2008a. Valor nutricional del pasto kikuyo (Pennisetum clandestinum Hoechst Ex Chiov.) para la producción de leche en Colombia (Una revisión): II. Contenido de energía, consumo, producción y eficiencia nutricional. Livest. Res. Rural Dev. 20.

Correa, H.., Pabón, M.., Carulla, J.., 2008b. Valor nutricional del pasto kikuyo (Pennisetum clandestinum Hoechst Ex Chiov.) para la producción de leche en Colombia (Una revisión): I - Composición química y digestibilidad ruminal y posruminal. Livest. Res. Rural Dev. 20.

De la Motte, L.G., Jérôme, E., Mamadou, O., Beckers, Y., Bodson, B., Heinesch, B., Aubinet, M., 2016. Carbon balance of an intensively grazed permanent grassland in southern Belgium. Agric. For. Meteorol. 228–229, 370–383. https://doi.org/10.1016/j.agrformet.2016.06.009

Delagarde, R., Peyraud, J.., Delaby, L., Faverdin, P., 2000. 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. Anim. Feed Sci. Technol. 84, 49–68. https://doi.org/10.1016/S0377-8401(00)00114-0

Difante, S., Júnior, N., Pacheco, V., Euclides, B., Carneiro, S., Barbosa, R.A., Gonçalves, W.V., 2009. Revista Brasileira de Zootecnia Sward structure and nutritive value of tanzania guineagrass subjected to rotational stocking managements 1 Estrutura do pasto e valor nutritivo do capim-tanzânia submetido a estratégias de pastejo rotativo 3598.

Dobos, R.C., Fulkerson, W.J., Sinclair, K., Hinch, G.N., 2009. Grazing behaviour and pattern of intake of dairy cows grazing kikuyu (Pennisetum clandestinum) grass pastures in relation to sward height and length of grazing session. Anim. Prod. Sci. 49, 233. https://doi.org/10.1071/ea08212

Durmic, Z., Hutton, P., Revell, D.K., Emms, J., Hughes, S., Vercoe, P.E., 2010. In vitro fermentative traits of Australian woody perennial plant species that may be considered as potential sources of feed for grazing ruminants. Anim. Feed Sci. Technol. 160, 98–109.

Elgersma, A., 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. Eur. J. Lipid Sci. Technol. 117, 1345–1369. https://doi.org/10.1002/ejlt.201400469

Fonseca, L., Mezzalira, J.C., Bremm, C., Filho, R.S.A., Gonda, H.L., Carvalho, P.C. d. F., 2012. Management targets for maximising the short-term herbage intake rate of cattle grazing in Sorghum bicolor. Livest. Sci. 145, 205–211. https://doi.org/10.1016/j.livsci.2012.02.003

French, P., O'Brien, B., Shalloo, L., 2015. Development and adoption of new technologies to increase the efficiency and sustainability of pasture-based systems. Anim. Prod. Sci. 55, 931. https://doi.org/10.1071/AN14896

Fulkerson, W.J., Neal, J.S., Clark, C.F., Horadagoda, A., Nandra, K.S., Barchia, I., 2006. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. Livest. Sci. 107, 253–264. https://doi.org/10.1016/j.livsci.2006.09.029

Garcia, S.C., Islam, M.R., Clark, C.E.F., Martin, P.M., 2014. Kikuyu-based pasture for dairy production: a review. Crop Pasture Sci. 65, 787. https://doi.org/10.1071/CP13414

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G., 2013. Tackling Climate Change Through Livestock - A global assessment of emissions and mitigation opportunities. FAO, Roma.

Gerssen-Gondelach, S.J., Lauwerijssen, R.B., Havlík, P., Herrero, M., Valin, H., Faaij, A.P.C., Wicke, B., 2017. Intensification pathways for beef and dairy cattle production systems: Impacts on GHG emissions, land occupation and land use change. Agric. Ecosyst. Environ. 240, 135–147. https://doi.org/10.1016/j.agee.2017.02.012

Gonçalves, E.N., Carvalho, P.C. de F., Kunrath, T.R., Carassai, I.J., Bremm, C., Fischer, V., 2009. Plant-animal relationships in pastoral heterogeneous environment: process of herbage intake. Rev. Bras. Zootec. 38, 1655–1662.

Herrero, M., Thornton, P.K., Notenbaert, A.M., Wood, S., Msangi, S., Freeman, H.A., Bossio, D., Dixon, J., Peters, M., van de Steeg, J., Lynam, J., Rao, P.P., Macmillan, S., Gerard, B., McDermott, J., Sere, C., Rosegrant, M., 2010. Smart Investments in Sustainable Food Production: Revisiting Mixed Crop-Livestock Systems. Science (80-.). 327, 822–825. https://doi.org/10.1126/science.1183725

Horrocks, C.A., Arango, J., Arevalo, A., Nuñez, J., Cardoso, J.A., Dungait, J.A.J., 2019. Smart forage selection could significantly improve soil health in the tropics. Sci. Total Environ. 688, 609–621. https://doi.org/10.1016/j.scitotenv.2019.06.152

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E., Waghorn, G., Dijkstra, J., Oosting, S., 2013. Mitigation of greenhouse gas emissions in livestock production. A review of technical options for non-CO2 emissions, Pierre J. ed. FAO, Roma.

Hristov, A. N., Ott, T., Tricarico, J., Rotz, A., Waghorn, G., Adesogan, A., Dijkstra, J., Montes, F., Oh, J., Kebreab, E., Oosting, S.J., Gerber, P.J., Henderson, B., Makkar, H.P.S., Firkins, J.L., 2013. SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options1. J. Anim. Sci. 91, 5095–5113. https://doi.org/10.2527/jas.2013-6585

Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle Methane Emissions from Cattle. J Anim Sci 73, 2483–2492.

Kurihara, M., Magner, T., Hunter, R.A., McCrabb, G.J., 1999. Methane production and energy partition of cattle in the tropics. Br. J. Nutr. 81, 227–234. https://doi.org/10.1017/S0007114599000422

Lobato, J.F.P., Freitas, Ak., Devincenzi, T., Cardoso, L.L., Tarouco, J.U., Vieira, R.M., Dillenburg, D.R., Castro, I., 2014. Brazilian beef produced on pastures: sustainable and healthy. Meat Sci. 98, 336–345.

Lopez, S., Newbold, C.J., 2007. Analysis of Methane. Meas. Methane Prod. From Ruminants 1–13. https://doi.org/10.1007/978-1-4020-6133-2_1

Lovett, D.K., Bortolozzo, A., Conaghan, P., O'Kiely, P., O'Mara, F.P., 2004. *In vitro* total and methane gas production as influenced by rate of nitrogen application, season of harvest and perennial ryegrass cultivar. Grass Forage Sci. 59, 227–232. https://doi.org/10.1111/j.1365-2494.2004.00421.x

Marín, A., Giraldo, L., Correa, G., 2014. Parámetros de fermentación ruminal *in vitro* del pasto Kikuyo (Pennisetum clandestinum). Liv. Res. Rural Dev. 26.

Mayland, H., Mertens, D., Taylor, T., Burns, J., Fisher, D., Gregorini, P., Ciavarella, T., Smith, K., Shewmaker, G., Griggs, T., 2005. Diurnal changes in forage quality and their effects on animal preference, intake, and performance, in: 35th California Alfalfa and Forage Symposium', Visalia, CA, USA. pp. 223–230.

McAllister, T.A., Newbold, C.J., 2008. Redirecting rumen fermentation to reduce methanogenesis. Aust. J. Exp. Agric. 48, 7. https://doi.org/10.1071/EA07218

McGinn, S.M., Beauchemin, K.A., Coates, T., McGeough, E.J., 2014. Cattle Methane Emission and Pasture Carbon Dioxide Balance of a Grazed Grassland. J. Environ. Qual. 43, 820. https://doi.org/10.2134/jeq2013.09.0371

Meale, S.J., Chaves, A. V, Baah, J., McAllister, T.A., 2012. Methane production of different forages *In vitro* Ruminal Fermentation. Asian-Australasian J. Anim. Sci. 25, 86–91.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93, 217–222.

Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid.

Mezzalira, J.C., De Faccio Carvalho, P.C., Fonseca, L., Bremm, C., Cangiano, C., Gonda, H.L., Laca, E.A., 2014. Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. Appl. Anim. Behav. Sci. 153, 1–9. https://doi.org/10.1016/j.applanim.2013.12.014

Moe, P.W., Tyrrell, H.F., 1979. Methane production in dairy cows. J. Dairy Sci. 62, 1583–1586.

Moss, Jouany, J.-P., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231–253. https://doi.org/10.1051/animres:2000119

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C., Gerber, P., 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. Glob. Food Sec. 14, 1–8. https://doi.org/10.1016/j.gfs.2017.01.001

Muñoz, C., Letelier, P.A., Ungerfeld, E.M., Morales, J.M., Hube, S., Pérez-Prieto, L.A., 2016. Effects of pregrazing herbage mass in late spring on enteric methane emissions, dry matter intake, and milk production of dairy cows. J. Dairy Sci. 99, 7945–7955. https://doi.org/10.3168/jds.2016-10919

Navarro-villa, A., Brien, M.O., 2011. *In vitro* rumen methane output of feeds consumed by housed cattle. Anim. Grassl. Innov. 1–4.

Navarro-Villa, A., O'Brien, M., López, S., Boland, T.M., O'Kiely, P., 2011. *In vitro* rumen methane output of red clover and perennial ryegrass assayed using the gas production technique (GPT). Anim. Feed Sci. Technol. 168, 152–164. https://doi.org/10.1016/j.anifeedsci.2011.04.091

Patra, A., Park, T., Kim, M., Yu, Z., 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J. Anim. Sci. Biotechnol. 8, 13. https://doi.org/10.1186/s40104-017-0145-9

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2018. nlme: linear and nonlinear mixed effects models. R package version 3.1-137. R Found. Stat. Comput. https//CRAN. R-project. org/package= nlme (accessed 6 Feb. 2019).

Purcell, P.J., O'Brien, M., Boland, T.M., O'Kiely, P., 2011. *In vitro* rumen methane output of perennial ryegrass samples prepared by freeze drying or thermal drying (40°C). Anim. Feed Sci. Technol. 166–167, 175–182. https://doi.org/10.1016/j.anifeedsci.2011.04.065

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

Ramírez, J., Posada, O., Noguera, R., 2015. Effects of Kikuyu grass (Pennisetum clandestinum) age and different forage: concentrate ratios on methanogenesis. Rev. MVZ Córdoba 20, 4726–4738.

Ribeiro, G.O., Teixeira, A.M., Velasco, F.O., Júnior, W.G.F., Pereira, L.G.R., Chaves, A. V, Gonçalves, L.C., McAllister, T.A., 2014. Production, nutritional quality and *in vitro* methane production from Andropogon gayanus grass harvested at different maturities and preserved as hay or silage. Asian-Australasian J. Anim. Sci. 27, 330.

Savian, J.V., Schons, R.M.T., Marchi, D.E., Freitas, T.S. de, Silva Neto, G.F., Mezzalira, J.C., Berndt, A., Bayer, C., Carvalho, P.C. de F., 2018. Rotatinuous stocking : A grazing management innovation that has high potential to mitigate methane emissions by sheep. J. Clean. Prod. 186, 602–608. https://doi.org/10.1016/j.jclepro.2018.03.162

Sbrissia, A.F., Duchini, P.G., Zanini, G.D., Santos, G.T., Padilha, D.A., Schmitt, D., 2018. Defoliation Strategies in Pastures Submitted to Intermittent Stocking Method: Underlying Mechanisms Buffering Forage Accumulation over a Range of Grazing Heights. Crop Sci. 58, 945. https://doi.org/10.2135/cropsci2017.07.0447

Sbrissia, A.F., Euclides, V.P.B., Barbosa, R.A., Montagner, D.B., Padilha, D.A., Santos, G.T., Zanini, G.D., Duchini, P.G., Silva, S.C., 2013. Grazing management flexibility in pastures subjected to rotational stocking management: herbage production and chemical composition of kikuyu-grass swards, in: Revitalising Grasslands to Sustain Our Communities: Proccedings of the 22th International Grassland Congress. pp. 1038–1040.

Schmitt, D., Padilha, D.A., Dias, K.M., Santos, G.T., Rodolfo, G.R., Zanini, G.D., Sbrissia, A.F., 2019a. Chemical composition of two warm-season perennial grasses subjected to proportions of defoliation. Grassl. Sci. 65, 171–178. https://doi.org/10.1111/grs.12236

Schmitt, D., Padilha, D.A., Medeiros-Neto, C., Ribeiro Filho, H.M.N., Sollenberger, L.E., Sbrissia, A.F., 2019b. Herbage intake by cattle in kikuyugrass pastures under intermittent stocking method. Rev. CIÊNCIA AGRONÔMICA 50. https://doi.org/10.5935/1806-6690.20190058

Tagliapietra, F., Cattani, M., Bailoni, L., Schiavon, S., 2010. *In vitro* rumen fermentation: effect of headspace pressure on the gas production kinetics of corn meal and meadow hay. Anim. Feed Sci. Technol. 158, 197–201.

Teague, W.R., Apfelbaum, S., Lal, R., Kreuter, U.P., Rowntree, J., Davies, C.A., Conser, R., Rasmussen, M., Hatfield, J., Wang, T., Wang, F., Byck, P., 2016. The role of ruminants in reducing agriculture's carbon footprint in North America. J. Soil Water Conserv. 71, 156–164. https://doi.org/10.2489/jswc.71.2.156

Theodorou, M.K., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197. https://doi.org/https://doi.org/10.1016/0377-8401(94)90171-6

Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. Grass forage Sci. 18, 104–111.

Ungerfeld, E.M., 2015. Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis. Front. Microbiol. 6, 37.

Ungerfeld, E.M., Kohn, R.A., 2006. The role of thermodynamics in the control of ruminal fermentation. Rumin. Physiol. Dig. Metab. impact Nutr. gene expression, Immunol. Stress 55–85.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., Perdok, H.B., 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93, 5856–5866.

Vargas, J.J., Pabón, M.L., Carulla, J.E., 2018. Methane production from four forages at three maturity stages in a ruminal *in vitro* system. Rev. Colomb. Ciencias Pecu. 31, 120–129. https://doi.org/10.17533/udea.rccp.v31n2a05

Werling, B.P., Dickson, T.L., Isaacs, R., Gaines, H., Gratton, C., Gross, K.L., Liere, H., Malmstrom, C.M., Meehan, T.D., Ruan, L., Robertson, B.A., Robertson, G.P., Schmidt, T.M., Schrotenboer, A.C., Teal, T.K., Wilson, J.K., Landis, D.A., 2014. Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. Proc. Natl. Acad. Sci. 111, 1652–1657. https://doi.org/10.1073/pnas.1309492111

Yang, C., Rooke, J.A., Cabeza, I., Wallace, R.J., 2016. Nitrate and Inhibition of Ruminal Methanogenesis: Microbial Ecology, Obstacles, and Opportunities for Lowering Methane Emissions from Ruminant Livestock. Front. Microbiol. 7, 132. https://doi.org/10.3389/fmicb.2016.00132

4 CHAPTER IV.

FINAL CONCLUSIONS

Nowadays, the pasture-based livestock systems are of particular importance given the growing demands for sustainable production. In this sense, the definition of grazing management targets leading to maximizing quantity and quality of herbage consumed by the animals maintaining low CH_4 production, both total and per unit of dry matter intake or digested, are key and represent one of the biggest challenges for farmers and professionals. This thesis purposed two objectives: (i) Defining the sward height of the kikuyu grass that allows dairy heifers to maximize their herbage and total digestible nutrients intake per unit of eating, and (ii) Assess the effect of the sward height of the kikuyu grass on the chemical composition and the main *in vitro* ruminal fermentation parameters of the potentially grazed stratum and identify the sward structures with the greatest potential for mitigating methane emissions.

From the perspective of the animal, defining very low sward heights (i.e 10 cm) of the kukuyu grass as a goal of grazing management can be constrained more the bite mass, and thus, the STIR, than sward heights managed between 20 cm to 30 cm, although defining sward heights above 25 cm would also reduce the STIR mainly due to the decrease of the herbage and leaves bulk density. From nutritional approach, in tall pastures the IVDMD decrease and the *in vitro* ruminal VFA profile suggest negative digestive effects besides of the greater methanogenic potential per unit of dry matter digested. Additionally, it can be highlighted that the better nutritive characteristics of herbage at lower sward heights is not a determining factor of the animal response, at least in a short term, since the sward structure could have a negative effect on bite mass, and consequently, on the STIR.

In this study, it was also found that the herbage mass of kikuyu grass remained constant between 20 to 30 cm which was attributed to a mechanism of tiller size/number compensation previously reported for this species in the literature (Sbrissias et al 2018). In other words, the fact that having higher sward heights does not necessarily mean greater herbage mass accumulation. This has important implications in the Colombian context where it is common to determine grazing management targets prioritize the maximum herbage mass accumulation (i.e. forage harvest efficiency), that does not correspond with animal goals or efficient strategies to mitigate CH_4 production.

In conclusion, define very low (10 cm) or very tall (30 cm) sward heights of kikuyu grass as a grazing management target, even if only access the top stratum was considered, would have a negative impact on the herbage and nutrient intake rate which will probably affect the animal productivity. Despite *in vitro* ruminal fermentation parameters indicate that the sward heights between 10 to 25 cm had the greatest methane mitigation potential, the short-term animal response suggests that to optimize the STIR and TDN-STIR of dairy heifers, the sward height of kikuyu grass should be managed around to 20 cm.

The improvement in the understanding of the relationship between sward structure (studied through the sward height) and grazing behavior components, as well as the its implications on the nutritive characteristics and the *in vitro* ruminal fermentation parameters, are important to aid decision making of the sward height of the kikuyu grass for being grazing by animals.

REFERENCES

ALLDEN, W. G.; WHITTAKER, I. A. The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. **Australian Journal of Agricultural Research**, East Melbourne, v. 21, n.5, p. 755–766, 1970.

ALLISON, C. D. Factors affecting forage intake by range ruminants: a review. **Journal of Range Management**, Denver, v. 38, n. 4, p. 305-311, 1985. Available in: https://www.jstor.org/stable/3899409?origin=crossref. Access in: 18 Feb. 2019.

AMARAL, M. F. *et al*. Sward structure management for a maximum short-term intake rate in annual ryegrass. Grass and Forage Science, Oxford, v. 68, n. 2, p. 271–277, 2013. Available in: http://doi.wiley.com/10.1111/j.1365-2494.2012.00898.x. Access in: 25 July 2017.

ANDRIAMANDROSO, A. L. H. *et al*. A review on the use of sensors to monitor cattle jaw movements and behavior when grazing. **Biotechnology, Agronomy, Society and Environment**, Gembloux, v. 20, n. 2, p. 273–286, 2016.

BAILEY, D. W. Daily selection of feeding areas by cattle in homogeneous and heterogeneous environments. **Applied Animal Behaviour Science**, Amsterdam, v. 45, n. 3/4, p. 183–200, 1995. Available in: https://linkinghub.elsevier.com/retrieve/pii/016815919500586H. Access in: 22 July 2017.

BAILEY, D. W. *et al.* Mechanisms That Result in Large Herbivore Grazing Distribution Patterns. **Journal of Range Management**, Denver, v. 49, n. 5, p. 386-400, 1996. Available in: https://www.jstor.org/stable/4002919?origin=crossref. Access in: 13 Aug. 2017.

BAILEY, D. W.; PROVENZA, Frederick D. Mechanisms Determining Large-Herbivore Distribution. *In:* PRINS, H. H. T.; VAN LANGEVELDE, F. (ed.). **Resource ecology**. Dordrecht: Springer, 2008. p. 7–28. E-book. Available in: http://dx.doi.org/10.1007/978-1-4020-6850-8_2. 13. Access in: 13 Aug. 2017.

BAUMONT, R. *et al.* A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. **Animal Feed Science and Technology**, Amsterdam, v. 112, n. 1/4, p. 5–28, 2004. Available in: http://linkinghub.elsevier.com/retrieve/pii/S0377840103002967. Access in: 13 Aug. 2017.

BEAUCHEMIN, K. A. *et al.* Nutritional management for enteric methane abatement: a review. **Australian Journal of Experimental Agriculture**, East Melbourne, v. 48, n. 1/2, p. 21–27, 2008.

BEAUCHEMIN, K.A.; MCGINN, S. M.; GRAINGER, C. Reducing methane emissions from dairy cows. *In*: INTERMOUNTAIN NUTRITION CONFERENCE: DAIRY NUTRITIONAL STRATEGIES TO MEET ECONOMIC AND ENVIRONMENTAL CHALLENGES, 10., 2008, Salt Lake City. **Proceedings** […]. Logan: Utah State University, 2008. p. 237-249.

BEGGS, D. S. *et al.* Implications of prolonged milking time on time budgets and lying behavior of cows in large pasture-based dairy herds. **Journal of Dairy Science**, Champaign, v. 101, n. 11, p. 10391–10397, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030218308543. Access in: 18 Feb. 2019.

BENVENUTTI, M. A. *et al.* Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. **Grass and Forage Science**, Oxford, v. 71, n. 3, p. 424–436, 2016. Available in: http://doi.wiley.com/10.1111/gfs.12186. Access in: 13 Aug. 2017.

BENVENUTTI, M.A.; GORDON, Iain J.; POPPI, Dennis P. 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**, Oxford, v. 61, n. 3, p. 272–281, 2006. Available in: http://doi.wiley.com/10.1111/j.1365-2494.2006.00531.x. Access in: 12 Jan. 2017.

BENVENUTTI, M. A.; GORDON, Iain J.; POPPI, Dennis P. The effects of stem density of tropical swards and age of grazing cattle on their foraging behaviour. **Grass and forage Science**, Oxford, v. 63, n. 1, p. 1–8, 2008. Available in: http://doi.wiley.com/10.1111/j.1365-2494.2007.00609.x. Access in: 12 Jan. 2017.

BERGMAN, Carita M. *et al.* Ungulate foraging strategies: energy maximizing or time minimizing? **Journal of Animal Ecology**, London, v. 70, n. 2, p. 289–300, 2001. Available in: http://doi.wiley.com/10.1046/j.1365-2656.2001.00496.x. Access in: 13 Aug. 2017.

BLACK, J. L.; KENNEY, P. A. Factors affecting diet selection by sheep. 2. Height and density of pasture. **Australian Journal of Agricultural Research**, East Melbourne, v. 35, n. 4, p. 565–578, 1984.

BOVAL, M. *et al.* Effect of structure of a tropical pasture on ingestive behaviour, digestibility of diet and daily intake by grazing cattle. **Grass and Forage Science**, Oxford, v. 62, n. 1, p. 44–54, 2007. Available in: http://doi.wiley.com/10.1111/j.1365-2494.2007.00560.x. Access in: 22 Sept. 2016.

BOVAL, M.; DIXON, R. M. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. **Animal**, Cambridge, v. 6, n. 5, p. 748–762, 2012.

BREMM, Carolina *et al.* Foraging behaviour of beef heifers and ewes in natural grasslands with distinct proportions of tussocks. **Applied Animal Behaviour Science**, Amsterdam, v. 141, n. 3/4, p. 108–116, 2012. Available in: http://dx.doi.org/10.1016/j.applanim.2012.08.008. Access in: 22 Sept. 2016.

CANGIANO, C. A. **Conpast 3.0**. Programa de computación para la estimación del consumo de bovinos en pastoreo. Balcarce: INTA, 1999.

CARVALHO, P. C. F. *et al.* A estrutura do pasto como conceito de manejo: reflexos sobre o consumo e a produtividade. *In*: Reis, R. A. *et al*. (org.). **Volumosos na produção de ruminantes**. Jaboticabal: Funep, 2005. p. 107-124.

CARVALHO, P. C. F. Harry Stobbs memorial lecture: can grazing behaviour support innovations in grassland management? **Tropical Grasslands**, Cali, v. 1, n. 2, p. 137-155, 2013.

CARVALHO, P. C. F. A estrutura da pastagem e o comportamento ingestivo de ruminantes em pastejo. *In*: SIMPÓSIO SOBRE AVALIAÇÃO DE PASTAGENS COM ANIMAIS, 1., 1997, Maringá-PR. **Anais** [...]. Maringá: UEM, 1997. p. 25-52.

CARVALHO, P. C. F.; MORAES, Anibal. Comportamento ingestivo de ruminantes: bases para o manejo sustentável do pasto. *In*: CECATO, U.; JOBIM, C. C. (org.). **Manejo sustentável em pastagem**. Maringá: UEM, 2005. v. 1, p. 1-20.

CARVALHO, P. C. F. *et al.* Como a estrutura do pasto influencia o animal em pastejo? Exemplificando as interações planta-animal sob as bases e fundamentos do pastoreio “rotatínuo”. *In:* SIMPÓSIO SOBRE MANEJO ESTRATÉGICO DA PASTAGEM, 8., 2016, Viçosa. **Anais** [...]. Viçosa: UFV, 2016. p. 309 - 333.

CARVALHO, P. C. F. *et al.* Como otimizar a ingestão de forragem por vacas leiteiras em pastejo? *In:* SIMPÓSIO MINEIRO DE NUTRIÇÃO DE GADO DE LEITE, 8.; SIMPÓSIO NACIONAL DE PRODUÇÃO E NUTRIÇÃO DE GADO DE LEITE, 3., 2017, Uberlândia. **Anais** [...]. [Uberlândia: FAMEV], 2017. Palestra apresentada no evento.

CARVALHO, P. C. F *et al.* Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo. *In*: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 38., 2001, Piracicaba. **Anais** [...]. Piracicaba: ESALQ, 2001. p. 853–871. Available in: http://www.ufrgs.br/gpep/documents/capitulos/Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo.pdf. Access in: 22 Sept. 2016.

CHACON, E.; STOBBS, T. H. Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. **Australian Journal of Agricultural Research**, East Melbourne, v. 27, n. 5, p. 709-727, 1976. Available in: http://www.publish.csiro.au/?paper=AR9760709. Access in: 15 Oct. 2016.

CHILIBROSTE, P. *et al.* Behavioural adaptation of grazing dairy cows to changes in feeding management: do they follow a predictable pattern? **Animal Production Science**, Collingwood, v. 55, n. 3, p. 328-338, 2015. Available in: http://www.publish.csiro.au/?paper=AN14484. Access in: 2 May 2019.

CHILIBROSTE, P. **Grazing time:** the missing link: a study of the plant-animal interface by integration of experimental and modelling approaches. 1999. Thesis (PhD in Animal Nutrition) - Wageningen Agricultural University, Wageningen, 1999.

DELAGARDE, R.; LAMBERTON, Philippe. Daily grazing time of dairy cows is recorded accurately using the Lifecorder Plus device. **Applied Animal Behaviour Science**, Amsterdam, v. 165, p. 25–32, 2015. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0168159115000325. Access in: 2 Feb. 2019.

DEMMENT, M. W.; LACA, E. A. The grazing ruminant: models and experimental techniques to relate sward structure and intake. *In:* WORLD CONFERENCE ON ANIMAL PRODUCTION, 7., 1993, Edmonton. **Proceedings** [...]. Edmonton: Keeling & Mundi, 1993. p. 439–460.

DEMMENT, M. W.; PEYRAUD, J. L.; LACA, E. A. Herbage intake at grazing: a modelling approach. *In:* INTERNATIONAL SYMPOSIUM ON THE NUTRITION OF HERBIVORES, 4., 1995, Paris. **Proceedings** […]. Paris: INRA, 1995. p. 121–141.

DERAMUS, H. Alan *et al.* Methane emissions of beef cattle on forages. **Journal of Environment Quality**, Madison, v. 32, n. 1, p. 269-277, 2003. Available in: https://www.ncbi.nlm.nih.gov/pubmed/12549566. Access in: 22 Sept. 2016.

DIFANTE, G. *et al.* Sward structure and nutritive value of tanzania guineagrass subjected to rotational stocking managements. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 38, n. 1, p. 9–19, 2009.

DOBOS, R. C. *et al.* Grazing behaviour and pattern of intake of dairy cows grazing kikuyu (*Pennisetum clandestinum*) grass pastures in relation to sward height and length of grazing session. **Animal Production Science**, Collingwood, v. 49, n. 3, p. 233-238, 2009.

DOHME-MEIER, F. *et al.* Comparison of energy expenditure, eating pattern and physical activity of grazing and zero-grazing dairy cows at different time points during lactation. **Livestock Science**, Amsterdam, v. 162, p. 86–96, 2014.

DRESCHER, M. **Grasping complex matter:** large herbivore foraging in patches of heterogeneous resources. 2003. 184 f. Thesis (PhD) - Department of Environmental Sciences, Tropical Nature Conservation and Vertebrate Ecology Group, University of Netherlands, Wageningen, 2003.

ELGERSMA, A. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives.

European Journal of Lipid Science and Technology, Weinheim, v. 117, n. 9, p. 1345–1369, 2015. Available in: http://doi.wiley.com/10.1002/ejlt.201400469. Access in: 2 May 2019.

ELLIS, J. L. *et al.* Prediction of methane production from dairy and beef cattle. **Journal of Dairy Science**, Champaign, v. 90, n. 7, p. 3456–3466, 2007.

EUCLIDES, V. P. B. *et al.* Maintaining post-grazing sward height of *Panicum maximum* (cv. Mombaça) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. **Grass and Forage Science**, Oxford, v. 73, n. 1, p. 174–182, 2018. Available in: https://doi.org/10.1111/gfs.12292. Access in: 22 Feb. 2019.

FAO. **The future of food and agriculture:** trends and challenges. Rome: FAO 2017. E-book. Available in: http://www.fao.org/3/a-i6583e.pdf. Access in: 9 Aug. 2018.

FLORES, E. R. *et al.* Sward height and vertical morphological differentiation determine cattle bite dimensions. **Agronomy Journal**, Madison, v. 85, n. 3, p. 527-532, 1993. Available in: https://dl.sciencesocieties.org/publications/aj/abstracts/85/3/AJ0850030527?access=0&view=article. Access in: 22 Sept. 2016.

FONSECA, L. *et al.* Management targets for maximising the short-term herbage intake rate of cattle grazing in Sorghum bicolor. **Livestock Science**, Amsterdam, v. 145, n. 1/3, p. 205–211, 2012. Available in: http://linkinghub.elsevier.com/retrieve/pii/S1871141312000595. Access in: 16 July 2016.

FONSECA, L. *et al.* Effect of sward surface height and level of herbage depletion on bite features of cattle grazing Sorghum bicolor swards. **Journal of Animal Science**, Champaign, v. 91, n. 9, p. 4357–4365, 2013. Available in: https://academic.oup.com/jas/article/91/9/4357/4717390. Access in: 16 July 2016.

FORBES, J. M.; BARRIO, J. P. Abdominal chemo-and mechanosensitivity in ruminants and its role in the control of food intake. **Experimental Physiology**: Translation and Integration, Cambridge, v. 77, n. 1, p. 27–50, 1992. Available in: http://doi.wiley.com/10.1113/expphysiol.1992.sp003581. Access in: 2 July 2017.

FORBES, J. M. **Voluntary food intake and diet selection in farm animals**. [Wallingford]: Cabi, 1995.

FORBES, T. D. Researching the plant-animal interface: the investigation of ingestive behavior in grazing animals. **Journal of Animal Science**, Champaign, v. 66, n. 9, p. 2369–2379, 1988.

FRENCH, P.; O'BRIEN, B.; SHALLOO, L. Development and adoption of new technologies to increase the efficiency and sustainability of pasture-based systems. **Animal Production Science**, Collingwood, v. 55, n. 7, p. 931-935, 2015. Available in: http://www.publish.csiro.au/?paper=AN14896. Access in: 22 Jan. 2019.

GALLI, J. R.; CANGIANO, C. A.; FERNANDEZ, H. H. Comportamiento Ingestivo y Consumo de bovinos en pastoreo. **Revista Argentina de Producción Animal**, Buenos Aires, v. 16, n. 2, p. 119–42, 1996.

GARCIA, S. C. *et al.* Kikuyu-based pasture for dairy production: a review. **Crop and Pasture Science**, Collingwood, v. 65, n. 8, p. 787, 2014. Available in: http://www.publish.csiro.au/?paper=CP13414. Access in: 26 July 2018.

GARNETT, T. *et al.* Grazed and confused? Ruminating on cattle, grazing systems, methane, nitrous oxide, the soil carbon sequestration question-and what it all means for greenhouse gas emissions. **Food Climate Research Network**, Oxford, p. 1–127, 2017. Available in: https://www.fcrn.org.uk/sites/default/files/project-files/fcrn_gnc_report.pdf. Access in: 12 July 2019.

GERBER, P. J. *et al.* **Tackling climate change through livestock:** a global assessment of emissions and mitigation opportunities. Rome: FAO, 2013.

GERSSEN-GONDELACH, S. J. *et al.* Intensification pathways for beef and dairy cattle production systems: Impacts on GHG emissions, land occupation and land use change. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 240, p. 135–147, 2017. Available in: http://dx.doi.org/10.1016/j.agee.2017.02.012. Access in: 26 July 2018.

GIBB, M. J.; HUCKLE, C. A.; NUTHALL, R. Effect of time of day on grazing behaviour by lactating dairy cows. **Grass and Forage Science**, Oxford, v. 53, n. 1, p. 41–46, 1998. Available in: http://doi.wiley.com/10.1046/j.1365-2494.1998.00102.x. Access in: 16 July 2016.

GIBB, M. J. Animal grazing/intake terminology and definitions. *In*: PASTURE ECOLOGY AND ANIMAL INTAKE, 3., 1996, Dublin. **Proceedings** […]. Dublin: [*s.n.*], 1998. v. 3, p. 21-35.

GIBB, M. J. *et al.* Can grazed pasture meet the needs of the high genetic merit dairy cow? *In:* GRASS AND FORAGE FOR CATTLE OF HIGH GENETIC MERIT, 1996, Reading. [**Proceedings …**]. Reading: British Grassland Society, 1996. p. 2.

GIBB, M. J. *et al.* Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. **Grass and Forage Science**, Oxford, v. 52, n. 3, p. 309–321, 1997. Available in: http://doi.wiley.com/10.1111/j.1365-2494.1997.tb02361.x. Access in: 2 July 2017.

GONÇALVES, E. N. *et al.* Plant-animal relationships in pastoral heterogeneous environment: process of herbage intake. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 38, n. 9, p. 1655–1662, 2009.

GREGORINI, P. *et al.* The interaction of diurnal grazing pattern, ruminal metabolism, nutrient supply, and management in cattle. **The Professional Animal Scientist**, Champaign, v. 24, n. 4, p. 308–318, 2008. Available in: http://dx.doi.org/10.15232/S1080-7446(15)30861-5. Access in: 2 July 2017.

GREGORINI, P. *et al.* Effect of herbage depletion on short-term foraging dynamics and diet quality of steers grazing wheat pastures1. **Journal of Animal Science**, Champaign, v. 89, n. 11, p. 3824–3830, 2011. Available in: http://academic.oup.com/jas/article/89/11/3824/4789663. Access in: 2 July 2017.

GREGORINI, P.; GUNTER, S. A.; BECK, P. A. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: timing of herbage and fasting allocation. **Journal of Animal Science**, Champaign, v. 86, n. 4, p. 1006–1020, 2008.

GREGORINI, P.; TAMMINGA, S.; GUNTER, S. A. Behavior and daily grazing patterns of cattle. **The Professional Animal Scientist**, Champaign, v. 22, n. 3, p. 201–209, 2006. Available in: http://dx.doi.org/10.15232/S1080-7446(15)31095-0. Access in: 2 July 2017.

HAMMOND, K. J. *et al.* Effects of feed intake on enteric methane emissions from sheep fed fresh white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) forages. **Animal Feed Science and Technology**, Amsterdam, v. 179, n. 1/4, p. 121–132, 2013. Available in: http://dx.doi.org/10.1016/j.anifeedsci.2011.04.028. Access in: 15 Oct. 2016.

HARDY, M. B.; MEISSNER, H. H.; O'REAGAIN, P. J. Forage intake and free-ranging ruminants: a tropical perspective. *In*: INTERNATIONAL GRASSLAND CONGRESS, 18., 1997, Winnepeg. **Proceedings** […]. Calgary: The Congress, 1997. p. 45–52.

HARPER, K.; MCNEILL, D. The role iNDF in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems (the role of iNDF in the regulation of forage intake). **Agriculture**, Basel, v. 5, n. 3, p. 778–790, 2015.

HEGARTY, R. S. Nutritional management options to reduce enteric methane emissions from NSW beef and dairy herds. *In*: ANNUAL CONFERENCE OF THE GRASSLAND SOCIETY OF NSW, 24., 2009, Orange. **Proceedings** […].Orange: Grassland Society of NSW, 2009. p. 40–45.

HERRERO, Mario *et al.* Greenhouse gas mitigation potentials in the livestock sector. **Nature Climate Change**, London, v. 6, n. 5, p. 452–461, 2016. Available in: http://dx.doi.org/10.1038/nclimate2925. Access in: 13 Aug. 2017.

HIXON, Mark A. Food production and competitor density as the determinants of feeding territory size. **The American Naturalist**, Chicago, v. 115, n. 4, p. 510–530, 1980. Available in: https://www.journals.uchicago.edu/doi/abs/10.1086/283577 Access in: 10 Jan. 2020.

HIXON, Mark A. Energy maximizers and time minimizers: theory and reality. **The American Naturalist**, Chicago, v. 119, n. 4, p. 596–599, 1982.

HODGSON, J. The control of herbage intake in the grazing ruminant. **The Proceedings of the Nutrition Society**, Cambridge, v. 44, n. 2, p. 339–346, 1985.

HODGSON, J. Management of grazing systems. **Proceedings of the New Zealand Grassland Association**, Balchuta, v. 50, p. 117–122, 1989.

HODGSON, John. **Grazing management:** science into practice. London: Longman Group, 1990.

HODGSON, John; CLARK, D. A.; MITCHELL, R. J. Foraging behavior in grazing animals and its impact on plant communities. *In*: FAHEY JR., G. C. (ed.). **Forage quality, evaluation, and utilization**. Madison: Wiley, 1994. cap. 19, p. 796–827.

HOOK, Sarah E.; WRIGHT, André-Denis G.; MCBRIDE, Brian W. Methanogens: methane producers of the rumen and mitigation strategies. **Archaea**, London, v. 2010, [art.] ID 945785, [p. 1-11], 2010. Available in: http://www.hindawi.com/journals/archaea/2010/945785/. Access in: 15 Oct. 2016.

HRISTOV, A. N. *et al.* **Mitigation of greenhouse gas emissions in livestock production:** a review of technical options for non-CO2 emissions. Rome: FAO, 2013a. Available in: http://www.fao.org/3/i3288e/i3288e.pdf. Access in: 26 July 2018.

HRISTOV, A. N. *et al.* Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. **Journal of Animal Science**, Champaign, v. 91, n. 11, p. 5045–5069, 2013b. Available in: http://www.animalsciencepublications.org/publications/jas/abstracts/91/11/5045. Access in: 26 July 2018.

ILLIUS, A. W.; CLARK, D. A.; HODGSON, J. Discrimination and patch choice by sheep grazing grass-clover swards. **The Journal of Animal Ecology**, London, v. 61, n. 1, p. 183-194, 1992. Available in: https://www.jstor.org/stable/5521?origin=crossref. Access in: 2 July 2017.

ILLIUS, A. W.; GORDON, I. J. The allometry of food intake in grazing ruminants. **Journal of Animal Ecology**, London, v. 56, n. 3, p. 989-999, 1987. Available in: https://www.jstor.org/stable/4961?origin=crossref. Access in: 2 July 2017.

ISLAM, M. R. *et al.* Modelling pasture-based automatic milking system herds: system fitness of grazeable home-grown forages, land areas and walking distances. **Asian-Australasian Journal of Animal Sciences**, Seoul, v. 28, n. 6, p. 903–910, 2015. Available in: http://ajas.info/journal/view.php?doi=10.5713/ajas.14.0385. Access in: 18 Feb. 2019.

JANSSEN, P. H. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. **Animal Feed Science and Technology**, Amsterdam, v. 160, n. 1–2, p. 1–22, 2010. Available in: http://linkinghub.elsevier.com/retrieve/pii/S0377840110002087. Access in: 16 July 2017.

JOHNSON, K. A.; JOHNSON, D. E. Methane emissions from cattle methane emissions from cattle. J**ournal of Animal Science**, Champaign, v. 73, p. 2483–2492, 1995. Available in: https://academic.oup.com/jas/article/73/8/2483-2492/4632901. Access in: 22 Sept. 2016.

KURIHARA, M. *et al.* Methane production and energy partition of cattle in the tropics. **The British Journal of Nutrition**, Wallingford, v. 81, n. 3, p. 227–234, 1999.

LACA, E. A. *et al.* Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. **Grass and Forage Science**, Oxford, v. 47, n. 1, p. 91–102, 1992. Available in: http://doi.wiley.com/10.1111/j.1365-2494.1992.tb02251.x. Access in: 2 July 2017.

LACA, E. A.; DEMMENT, M. W. Herbivory: the dilemma of foraging in a spatially heterogeneous food environment. *In:* PALO, R.T.; ROBBINS, C. T. (org.). **Plant defenses against mammalian herbivory**. Boca Raton: CRC, 1991. p. 29–44.

LACA, E. A.; LEMAIRE, G. Measuring sward structure. *In:* T´MANNETJE, L.; JONES, R. M. (ed.). **Field and laboratory methods for grassland and animal production research**. Wallingford: CABI International, 2000. p. 103-122.

LACA, E. A.; ORTEGA, I. M. Integrating foraging mechanisms across spatial and temporal scales. *In:* INTERNATIONAL RANGELAND CONGRESS, 5., 1995, Salt Lake City. **Proceedings** [...]. Denver: Society for Range Management, 1996. p. 129-132.

LEMAIRE, G.; CHAPMAN, D. Tissue flows in grazed plant communities. *In*: HODGSON, J.; ILLIUS, A.W. (ed.). **The ecology and management of grazing systems**. Oxon: CAB International, 1996. p. 3-36.

LENG, R. A. Factors affecting the utilization of ‘poor-quality’forages by ruminants particularly under tropical conditions. **Nutrition Research Reviews**, Cambridge, v. 3, n. 1, p. 277–303, 1990.

MCALLISTER, T. A. *et al.* Dietary, environmental and microbiological aspects of methane production in ruminants. **Canadian Journal of Animal Science**, Ottawa, v. 76, n. 2, p. 231–243, 1996.

MCGINN, S. M. *et al.* Cattle methane emission and pasture carbon dioxide balance of a grazed grassland. **Journal of Environment Quality**, Madison, v. 43, n. 3, p. 820, 2014. Available in: https://dl.sciencesocieties.org/publications/jeq/abstracts/43/3/820. Access in: 13 Aug. 2017.

MERTENS, David R. Regulation of forage intake. *In*: FAHEY JR., G. C. (ed.). **Forage quality, evaluation, and utilization**. Madison: Wiley, 1994. p. 450–493.

MEZZALIRA, J. C. **Taxa de ingestão potencial em pastejo:** um estudo contrastando pastos de clima temperado e tropical. 2012. 168 f. Tese (Doutorado) - Programa de Pós-Graduação em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande de Sul, Porto Alegre, 2012. Available in: http://goo.gl/cBxFSf. Access in: 16 July 2016.

MEZZALIRA, J. C. *et al.* Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. **Applied Animal Behaviour Science**, Amsterdam, v. 153, p. 1–9, 2014. Available in: http://linkinghub.elsevier.com/retrieve/pii/S0168159113003213. Access in: 16 July 2016.

MONTES, F. *et al.* Special topics -- Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. **Journal of Animal Science**, Champaign, v. 91, p. 5070–5094, 2013. Available in: http://www.ncbi.nlm.nih.gov/pubmed/24045493. Access in: 22 Sept. 2016.

MOSS, Angela R.; JOUANY, Jean-Pierre; NEWBOLD, John. Methane production by ruminants: its contribution to global warming. **Annales de Zootechnie**, Les Ulis, v. 49, p. 231–253, 2000. Available in: http://hal.archives-ouvertes.fr/docs/00/88/98/94/PDF/hal-00889894.pdf. Access in: 22 Sept. 2016.

MOTTET, Anne *et al.* Livestock: on our plates or eating at our table? A new analysis of the feed/food debate. **Global Food Security**, Amsterdam, v. 14, p. 1–8, Sept. 2017. Available in: http://dx.doi.org/10.1016/j.gfs.2017.01.001. Access in: 26 July 2018.

O'REAGAIN, P. J.; MENTIS, M. T. Seasonal changes in the quality of diet selected by cattle grazing the natal sour sandveld. **Journal of the Grassland Society of Southern Africa**, Sunnyside, v. 5, n. 1, p. 48–51, 1988. Available in: http://www.tandfonline.com/doi/abs/10.1080/02566702.1988.9648108. Access in: 13 Aug. 2017.

O'REAGAIN, P. J.; MENTIS, M. T. The effect of plant structure on the acceptability of different grass species to cattle. **Journal of the Grassland Society of Southern Africa**, Sunnyside, v. 6, n. 3, p. 163–170, 1989.

O'REAGAIN, Peter J. Plant structure and the acceptability of different grasses to sheep. **Journal of Range Management**, Denver, v. 46, n. 3, p. 232-236, 1993. Available in: https://www.jstor.org/stable/4002612?origin=crossref. Access in: 13 Aug. 2017.

ORR, R. J. *et al.* Sward state and ingestive behaviour by Friesian dairy heifers under rotational grazing. *In:* RESEARCH MEETING OF THE BRITISH GRASSLAND SOCIETY, 5., 1997, Seale Hayne. **Proceedings** [...]. [Reading]: British Grassland Society, 1998. p. 51-52.

PALHANO, A. L. *et al.* Características do processo de ingestão de forragem por novilhas holandesas em pastagens de capim-mombaça. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 36, n. 4, p. 1014–1021, 2007. Available in:

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982007000500005&lng=pt&nrm=iso&tlng=pt. Access in: 13 Aug. 2017.

PARSONS, A. J. *et al.* A mechanistic model of some physical determinants of intake rate and diet selection in a two-species temperate grassland sward. **Functional Ecology**, Oxford, v. 8, p. 187–204, 1994.

PENNING, P. D. *et al.* Intake and behaviour responses by sheep to changes in sward characteristics under rotational grazing. **Grass and Forage Science**, Oxford, v. 49, n. 4, p. 476–486, 1994. Available in: http://doi.wiley.com/10.1111/j.1365-2494.1994.tb02025.x. Access in: 13 Aug. 2017.

PIANKA, E. R. Natural selection of optimal reproductive tactics. **American Zoologist**, Utica, v. 16, n. 4, p. 775–784, 1976.

POPPI, D. P.; HUGHES, T. P.; L'HUILLIER, P. J. Intake of pasture by grazing ruminants. Livestock feeding on pasture. **New Zealand Society of Animal Production**, Hamilton, v. 7, 1987. v. 7, p. 55–64, 1987.

PRACHE, S. Intake rate, intake per bite and time per bite of lactating ewes on vegetative and reproductive swards. **Applied Animal Behaviour Science**, Amsterdam, v. 52, n. 1/2, p. 53–64, 1997. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0168159196011392. Access in: 13 Aug. 2017.

PRACHE, S.; GORDON, I. J.; ROOK, A. J. Foraging behaviour and diet selection in domestic herbivores. **Annales de Zootechnie**, Les Ulis, v. 47, n. 5/6, p. 335–345, 1998. Available in: https://hal.archives-ouvertes.fr/hal-00889735/document. Access in: 13 Aug. 2017

PYKE, G. H.; PULLIAM, H. Ronald; CHARNOV, Eric L. Optimal foraging: a selective review of theory and tests. **The Quarterly Review of Biology**, Chicago, v. 52, n. 2, p. 137–154, 1977.

RAO, I. *et al.* LivestockPlus - The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics. **Tropical Grasslands - Forrajes Tropicales**, Cali, v. 3, n. 2, p. 59-82, 2015. Available in: http://www.tropicalgrasslands.info/index.php/tgft/article/view/262. Access in: 22 Sept. 2016.

REIS, R. A.; DA SILVA, S. C. Consumo de forragens. *In:* BERCHIELLI, T. T.; PIRES, A. V.; OLIVEIRA, S. G. (ed.). **Nutrição de ruminantes**. Jaboticabal: FUNEP, 2011. cap. 4, p. 83–109.

RHODES, I.; COLLINS, R. P. Canopy structure. *In:* DAVIES, A. *et al*. (org.). **Sward measurement handbook**. 2nd ed. Reading: British Grassland Society, 1993. p. 139–156.

ROOK, A. J.; HUCKLE, C. A.; PENNING, P. D. Effects of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. **Applied Animal Behaviour Science**, Amsterdam, v. 40, n. 2, p. 101–112, 1994. Available in: https://linkinghub.elsevier.com/retrieve/pii/0168159194900752. Access in: 13 Aug. 2017.

RUTTER, S. M.; CHAMPION, R. A.; PENNING, P. D. An automatic system to record foraging behaviour in free-ranging ruminants. **Applied Animal Behaviour Science**, Amsterdam, v. 54, n. 2/3, p. 185–195, 1997.

RUTTER, S. M. Graze: a program to analyze recordings of the jaw movements of ruminants. **Behavior Research Methods, Instruments, & Computers**, Austin, v. 32, n. 1, p. 86–92, 2000.

SAVIAN, J. V. *et al.* Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: implications for integrated crop–livestock system. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 190, p. 112–119, 2014. Available in: http://linkinghub.elsevier.com/retrieve/pii/S0167880914000887. Access in: 22 Sept. 2016.

SAVIAN, J. V. *et al.* Rotatinuous stocking : a grazing management innovation that has high potential to mitigate methane emissions by sheep. **Journal of Cleaner Production**, Amsterdam, v. 186, p. 602–608, 2018. Available in: http://linkinghub.elsevier.com/retrieve/pii/S0959652618308291. Access in: 18 Dec. 2018.

SBRISSIA, A. F. *et al.* Defoliation strategies in pastures submitted to intermittent stocking method: underlying mechanisms buffering forage accumulation over a range of grazing heights. **Crop Science**, Madison, v. 58, n. 2, p. 945-954, 2018. Available in: https://dl.sciencesocieties.org/publications/cs/abstracts/58/2/945. Access in: 18 Feb. 2019.

SCHMITT, D. *et al.* Pre-grazing heights do not change the morphological and chemical composition of pastures subjected to the same level of defoliation. *In:* REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 50., 2013, Campinas. **Anais** […]. Campinas: SBZ, 2013. p. 63.

SCHMITT, D. *et al.* Herbage intake by cattle in kikuyugrass pastures under intermittent stocking method. **Revista Ciência Agronômica**, Fortaleza, v. 50, n. 3, p. 493-501, 2019. Available in: http://ccarevista.ufc.br/seer/index.php/ccarevista/article/view/6034. Access in: 18 Feb. 2019.

SCHOENER, T. W. Theory of feeding strategies. **Annual Review of Ecology and Systematics**, Palo Alto, v. 2, n. 1, p. 369–404, 1971.

SEARLE, K. R.; SHIPLEY, Lisa A. The comparative feeding bahaviour of large browsing and grazing herbivores. *In:* GORDON, I. J.; PRINS, H. H. T. (org.). **The ecology of browsing and grazing**. Berlin: Springer, 2008. p. 117–148.

SILVA, S. C.; CARVALHO, P. C. F. Foraging behaviour and herbage intake in the favourable tropics/sub-tropics. *In*: MCGILLOWAY, D.A. (ed.). **Grassland:** a global resource. Wageningen: Wageningen Academic, 2005. p. 81–95.

SOLLENBERGER, L. E.; BURNS, J. C. Canopy characteristics, ingestive behaviour and herbage intake in cultivated tropical grasslands. *In:* INTERNATIONAL GRASSLAND CONGRESS, 19., 2001, São Pedro. **Anais** […]. São Pedro: Fealq, 2001. p. 321-327.

SOUZA FILHO, W. *et al.* Mitigation of enteric methane emissions through pasture management in integrated crop-livestock systems: trade-offs between animal performance and environmental impacts. **Journal of Cleaner Production**, Amsterdam, v. 213, p. 968–975, 2019. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0959652618339672. Access in: 22 July 2019.

STOBBS, T. H. The effect of plant structure on the intake of tropical pastures - II. Differences in sward structure, nutritive value, and bite size of animals grazing Setaria anceps and Chloris gayana at various stages of growth. **Australian Journal of Agricultural Research**, East Melbourne, v. 24, p. 821–829, 1973a.

STOBBS, T. H. The effect of plant structure on the intake of tropical pastures - I. Variation in the bite size of grazing cattle. **Australian Journal of Agricultural Research**, East Melbourne, v. 24, p. 809–819, 1973b.

TEAGUE, W. R. *et al.* The role of ruminants in reducing agriculture's carbon footprint in North America. **Journal of Soil and Water Conservation**, Ankeny, v. 71, n. 2, p. 156–164, 2016. Available in: http://www.jswconline.org/cgi/doi/10.2489/jswc.71.2.156. Access in: 18 Feb. 2019.

THOMPSON, A. J.; WEARY, D. M.; VON KEYSERLINGK, M. A. G. Technical note: Mining data from on-farm electronic equipment to identify the time dairy cows spend away from the pen. **Journal of Dairy Science**, Champaign, v. 100, n. 5, p. 3975–3982, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030217302357. Access in: 18 Jan. 2019.

ULYATT, M. J.; LASSEY, K. R. Methane emissions from pastoral systems: the situation in New Zealand. **Archivos Latinoamericanos de Producción Animal**, Mayaguez, v. 9, n. 1, p. 118–126, 2001.

UTSUMI, S. A. *et al.* Resource heterogeneity and foraging behaviour of cattle across spatial scales. **BMC Ecology**, London, v. 9, n. 9, p. [1-10], 2009. Available in: http://bmcecol.biomedcentral.com/articles/10.1186/1472-6785-9-9. Access in: 13 Aug. 2017.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca: Cornell University, 1994.

VISSCHER, D. R.; MERRILL, Evelyn H. Functional connectivity in ruminants: A generalized state-dependent modelling approach. **PLoS ONE**, San Francisco, v. 13, n. 6, [art.] e0199671, p. [1-22], 2018. Available in: https://dx.plos.org/10.1371/journal.pone.0199671. Access in: 18 Feb. 2019.

WADE, M. H.; CARVALHO, P. C. F. Defoliation patterns and herbage intake on pastures. **Grassland ecophysiology and grazing ecology**. Wallingford: CABI, 2000. p. 233–248.

WADE, M. H. **Factors affecting the availability of vegetative *Lolium perenne* to grazing dairy cows with special reference to sward characteristics, stocking rate and grazing method**. 1991. 53 f. Thèse (Doctorat) - Université de Rennes, Rennes, 1991.

WERLING, B. P. *et al.* Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, DC, v. 111, n. 4, p. 1652–1657, 2014. Available in: http://www.pnas.org/cgi/doi/10.1073/pnas.1309492111. Access in: 18 July 2016.

ZANINI, G. D. *et al.* Distribuição de colmo na estrutura vertical de pastos de capim Aruana e azevém anual submetidos a pastejo intermitente por ovinos. **Ciência Rural**, Santa Maria, v. 42, n. 5, p. 882–887, 2012.

APPENDIX 1

J. Dairy Sci. 101:Instructions 1–14

Journal of Dairy Science® Instructions to Authors: Style and Form[1]

Journal Policies and Procedures

The American Dairy Science Association® (ADSA®) invites scientists from the global community to submit papers for consideration to the *Journal of Dairy Science* (JDS). Authors need not be members of ADSA. These instructions detail editorial policies and style and form for publishing in JDS. We recommend that authors refer to these instructions, as well as the **Instructions to Authors: Policies**, during submission, peer review, acceptance, proof correction, and final publication phases.

Contact Information for Journal Staff

For information on the scientific content of the journal, contact the editor-in-chief, Dr. Matthew C. Lucy; phone: (573) 882-9897; e-mail: lucym@missouri.edu.

For assistance with Scholar One (Manuscript Central) and Manuscript Submission/Copyright forms, contact Shauna Miller, editorial assistant, Headquarters Office, 1800 S. Oak St., Suite 100, Champaign, IL 61820; phone (217) 239-3339; fax (217) 378-4083; shaunam@assochq.org.

For questions about manuscript preparation, journal style and form, and proofs, contact Louise Adam, lead technical editor, at loua@assochq.org or journals@assochq.org.

For other information, contact Susan Pollock, managing editor, Headquarters Office, American Dairy Science Association, 1800 S. Oak St., Suite 100, Champaign, IL 61820; phone (217) 356-7641; journals@assochq.org.

Aims and Scope

The *Journal of Dairy Science* publishes original research, invited review articles, and other scholarly work that relates to the production and processing of milk or milk products intended for human consumption. The journal is broadly divided into dairy foods and dairy production sections. The Resources and Environment section may include papers from either Dairy Foods or Dairy Production.

Dairy Foods Sections
- Bioactivity and Human Health
- Chemistry and Materials Science
- Microbiology and Safety
- Processing and Engineering
- Resources and Environment
- Sensory Analysis

Dairy Production Sections
- Animal Nutrition
- Genetics and Genomics
- Health, Behavior, and Well-being
- Management and Economics
- Physiology
- Resources and Environment

In addition to the above sections, interpretive applied summaries and recommendations may be submitted to the Dairy Industry Today section. Syntheses and applications from technical reports that contribute to solutions to problems in the dairy industry are especially solicited. Authors of reports for extension education of the nonscientist are encouraged to share their contributions with colleagues and to achieve wider circulation of their conclusions and recommendations through this section. In addition, papers that report on advances in teaching and outreach techniques are suitable for this section.

Types of Articles

Full-Length Research Papers. The majority of papers published in JDS are full-length research articles. The journal emphasizes the importance of high-quality scientific writing and clarity in presentation of the concepts and methods, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. The results of experiments published in the journal must be replicated, either by replicating treatments within experiments or by repeating experiments. Studies using commercial products should address a hypothesis-based question relevant to the biology or mechanism of action of the product.

In addition to full-length research papers, the following types of articles appear in the journal:

[1]Revised April 2018.

Hot Topics. Papers submitted for this section must report on a completed experiment testing a timely, original hypothesis of importance to an area of dairy science. The work may be preliminary in nature, but with sufficient data so that the hypothesis is clearly tested. Results may point to avenues for fruitful, in-depth analyses. Reports must contain an explicitly stated hypothesis and objectives, with sufficient detail in methodology for repetition of the work, as well as results, a brief discussion, and references. Total page limits for text, tables, figures, and references must be no more than 5 journal pages (approximately 10 manuscript pages minus space for tables and figures). Hot topics should not contain main headings or subheadings. The total number of tables and figures should be no more than 3; references should be minimal.

Hot topics papers will be given priority for publication. An effort will be made to notify authors of a decision within 1 mo of the date of receipt. Once accepted, the paper should be published within 3 mo.

Short Communications. Short communications are reports of limited experiments that test a timely, original hypothesis of importance to some area of dairy science. The manuscript should be no more than 5 journal pages in length and the total number of tables and figures should be no more than 3 (approximately 10 manuscript pages minus space for tables and figures); "*Short communication:*" should precede the title on the title page of the manuscript. Short communications should not contain main headings or subheadings. The manuscript may report negative results. Reports must contain a hypothesis, objectives, sufficient detail in methodology for repetition of the work, results with brief discussion, and references.

Technical Notes. Papers in this section should report a method that is useful to some aspect of dairy science. Submissions should include a brief justification for the technique, be it new or an improvement on a previously published technique. The report should state a hypothesis, include a full description of procedures that can be repeated by researchers, and include explicit controls to indicate sensitivity, precision, and accuracy of the technique. Technical notes should not contain main headings or subheadings.

If the technique is an improvement on an existing technique, sufficient comparison of the previous technique should be included, and mean and dispersion information must be included. The page limit is 5 journal pages (approximately 10 manuscript pages minus space for tables and figures). Use of tables, figures, and references should be minimized. Requests for longer technical notes may be made to the senior editor and editor-in-chief, but justification for a longer report will be required.

Invited Reviews. The journal publishes invited reviews in all scientific sections of the journal. Authors interested in writing a review should contact the invited reviews editor, Filippo Miglior (miglior@cdn.ca) with justification for the review. Ultimately, the invitation for submissions and overseeing of the peer-review process are the responsibility of the Invited Reviews Editor; authors should not submit an Invited Review without first receiving an invitation letter. The Invited Reviews Editor may also solicit reviews on topics of interest to the journal. The first 10 printed pages of an invited review are published at no cost to the author.

Symposium Reviews. The editor-in-chief invites selected topics from the ADSA annual meeting program to be published in the journal. Symposium reviews must be prepared according to JDS Style and Form and submitted to the appropriate scientific section (not as invited reviews). These papers will undergo the same review and editing process as other papers submitted to the journal. The first 10 printed pages of a symposium review are published at no cost to the author.

Graduate Student Literature Reviews. Graduate students may submit their literature reviews (as Grad Student Lit Review) to be evaluated by the journal for publication as review papers. Papers must be prepared according to JDS Style and Form, contain no more than 30 double-spaced pages and 75 references, and be submitted to the appropriate scientific section of the journal (not as invited reviews). Students submitting papers should note in the cover letter that the paper is a graduate student literature review, that they are competing for the Graduate Student Literature Review Award and indicate the category in which they are competing (PhD Production Division, MS Production Division, PhD Dairy Foods Division, MS Dairy Foods Division). A full description of the award can be found on the ADSA website (https://www.adsa.org/Membership/ADSAAwards.aspx).

Letters to the Editor. Short (300 words) letters to the editor on topics of concern to readers, including comment on publications with rebuttals from authors if needed, may be submitted to the editor-in-chief or to any of the editors. The letters should be titled and the title and running head should include "Letter to the editor." Letters will be published at the discretion of the editor-in-chief. Authors of letters are subject to the same copyright release requirements as other authors. Letters are published at no charge to the author(s).

MANUSCRIPT PREPARATION

Writing Style

Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in *Merriam-Webster's Collegiate Dictionary,* 11th ed., *Webster's Third International Dictionary,* or the *New Oxford American English Dictionary*, 3rd ed.

Today, most medical and scientific style manuals support the active over the passive voice. Use of the active voice results in lively, clear, and concise writing. Passive voice may still be appropriate in the Materials and Methods section, for example, where the actor is unimportant and the writer wishes to focus on the action or the recipient of the action. The active voice and first-person pronouns (I, we) should be used in the Results, Discussion, and Conclusions sections. For example, "we observed a difference…," "we concluded that …," or "Treatment A affected dry matter intake . . ." rather than "There was a difference . . .," "It was concluded that …," or Dry matter intake was affected by treatment A …"

For scientific conventions, authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers,* 8th ed., published by the Council of Science Editors in cooperation with University of Chicago Press (www.scientificstyleandformat.org/).

Preparing the Manuscript File

Manuscripts should be typed double-spaced (in Microsoft Word) with lines and pages numbered consecutively, using Times New Roman font at 12 points. Special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType from Design Science (www.dessci.com). Note that equations created using the Equation Builder in Microsoft Word 2007 (and later versions) may not be compatible with earlier versions of Word or other software used in our composition system. Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text). Failure to follow these instructions may result in immediate rejection of the manuscript.

Interpretive Summary

All authors of JDS papers should provide an interpretive summary (IS) of 100 words or less that has been written for nonspecialist readers. The summary should consist of a title, the first author's last name, and a summary, which must include a sentence or two to summarize the project's expected importance, or its economic, environmental, and/or social impact. Common abbreviations are permitted (those from the JDS Unrestricted list). The summary should appear at the top of the first page of the manuscript, before the running head and title. Interpretive summaries will be peer reviewed. At publication, interpretive summaries will appear in a section at the beginning of the journal. The summaries are intended for an audience who may not be familiar with work in the authors' area of expertise and for government or media researchers, and they will provide JDS readers with a brief overview of the research presented in each issue.

Headings

Major Headings. Major headings are centered, in all capitals and boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), CONCLUSIONS (optional), ACKNOWLEDGMENTS, APPENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are boldface and italic. The heading is not followed by punctuation. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Short Communications, Technical Notes, and Hot Topics do not use headings except for ACKNOWLEDGMENTS, REFERENCES, and APPENDIX.

Title Page

Across the top of the title page (first page), indicate a running head (abbreviated title) of no more than 45 characters. The running head is centered and uppercase. Dairy Industry Today and Hot Topic serve as the running heads for those respective article types. Short Communications, Technical Notes, Invited Reviews, and Letters to the Editor use a running head beginning with the appropriate designation (i.e., SHORT COMMUNICATION:) followed by a short title.

The title should be in boldface; the first letter of the article title (and subtitle, if present) and proper names are capitalized and the remainder of the title is lowercase. The title should contain words or phrases used for indexing the article.

Under the title, names of authors should be given in mixed case (e.g., T. E. Smith or Tom E. Smith). Institutional addresses are displayed below the author names; footnotes referring from author names to displayed addresses should be symbols **in the following order**: *, †, ‡, §, #, ‖, and ¶. The full name, mailing address, phone number, and e-mail address of the corresponding author should appear directly below the affiliation lines on the title page. The corresponding author will be identified by a numbered footnote and e-mail address below the accepted line on the first page of the published article (e.g., [1]Corresponding author: my.name@university.edu). Supplementary address information may be given in footnotes to the first page; use numerals for these footnotes. Acronyms (except USDA) for affiliations are discouraged unless the acronym is the official name. The state or provincial postal code abbreviation is not included between the city and postal code if the state or province is previously mentioned in the address (see example). Acceptable format is shown below:

J. E. Smith,* R. A. Jones,† and A. T. Peters‡
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Abstract. Abstracts should be limited to 2,500 keystrokes (i.e., characters plus spaces). The abstract should review important objectives, materials, results, conclusions, and applications as concisely as possible. The abstract disseminates scientific information through abstracting journals and is a convenience for readers. Open the abstract with objectives and make the abstract intelligible without reference to the manuscript. Use complete sentences and standard terms. Limit the use of abbreviations in the Abstract. Refer to the list on the inside front cover of JDS or Appendices 1 and 2 of this document for those terms that should be defined in the abstract. If a term is used fewer than 3 times in the abstract, it should be spelled out at each use.

Minimize the amount of data in the abstract and exclude statements of statistical probability (e.g., $P < 0.05$). Exclude references to other work because the abstracts will appear online and in indexing services without the accompanying reference list.

Key Words. After the abstract, list 2 to 5 key words or phrases; they should be typed in lowercase letters and separated by commas. Key words should be singular (e.g., "dairy cow" not "dairy cows").

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript, and in each table and figure in which they are used. Author-derived abbreviations will be shown in bold type at first use in the body of the manuscript. Refer to the "Miscellaneous Usage Notes" on page 10 for more information on abbreviations.

Body of the Paper

The body of the paper should contain an introduction to the problem (questions, objectives, reasons for research, and related literature); materials, methods, experimental design, and procedures; and results, discussion, conclusions, and applications.

The introduction should concisely describe the rationale for conducting the study, background, objectives, and hypotheses to be tested. The introduction should be no longer than 4,000 characters (words and spaces).

Results and Discussion may be combined into a single section. If not, the Results section should not contain discussion of previously published work. Results and references to tables and figures already described in the results section should not be repeated in the Discussion section. The conclusions section (optional) should consist of one brief paragraph summarizing only the main findings of the study. As such, it should not contain references to other works.

Appendix

A technical appendix may follow the References section. The appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The appendix will not be a repository for raw data.

References

List only pertinent references. No more than 3 references should be needed to support a specific concept. Research papers and reviews should cite a reasonable number of references. Abstracts and articles from non-peer-reviewed magazines and proceedings should be cited sparingly. Citation of abstracts published more than 3 yr ago is strongly discouraged.

Citations in Text. In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993), with citations listed chronologically (i.e., oldest first) and then alphabetically within a year. Where there are more than 2 authors, the first author's name is followed by the abbreviation "et al." in text (but all authors should be listed in the Reference section). Work that has not been accepted for publication should be listed in the text as follows: "J. E. Jones (institution, city, and state, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data (including papers under review) must not be included in the references section.

References Section. To be listed in the references section, papers must be published or accepted for publication. Manuscripts submitted for publication but not yet accepted can be cited as "unpublished data" in the text. In the references section, references are listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year should be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the reference section. Journals should be abbreviated according to the conventional ISO abbreviations used by PubMed (https://www.ncbi.nlm.nih.gov/nlmcatalog/journals). One-word journal names (e.g., Theriogenology) are not abbreviated.

For journal articles, include all authors (do not use "et al."), year, article title (lowercased except for first word and proper nouns), abbreviated journal name, volume, page range, and digital object identifier (DOI). Inclusive page numbers (or article identifiers) must be provided, and DOI should be given whenever possible, with the prefix "https://doi.org/".

For book references, include authors, year, chapter or section title, page range, book title, edition, book editors (if applicable), and publisher name and location.

For conference proceedings, include authors, year, abstract title, page number or abstract number, proceedings title, location of meeting, and name and location of proceedings publisher. For abstracts presented at ADSA or joint annual meetings, cite as a journal article but include the journal supplement number and the page of the supplement on which the abstract appeared. Include "(Abstr.)" at the end of the citation.

For patents, provide names of inventors, year, title, name of assignee, and US or other patent number.

For websites, provide authors (or organization name), year, page title, date accessed (in month, day, year format), and URL.

For theses, provide author, year, title, thesis type (PhD, MS, DVM), department name, and university name and location.

Sample references are given below.

Journals

Buch, L. H., A. C. Sorensen, J. Lassen, P. Berg, J.-A. Eriksson, J. H. Jakobsen, and M. K. Sorensen. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. J. Dairy Sci. 94:1540–1551. https://doi.org/10.3168/jds.2010-3137.

Chapinal, N., A. M. de Passille, D. M. Weary, M. A. Hayes, B. J., P. J. Bowman, A. C. Chamberlain, K. Savin, C. P. van Tassell, T. S. Sonstegard, and M. E. Goddard. 2009. A validated genome-wide association study to breed cattle adapted to an environment altered by climate change. PLoS ONE 4:e6676.

de Vries, M. J., and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83:62–69.

Jenkins, T. C., E. Block, and P. H. Morris. 2011. Potassium reduces the accumulation of *trans*-10, *cis*-12 conjugated linoleic acid and trans-18:1 in continuous cultures of mixed ruminal microorganisms regardless of dietary fat level. J. Dairy Sci. 94(E-Suppl. 1):509. (Abstr.)

VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91:4414–4423.

Books

AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International, Gaithersburg, MD.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Lengemann, F. W., R. A. Wentworth, and C. L. Comar. 1974. Physiological and biochemical aspects of the accumulation of contaminant radionuclides in milk. Pages 159–170 in Lactation: A Comprehensive Treatise. Nutrition and Biochemistry of Milk/Maintenance. Vol. 3. B. L. Larson and V. R. Smith, ed. Academic Press, London, UK.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Conferences

Barbano, D. M. 1996. Mozzarella cheese yield: Factors to consider. Page 29 in Proc. Wisconsin Cheese Makers Mtg., Madison. Ctr. Dairy Res., Univ. Wisconsin, Madison.

National Mastitis Council. 1995. Summary of peer-reviewed publications on efficacy of premilking and postmilking teat disinfections published since 1980. Pages 82–92 in Natl. Mastitis Counc. Reg. Mtg. Proc., Harrisburg, PA. Natl. Mastitis Counc., Inc., Madison, WI.

Table 1. Effect of garlic oil, diallyl disulfide, allyl mercaptan, monensin, and lovastatin on a 17-h in vitro batch culture rumen microbial fermentation trial

	Treatment[1]						
Item	Control	GAR300	DAD300	ALM300	MON	LOV	SEM
pH	6.6	6.7	6.7	6.6	6.6	6.6	0.01
Apparent disappearance of DM, %	61.0[a]	50.7[b]	51.2[b]	60.4[a]	53.9[b]	62.4[a]	1.11
Fiber digestibility							
NDF, %	56.8[a]	44.3[b]	41.4[b]	55.9[a]	39.3[b]	60.0[a]	1.73
ADF, %	53.7[a]	36.8[b]	34.9[b]	52.5[a]	30.7[b]	57.0[a]	2.03
Gas, µmol	4,674.8[a]	3,756.9[cd]	3,359.7[d]	4,388.2[ab]	4,009.6[bc]	4,673.1[a]	123.34
CH_4, µmol	417.3[a]	110.1[d]	131.3[d]	335.9[b]	241.7[c]	396.3[a]	21.56
Total VFA, m*M*	49.3[a]	39.7[c]	38.8[c]	45.4[b]	45.7[ab]	48.4[ab]	1.17
Individual, mol/100 mol							
Acetate	61.2[a]	54.3[d]	53.9[d]	58.3[b]	56.4[c]	61.1[a]	0.53
Propionate	22.6[d]	25.8[c]	28.3[b]	22.8[d]	34.2[a]	22.8[d]	0.78
Butyrate	12.5[c]	16.5[a]	14.0[bc]	15.0[ab]	6.6[d]	12.4[c]	0.60
Branched-chain VFA	2.0[a]	1.7[b]	1.7[b]	2.0[a]	1.4[c]	2.0[a]	0.10
C2:C3	2.7[a]	2.1[b]	1.9[c]	2.5[a]	1.6[d]	2.7[a]	0.07
CH_4 (µmol):VFA (µmol)	0.20[a]	0.05[d]	0.07[cd]	0.15[ab]	0.10[bcd]	0.17[ab]	0.00
N-NH_3, mg/100 mL	16.7[ab]	16.6[bc]	19.0[a]	17.2[ab]	14.4[c]	16.4[bc]	1.10

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: GAR300 = 300 mg/L *Allium sativa* (garlic oil); DAD300 = 300 mg/L diallyl disulfide; ALM300 = 300 mg/L allyl mercaptan; MON = 12.5 mg/L monensin; LOV = 5 mg/L lovastatin.

Other

Biernoth, G., and W. Merk, inventors. 1985. Fractionation of milk fat using a liquified gas or a gas in the supercritical state. Unilever NV-PLC, assignee. US Pat. No. 4,504,503.

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Federaton of Animal Science Societies, Champaign, IL.

Kelly, M. G. 1977. Genetic parameters of growth in purebred and crossbred dairy cattle. MS Thesis. North Carolina State Univ., Raleigh.

US Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137–10151.

Tables

The use of tables should be minimized; however, tables may be the most effective way to organize data. When used, tables should be self-explanatory and understandable without excessive reference to the text. Table 1 in this document may be used as an example.

Tables must be prepared using the table feature in Microsoft Word; tables prepared in other programs (e.g., Excel) or by using spaces, tabs, and hard returns will not convert accurately and errors can result. When possible, tables should be organized to fit across the page without running landscape. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns may create layout problems).

Place the table number and title on the same line above the table (as shown in sample table). The table title should describe concisely the data shown; it does not require an ending period. Do not use vertical rules and use few horizontal rules. Bold and italic typefaces should not be used in tables, but when it is necessary to do so, such use must be defined in a footnote. Limit the data field to the minimum needed for meaningful comparison within the accuracy of the methods.

For each table, define author-derived abbreviations in parentheses or in numbered footnotes. Abbreviations should conform to journal style and be consistent with those used in the text.

For differences among means within a row or column, superscript letters should be used as appropriate sequentially (e.g., a, ab, b, c, cd) consistently from largest to smallest means and defined in the footnote. Informational footnotes should be numbered and each footnote should begin a new line (see sample table). Probability may be indicated in a separate footnote following any informational footnotes thus: †$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the last name of the first author and figure number. Figure captions should be typed (double spaced) on a separate page.

- ***Figure size.*** Prepare figures at final size for publication. Figures should be prepared to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).

• ***Font size.*** Ensure that all type within the figure and axis labels is readable at final publication size. A minimum type size of 8 points (after reduction to publication size) should be used. The font size should be proportional to the overall size of the figure (within a range of 8 to 12 points at final publication size).

• ***Fonts.*** For best readability, use Helvetica, Times New Roman, Arial, and the symbols palette within those fonts only.

• ***Line weight.*** For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of gray lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.

• ***Axis labels.*** Each axis should have a descriptor and a unit. Units may be separated from the descriptor by a comma or parentheses.

• ***Shading and fill patterns.*** For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print. Complex patterns and 3-dimensional effects reproduce poorly. Remove unnecessary backgrounds and gridlines from graphs.

• ***Symbols.*** Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, △, ▽, ★, ☆, ◇, ◆, +, or ×. Symbols should be defined in the figure caption or in a key on the figure (but not both).

• ***File formats.*** Figures can be submitted in PDF, EPS, TIFF, and JPEG formats or pasted into Microsoft Word.

• ***Grayscale figures.*** If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in grayscale.

• ***Color figures.*** If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).

• ***Resolution.*** Minimum resolution is 600 dpi for grayscale and color figures, and 1,200 dpi for line art. Submitting figures that do not meet these requirements may delay publication of your article.

• ***Photomicrographs.*** Photomicrographs must have their unmagnified size designated with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.

• ***Captions.*** The caption should provide sufficient information that the figure can be understood without excessive reference to the text. All author-derived abbreviations and symbols used in the figure should be defined in the caption.

• ***General tips.*** Do not use three-dimensional bar charts unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily readable at final publication size.

Color Charge. The cost to publish each color figure in the print journal is $650; a surcharge for offprints will also be assessed. At the time of submission on Manuscript Central, authors will be asked to approve color charges for figures that they wish to have published in color in the print journal. Color versions of figures can be included in the online PDF and full-text article at no charge. Note that online color figures will be available in the final published version of the article (not in the galley proof for Articles in Press version).

Statistical Analysis

Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.

Experimental Unit. The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen or the paddock is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.

Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measurement should be included as a covariate.

A parameter [mean (μ), variance (σ^2)], which defines or describes a population, is estimated by a statistic ($\bar{x}$, s^2). The term *parameter* is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

Experimental Design. Standard designs are adequately described by name and size (e.g., "a randomized complete block design with 6 treatments in 5 blocks"). For a factorial set of treatments, an adequate description might be as follows: "Tryptophan at 0.05 or 0.10% of the diet and niacin at 5, 10, or 20 mg/kg of diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks, each block consisting of littermates." Note that a factorial arrangement is not a design; the term "design" refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

Variability. Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not "statistically significant" is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They are also useful to the reader as measures of efficiency of experimental techniques. **A value attached by "±" to a number implies that the second value is its standard error (not its standard deviation) unless otherwise specified.** Adequate reporting may require only (1) the number of observations, (2) arithmetic treatment means, and (3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated-measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is appropriate to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple comparison tests should be used only to compare means of treatments that are unstructured or not related. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. Means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

Significance. The terms *significant* and *highly significant* traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P-value is preferred to the use of these terms. For example, use "... we observed a difference ($P < 0.05$) between control and treated samples" rather than "...we observed a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.03$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (alpha) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 2 or 3 places after the decimal (2 significant digits are usually sufficient). Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of Type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a beta error, not an alpha error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. In most cases, 2 or 3 significant digits (not decimal places) are sufficient.

Nomenclature: Genes and Proteins

The journal recommends using internationally accepted symbols for genes and proteins; such symbols may be used without definition. Symbols for specific genes and proteins can be obtained by querying the gene database of PubMed (https://www.ncbi.nlm.nih.gov/pubmed). Nomenclature rules for humans, nonhuman primates, and livestock are available at https://www.genenames.org, and rules for mice and rats are at http://www.informatics.jax.org/mgihome//nomen/strains.shtml. Gene symbols should be shown in italics (e.g., *SERPINA14*) and proteins in roman text (e.g., SERPINA14). Gene symbols are generally shown in all uppercase letters (e.g., *LHB*), except in mice and rats, where only the first letter is capitalized (e.g., *Lhb*).

Nomenclature: Single Nucleotide Polymorphisms

The increasing number of SNP association studies and the improvements in bovine genome annotation require a standardized SNP nomenclature for unequivocal and correct SNP identification. Additionally, information regarding the SNP investigated should be easily accessible in a publicly available database. Therefore, all relevant SNP included in a study should be listed with their unique RefSNP (rs) or submitted SNP (ss) number (if rs number is not yet available) as indicated in the public domain NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/snp). If the SNP investigated do not yet have an entry in the NCBI dbSNP database, the authors of the manuscript are responsible for submitting all the required information to NCBI (see https://www.ncbi.nlm.nih.gov/projects/SNP/) for depositing the SNP into the database and obtaining a unique ss number for the SNP. In the text of the manuscript, use the rs/ss number of the SNP or an alternative standardized nomenclature.

Nomenclature: Microorganisms.

All microorganisms must be named by genus and species. The name of the genus must appear in full the first time that the microorganism is cited in the abstract, in the body of the paper, and in each table and figure legend. Thereafter, the genus can be abbreviated by its first initial unless it will be confused with other microorganisms cited in the paper, in which case each genus should be abbreviated to use enough letters to avoid confusion (e.g., *Strep.* vs. *Staph.*). The formal, binomial names of all microorganisms should be in italics. Specific strain designations and numbers should be used when appropriate.

For microorganisms that are genetic variants of a parent strain, the genotypic and phenotypic properties should be cited according to the procedures described by Demerec et al. (1966) in *Genetics* 54:61–76 (www.genetics.org/content/54/1/61.long). Phenotypes should be identified by 3 letters; the first is capitalized. Genotypes should be identified by 3 lowercase italic letters. Superscript plus (+) signs are used to refer to a wild-type. The serial isolation number is placed after the locus symbol for mutations. The delta symbol is used to indicate deletions. Nomenclature for bacterial plasmids should be cited according to Novick et al. (1976) in *Bacteriological Reviews* 40:168–189 (mmbr.asm.org/content/40/1/168.full.pdf+html).

Nomenclature: Enzymes

First mention of an enzyme within a manuscript should include the Enzyme Commission (EC) number (http://www.chem.qmul.ac.uk/iubmb/enzyme/).

In Vitro Antimicrobial Susceptibility Tests

Authors should avoid the use of the term “antibiotic” when referring to a specific agent unless that agent is naturally occurring and unmodified (e.g., penicillin). The broader term “antimicrobial agent” is preferred because it includes naturally produced agents, semisynthetic agents, and totally synthetic agents. The term “susceptibility” should be used instead of “sensitivity.” Authors unfamiliar with antimicrobial susceptibility testing should obtain CLSI (formerly NCCLS) document M31 (Clinical Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, PA 19087-1898) for specific information regarding antimicrobial susceptibility testing of veterinary pathogens. CLSI or NCCLS equivalent methods for antimicrobial susceptibility testing available outside the US are also acceptable.

Two methods are generally used to generate antimicrobial susceptibility data: the agar disk diffusion (ADD) method and the minimum inhibitory concentration (MIC) method. The use of the term “Kirby-Bauer” to refer to the ADD method is incorrect and should be avoided. The correct citation for this method is the “disk diffusion method of Bauer et al.” The ADD method is a qualitative method and results should be reported as susceptible, intermediate, or resistant (SIR). If zone of inhibition diameters are reported, these should be reported in millimeters.

The MIC method is quantitative and results should be reported in micrograms per milliliter (μg/mL). The minimum summary statistics for reporting MIC results from multiple strains of an organism are the MIC_{50}, the MIC_{90}, and the range. The MIC_{50} and MIC_{90} represent the concentrations required to inhibit 50 and 90% of the strains, respectively. The MIC_{50} and MIC_{90} reported should be the actual concentrations tested, not values calculated from the actual data obtained. When <10 isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested. If more than a single drug is studied, insert a column labeled "test agent" between the columns listing the organisms and the columns containing the numerical data, and record data for each agent in the same isolate order. In addition, the percentage of strains categorized as susceptible, intermediate, or resistant may be reported. If only one of these categories is to be reported, the percent susceptible value is preferred. If the percentage of resistant isolates is to be reported for an agent, it should include isolates categorized as intermediate.

The percentage of strains susceptible or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by CLSI. Given the paucity of approved breakpoints for mastitis pathogens, authors may use breakpoints from other species (e.g., human breakpoints for ampicillin or canine breakpoints for enrofloxacin). However, authors must clearly state that the breakpoints are not approved for mastitis pathogens. Moreover, authors cannot assign breakpoints or use breakpoints from related antibiotics (except for class testing purposes) or breakpoints developed for other methods.

Authors must indicate that the appropriate quality control tests were performed. Information regarding the frequency of testing and the specific strains tested should be provided. The frequency of quality control testing and organisms tested should conform to the recommendations in the CLSI standard (document M31) or equivalent. A single statement in the manuscript indicating that the results obtained for the quality control documents were within published ranges is acceptable. However, authors may be requested to provide the quality control information during the manuscript review cycle.

Sensory Data

Sensory data should comply with "*Invited Review:* Sensory Analysis of Dairy Foods," *Journal of Dairy Science* 90:4925–4937 (https://doi.org/10.3168/jds.2007-0332).

Miscellaneous Usage Notes

Abbreviations. Abbreviations should not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by their abbreviation (e.g., IgG, CD). Abbreviations may be used in heads within the paper if they have been first defined within the text. The inside front cover of every issue of the journal lists abbreviations that can be used without definition (see also Appendices 1 and 2). Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. Generally, author-derived abbreviations should be in all capital letters. Terms used fewer than 3 times after first use must be spelled out in full rather than abbreviated. Do not use capitalized whole words (e.g., CORN) as treatment abbreviations, or single-letter abbreviations that could be confused with chemical elements (e.g., P, C, S). All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter.

The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural forms of abbreviations do not require "s." Chemical symbols and 1-letter and 3-letter abbreviations for amino acids do not need definition. Bacterial genus names are abbreviated according to the guidelines recommended in *Scientific Style and Format* (8th ed.). Units of measure, except those in the standard JDS abbreviation list, should be abbreviated according to standard SI usage and do not need to be defined. See "Appendix 2" on page 14 for a list of commonly used terms.

Foreign and Latin Words and Phrases. Non-English words in common usage (i.e., given in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, ad libitum, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized; in addition, all taxa of bacteria should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions.

Capitalization. Breed and variety names are to be capitalized (e.g., Holstein, Danish Red). Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used. Proper nouns should be capitalized.

Numbers and Units. The *Journal of Dairy Science* uses the Council of Science Editors' number style given in the eighth edition of *Scientific Style and Format*.

Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits; a comma separator must be used in numbers greater than 999 (e.g., 2,478). Measures must be in the metric (SI) system; however, US equivalents may be given in parentheses. Units of measure not associated with a numeric value should be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use.

Monetary values should be presented in US dollars or euros. If other currencies are used, a conversion to US dollars should be given at first use (e.g., "1 NZ$ = 0.73 US$).

General Usage. Note that "and/or" is not permitted; choose the more appropriate meaning or use "x or y or both."

Use the slant line only when it means "per" with numbered units of measure or "divided by" in equations. Use only one slant line in a given expression: e.g., g/cow per day. The slant line may not be used to indicate ratios or mixtures.

Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <) when these signs occur between 2 items.

Items in a series should be separated by commas: a, b, and c.

Commercial Products. The use of names of commercial products should be minimized. When a commercial product is being tested as part of the experiment, the manufacturer and location should be given parenthetically at first mention in text, tables, and figures, but, when possible, the generic name should be used thereafter. Only generic names should be used in article titles. Trademark symbols and registration marks should not be used and will be removed.

Studies using commercial products should address a hypothesis-based question relevant to the biology or mechanism of action of the product. Avoid describing a method as "per manufacturer's instructions." If the product goes out of production, the method will be lost to readers. Many products come with literature references; try to use references that can be found by other researchers to describe a method being used.

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APPENDIX 2

ANIMAL FEED SCIENCE AND TECHNOLOGY

An International Scientific Journal Covering Research on Animal Nutrition, Feeding and Technology

AUTHOR INFORMATION PACK

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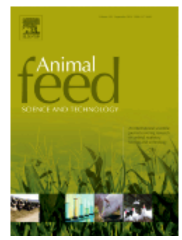

ISSN: 0377-8401

DESCRIPTION

Animal Feed Science and Technology is a unique journal publishing scientific papers of international interest focusing on **animal feeds** and their **feeding**.

Papers describing research on feed for ruminants and non-ruminants, including **poultry**, **horses**, **companion animals** and **aquatic animals**, are welcome.

The journal covers the following areas:
Nutritive value of feeds (e.g., assessment, improvement) Methods of **conserving** and **processing** feeds that affect their nutritional value **Agronomic** and **climatic** factors influencing the nutritive value of feeds **Utilization** of feeds and the improvement of such Metabolic, production, reproduction and **health responses**, as well as potential environmental impacts, of diet inputs and feed technologies (e.g., feeds, feed additives, feed components, mycotoxins) Mathematical models relating directly to **animal-feed interactions** Analytical and experimental methods for **feed evaluation** Environmental impacts of feed technologies in animal production

The journal does not encourage papers with emphasis on animal products, molecular biology, genetics or management, or the regulatory or legal aspects of feeds as well as animal production studies with a focus on animal nutrition that do not have a direct link to a feed or feed technology.

Manuscripts must be prepared in accordance with the journal's Guide for Authors.
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Editorial on terminology and analytical methods (Anim. Feed Sci. Technol. 118 (2005) 181-186)
Editorial on experimental design and statistical criteria (Anim. Feed Sci. Technol. 129 (2006) 1-11)
Editorial on general suggestions and guidelines (Anim. Feed Sci. Technol. 134 (2007) 181-188)
Editors comments on plagiarism (Anim. Feed Sci. Technol. 154 (2009) 292-293)
Editorial on review techniques and responding on editorial comments (Anim. Feed Sci. Technol. 155 (2010) 81-85)
Editorial on use of replicates in statistical analyses in papers submitted for publication in Animal Feed Science and Technology (Anim. Feed Sci. Technol. 171 (2012) 1-5)

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3. Short Communications
4. Book Reviews

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Professor G. Flachowsky
Federal Research Centre of Agriculture
Institute of Animal Nutrition
Bundesallee 50
D-38116 Braunschweig
Germany

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The following definitions should be used, as appropriate:

a. aNDFom-NDF assayed with a heat stable amylase and expressed exclusive of residual ash.
b. NDFom-NDF not assayed with a heat stable amylase and expressed exclusive of residual ash.
c. aNDF-NDF assayed with a heat stable amylase and expressed inclusive of residual ash.
d. NDF-NDF assayed without a heat stable amylase and expressed inclusive of residual ash.
e. ADFom-ADF expressed exclusive of residual ash.
f. ADF-ADF expressed inclusive of residual ash.
g. Lignin (sa)-Lignin determined by solubilization of cellulose with sulphuric acid.
h. Lignin (pm)-Lignin determined by oxidation of lignin with permanganate.

While expressions of NDF and ADF inclusive of residual ash will continue to be acceptable (i.e., the terms aNDF, NDF and ADF above), the Editors-in-Chief highly recommend reporting all fibre values, including digestibilities, on an OM basis. Silica is partially soluble in ND, is quantitatively recovered in AD, and so may contribute to the 'fibre' values and to subsequent digestibility coefficients.

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AUTHOR BIOGRAPHY

Alejandra Marín was born in 1985 in Medellín, Colombia. During her formative years in a high school, she developed an interest in the animal sciences, biology, nature, and environment which led her to pursue a career in animal science. Alejandra was admitted to the degree program of Animal Science at the National University of Colombia in 2005. In the early stages of her career, she discovered her interest in grazing ruminants and animal nutrition and began her research career as a young scientist and research assistant at the laboratory of Ruminal Biotechnology (BIORUM) of National University of Colombia. She obtained a BSc in Animal Science (2010) and an MSc in Agricultural Sciences at the National University of Colombia (2014). During her master's degree she received a scholarship called "young researchers" granted by Colciencias. She did an internship at University of Florida, North Florida Research and Education Center (NFREC), Marianna, Florida. In NFREC, she obtained training in SF_6 technique under supervision of Dr. Nicolas Dilorenzo. In August 2016, Alejandra started her PhD in Animal Science under the supervision of Dr. Paulo Carvalho and Dr. Jerome Bindelle, in a double degree program between the National University of Colombia and Federal University of Rio Grande do Sul (UFRGS). During her Ph.D., she was actively involved in the Grazing Ecology Research Group (GPEP) at the UFRGS in Brazil. In GPEP she worked on the plant-animal interface, in a grazing management concept oriented to optimize short term intake rate. She also worked on climate change, grazing management strategies for mitigating enteric methane emissions, and adaptation options for sustainable livestock production. In 2017, the Climate Food and Farming Network (CLIFF) and CGIAR centers provided Alejandra with a grant to support her PhD research. She was a visiting researcher at the International Center for Tropical Agriculture (CIAT). She attends the 23rd Conference of the Parties (COP23) to UNFCCC, held in Born, Germany. She got an academic scholarship of CIHEAM/IAM Zaragoza to attend the advanced course, Livestock and climate change: assessment of emissions, mitigation options and adaptation strategies Zaragoza (Spain), held in February 2019. In December 2019, she defended her Ph.D. thesis at the National University of Colombia in Medellín.