

**UNIVERSIDAD DE LA REPÚBLICA  
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**EFEECTO DE LA ALTURA POSTPASTOREO EN EL  
COMPORTAMIENTO INGESTIVO, CONSUMO DE MATERIA  
SECA Y PRODUCCIÓN DE LECHE DE VACAS HOLANDO**

**por**

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Dedico este trabajo a quienes sembraron en mí, el amor por la producción animal...



...Mis abuelos (*in memoriam*).

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## RESUMEN

Se realizó un experimento para evaluar el efecto de tres intensidades de defoliación en una pastura a base de *Festuca arundinacea* sobre el comportamiento, el consumo de materia seca y la producción de leche de vacas lecheras. Se aplicaron tres alturas post-pastoreo: 15 (TL), 12 (TM) y 9 (TC) cm. El criterio para comenzar el pastoreo fue cuando la pastura alcanzó el estado fisiológico de tres hojas. Treinta y seis vacas Holando paridas en otoño fueron bloqueadas por paridad ( $2,6 \pm 0,8$ ), peso vivo ( $618 \pm 48$  kg) y condición corporal ( $2,8 \pm 0,2$ ) y aleatorizadas en los tratamientos. Se accedió al pasto de 8:00 a 14:00 y de 17:00 a 3:00. Los datos de tiempos de comportamiento de pastoreo y rumia fueron recolectados con registradores automáticos. El número de estaciones alimentarias (EA) y parches visitados y los pasos de búsqueda fueron registrados. El consumo de materia seca se estimó por el método de alcanos. La producción de leche se midió diariamente. Las variables de respuesta se analizaron con PROC GLIMMIX de SAS. El tiempo de pastoreo no fue afectado por el tratamiento ( $508 \pm 15$  min). La reducción de la altura post-pastoreo aumentó la duración de la primera sesión de pastoreo en la mañana y en la tarde. El número de sesiones de pastoreo fue mayor en TL que en TM sin diferenciarse de TC. El número de EA visitadas fue mayor en TL que en TC y ambos no difirieron de TM. El consumo de materia seca fue mayor en TL y TM que en TC (17,8 vs. 14,7 kg de MS). La producción de leche durante el período de ocupación fue de 13,1, 16,2 y 18,7 kg/día para TC, TM y TL, respectivamente. El contenido de sólidos en leche no difirió entre tratamientos. La menor intensidad de defoliación logró un comportamiento selectivo que resultó en un alto consumo de materia seca digestible y, en consecuencia, en la producción de leche. Las vacas en TM adoptaron un mecanismo de compensación que permitió lograr el mismo consumo de materia seca que las vacas en TL, pero menor producción de leche. Las vacas en TC no pudieron compensar la tasa de consumo más baja, impuesta por la altura y la estructura del pasto, aunque fueron menos selectivas, probablemente con ese propósito.

**Palabras clave:** interacción planta-animal, selectividad, cosecha de forraje

# **EFFECT OF POST-GRAZING SWARD HEIGHT ON INGESTIVE BEHAVIOUR, DRY MATTER INTAKE AND MILK PRODUCTION OF HOLSTEIN DAIRY COWS**

## **SUMMARY**

An experiment was carried to evaluate the effect of three defoliation intensities on a *Festuca arundinacea* pasture on the behavior, dry matter intake and milk production of dairy cows. Three post-grazing sward height were tested: 15 (TL), 12 (TM) and 9 (TC) cm. Thirty-six autumn-calving Holstein cows were blocked by parity ( $2.6 \pm 0.8$ ), body weight ( $618 \pm 48$  kg) and body condition score ( $2.8 \pm 0.2$ ), and randomized to the treatments. Grazing method was rotational and the pasture accessed from 0800 to 1400 h and 1700 to 0300 h. The grazing and ruminating behavior times data were collected with automatic recorders. The number of feeding stations and patches visited and search steps were recorded. Dry matter intake was estimated by alkane method. Response variables were analyzed with PROC GLIMMIX of SAS and mean values were declared different when Tukey test  $<0.05$ . Daily grazing time averaged  $508 \pm 15$  min. The reduction of post-grazing sward height increased the length of the first grazing session in the morning and in the afternoon. The number of grazing sessions was greater on TL than on TM, with no difference in TC. The number of feeding stations visited was greater on TL than on TC, and neither of them differed from TM. Dry matter intake was greater on TL and TM than on TC (17.8 vs. 14.7 kg DM). Milk production during the paddock occupation period was 13.1, 16.2 and 18.7 kg/day for TC, TM and TL, respectively. The milk concentration did not differ between treatments. The cows on the lower defoliation intensity achieved a selective behavior resulting in high digestible dry matter intake and consequently higher milk production. The cows on TM adopted a compensation mechanism which allowed them to achieve the same dry matter intake than cows on TL, but lower milk production. The height and structure of the pasture on TC imposed the cows for a lower intake rate, although they were less selective, probably trying to compensate the low ingestion.

**Keywords:** animal-plant interaction, selectivity, forage harvest



## **1. INTRODUCCIÓN**

La lechería uruguaya exporta uno 70% de la leche producida, representando un 10% de las exportaciones del país ocupando el cuarto lugar en orden de importancia nacional (DIEA, 2018). El énfasis exportador exige del sector lechero que la leche sea producida con alta calidad y a bajo costo, ya que está frente a un mercado altamente competitivo en el que se definen los precios según la oferta y demanda mundial del producto y las políticas proteccionistas de los diferentes bloques regionales y/o países.

En Uruguay, los sistemas de producción de leche se han intensificado en base a aumentos de carga y de productividad individual, siendo los sistemas más intensos, también los que cosechan más forraje por hectárea (Chilibroste y Battezzore, 2014; 2019). A su vez, se encontró una asociación positiva entre cosecha directa de forraje y margen de alimentación (Chilibroste y Battezzore, 2014; 2019), por lo que, sistemas de base pastoril son los que han demostrado mejor estabilidad frente a los diferentes escenarios de precios y clima a los cuales han estado expuesto los sistemas de producción.

El consumo de pasto por animales en pastoreo es afectado por la altura y masa de forraje disponible para los animales (Chilibroste et al., 2004), lo que influye significativamente en su performance productiva (Chilibroste et al., 2012) y reproductiva (Meikle et al., 2013). Las estrategias de pastoreo tienen efectos directos sobre el crecimiento de la pastura, donde bajas alturas remanentes (Soca et al., 2009) o bien sin control (Zibil et al., 2016) repercuten de manera desfavorable en la tasa de rebrote de la pastura y en la producción total de forraje, amenazando la base pastoril y la productividad de los sistemas lecheros uruguayos, ya que estos, en su gran mayoría, no han controlado, sobretodo, la altura remanente post pastoreo (Zibil et al., 2016).

La comprensión del proceso de pastoreo es fundamental para lograr mayor eficiencia en el uso de los recursos en la producción de leche. Considerando que existe una brecha de mejora del consumo de materia seca en la mayoría de los predios productores de leche (Fariña y Chilibroste, 2019) es conveniente el estudio de

estrategias de pastoreo, mediante el control de altura de entrada y salida, que permitan incrementar el consumo de forraje.

### **1.1. COMPORTAMIENTO Y CONSUMO DE MATERIA SECA BAJO PASTOREO**

El estudio del comportamiento de rumiantes abarca tres actividades principales como el consumo o pastoreo, rumia y descanso, que en conjunto representan prácticamente la totalidad de las actividades desarrolladas por el animal a lo largo del día. Entendiéndose por consumo los movimientos de prehensión, masticación y deglución del alimento, mientras que pastoreo comprende pequeñas caminatas de búsqueda, períodos cortos (<4 min) de inactividad en sesiones largas de pastoreo y el consumo, propiamente dicho (Gibb, 1998). En condiciones de pastoreo, cerca de 85% del tiempo diario dedicado al pastoreo está ubicado en el período con presencia de luz, divididos en dos sesiones principales, una al amanecer y otra, más importante, en el atardecer (Albright, 1993).

La rumia, contrariamente a las actividades de pastoreo, ocurre mayoritariamente en la noche con un promedio de 7 horas diarias, teniendo un pico aproximadamente 4 horas después de una sesión de consumo (Schirrmann et al., 2012). Además, la rumia tiene un papel clave en el mantenimiento del pH óptimo del rumen para permitir la digestión microbiana, además de la descomposición física del alimento durante la masticación. Es necesario un tiempo de masticación óptimo para minimizar riesgos de acidosis ruminal, mejorar la digestión de las fibras y promover altos niveles de consumo por vacas lecheras (Church, 1975).

El tiempo destinado a rumia y consumo pueden tener una relación compensatoria, es decir, cuando el tiempo de consumo es menor, el tiempo de rumia tiende a incrementarse (Dado y Allen, 1994). Este último es afectado sobre todo por el consumo de fibra en detergente neutro y partículas de tamaño medio (4 – 19 mm), mientras que el tiempo de consumo sufre influencia de partículas largas ( $\geq 19$  mm) y tiempo de acceso limitado al alimento (White et al., 2017). Sin embargo, el tiempo

total de masticación, siendo la suma del tiempo de consumo y de rumia, es menos variable que estos dos, con un máximo de 16 horas diarias y juega un papel vital en el mantenimiento de altos niveles de consumo de materia seca y una función digestiva eficiente en vacas lecheras de alta producción (Beauchemin, 2018).

El tiempo de descanso es comúnmente referido al momento de menor actividad de la vaca, aunque en ese período importantes funciones acontezcan, como la estratificación de las partículas en el rumen y la eructación de los gases productos de la fermentación, siendo Gibb (1998) quien sugirió que “descanso” fuera llamado de “otras actividades”. La distribución de esta actividad durante el día, normalmente tiene una relación inversa respecto a las actividades de pastoreo y correlativa respecto a la rumia (Fregonesi et al., 2007).

Las acciones de los animales son organizadas en forma jerárquica y en distintas escalas: paisaje, comunidad, parche, estación alimentaria y bocado (Carvalho, 1997). Estación alimentaria se define como el semicírculo hipotético donde el animal toma uno o más bocados sin la necesidad de mover las patas delanteras (Ruyle y Dwyer, 1985). Considerando la estación alimentaria como referencia de estudio, el comportamiento de los animales en pastoreo puede ser resumido a tiempo de búsqueda y desplazamiento entre estaciones alimentarias, tamaño de bocado en cada estación (Griffiths et al., 2003) y tiempo de permanencia en las estaciones alimentarias (Gonçalves et al., 2009).

El consumo diario de forraje por un animal en pastoreo, en un sentido más amplio, puede ser definido como el efecto combinado del comportamiento ingestivo, masa y tasa de bocado y tiempo de pastoreo (Allden y Whittaker, 1970). Entretanto, cada uno de estos factores que definen el consumo de forraje diario son resultados de características de la pastura, como altura (Gibb et al., 1997), disponibilidad y densidad (Laca et al., 1992); del animal, como estado fisiológico (Penning et al., 1996; Gibb et al., 1999) y tiempo de ayuno previo (Chilibroste et al., 1997; 2007); de factores abióticos, como condiciones ambientales (Chilibroste et al., 2010; Kamal et al., 2018) y del manejo del pastoreo y nutricional, como tiempo y momento de acceso a la pastura (Mattiuda et al., 2013; Soca et al., 2014), asignación de forraje (Bargo et al., 2002;

Chilibroste et al., 2005; 2007) y fornecimiento de suplementos (Bargo et al., 2002; Gibb et al., 2002; Soca et al., 2014). La ingestión de materia seca por animales en pastoreo es resultado de un proceso complejo y que tiene como unidad básica el bocado (Carvalho et al., 2001).

Hay una conocida relación entre la altura de la pastura y masa de bocado (Laca et al., 1992; Penning et al., 1994; Mezzalira et al., 2014), que es uno de los principales componentes del consumo diario de materia seca (Hodgson et al., 1994; Gibb et al., 1997; Penning et al., 1996; Carvalho, 1997; Carvalho et al., 2001; Cosgrove, 1997; Chilibroste et al., 2015). Tanto que, en la medida que disminuye la masa de forraje, la masa de cada bocado también tiende a ser menor y en esas situaciones los animales aumentan el tiempo de pastoreo y la tasa de bocado. Aunque se expresen los mecanismos de compensación, el consumo diario va a disminuir cuando el menor peso de bocado no pueda ser compensado por la mayor frecuencia de los mismos (Carvalho et al., 2001), ya que, en términos de tiempo y costo energético, los bocados más grandes se manejan más eficientemente que los bocados más chicos, por haber un costo fijo de la prehensión del bocado, independiente de la masa del mismo (Tharmaraj et al. 2003).

La performance animal es resultante de la concentración de energía y nutrientes, consumo, digestibilidad y metabolismo de los alimentos ofrecidos a los animales (Mertens, 1994), aunque sea el consumo el componente más importante y determinante de la producción animal (Cosgrove, 1997; Bargo et al., 2002).

## **1.2. INTENSIDAD DE DEFOLIACIÓN Y RESPUESTA ANIMAL Y VEGETAL**

A nivel mundial, la utilización de pasturas como base de la alimentación de rodeos lecheros no es novedad. Entretanto, estos sistemas han evolucionado en el último siglo, pasando de sistemas de pastoreo sin control y sin animales apropiados a esa finalidad para sistemas intensivos con animales de alto nivel productivo y que generan altas producciones por unidad de área (Roche et al., 2017).

La interacción entre el pastoreo y la vegetación, es dinámica y bidireccional. La estructura, la calidad y la distribución de la planta influyen a nivel cualitativo y cuantitativo en la dieta de animales en pastoreo, mientras que el pastoreo también tiene efecto en la estructura y la composición de la vegetación (Baumont et al., 2004). Siendo así, la estructura de las pasturas son tanto la causa como la consecuencia del proceso de pastoreo (Carvalho et al., 2009).

El manejo empleado sobre la pastura impacta en el comportamiento de pastoreo y en el consumo de forraje, que determinan la respuesta animal al ambiente pastoril que está sometido (Bailey, 2005; Carvalho, 2005). Por otro lado, es uno de los principales responsables del dinamismo de las pasturas, ya que ejerce impacto sobre el crecimiento vegetal, estructura de la vegetación y valor nutritivo del forraje producido (Pavlu et al., 2006).

Hay distintas maneras de controlar y expresar la presión de pastoreo en sistemas de producción, pero la intensidad de pastoreo expresada en altura de forraje es una manera utilizada en la investigación (Orr et al., 2004; Mattiauda et al., 2009; Faber, 2012; Amaral et al., 2013, Zibil et al., 2016) y de fácil implementación a nivel productivo (Mattiauda et al., 2009), acercando los hallazgos científicos del campo. Laca et al. (1992) demostró que hay una estrecha relación entre altura y densidad de forraje, variables que en conjunto explican grande parte del comportamiento y consumo bajo pastoreo.

Al estudiar el efecto del control de la intensidad de defoliación en la producción de forraje y en su utilización por los animales en tambos comerciales, Zibil et al. (2016) verificaron aumentos de 26,5% en la tasa de acúmulo diaria en el tratamiento con control de altura (entrada 15-20 y salida 5-7 cm) en relación al sin control (manejo rutinario de los tambos), habiendo así incrementos en la producción de forraje total y mejor utilización de la materia seca producida en función del control del pastoreo.

Mattiauda et al. (2009), en un trabajo nacional realizado en una pastura a base de festuca manejada con distintas alturas remanentes (3, 6, 9 y 12cm) verificaron una tendencia de incremento en la producción individual de leche en menores intensidades de pastoreo, así como en la producción de forraje, sumado a un menor uso de

suplementación concentrada. Por otra parte, los resultados demuestran que alturas remanentes de 3 cm no son viables a nivel de sistema. Resultados similares fueron observados por Soca et al. (2009). Pasturas de la misma especie manejadas a 3 cm de altura remante tenían una elevada proporción de suelo desnudo, mientras que con manejo de 12 cm hubo una tendencia de incrementar la producción de forraje y leche. En concordancia, Chico (2007) reporta que con alturas de manejo por debajo de 6 cm, las vacas no pudieron compensar con mayor tiempo de pastoreo la menor oferta de forraje. Por otro lado, la menor intensidad (12 cm) de defoliación proporcionó estabilidad en la producción de forraje y leche.

A nivel internacional (Pérez-Prieto y Delagarde, 2013) y nacional (Chilibroste et al., 2004; 2012) se han estudiado la relación de la asignación de forraje con la respuesta productiva del animal, pero es escasa la literatura nacional en estudios que traten de entender las relaciones entre consumo, comportamiento y producción de leche a distintas alturas de manejo de las pasturas con un enfoque sistémico, y con el propósito de encontrar alternativas para lograr aumentar el consumo de pasto e intensificar la producción de forraje, encontrando un punto óptimo en la interface planta-animal, reduciendo costos y mejorando la performance animal.

Por lo expuesto, es pertinente conocer cuáles son las estrategias comportamentales adoptadas por los animales que llevan a mayor eficiencia del punto de vista consumo y en que altura del pasto eso ocurre, es decir, identificar la altura de manejo que posibilite cosechar más pasto y lograr mayor producción de leche.

### **1.3. HIPÓTESIS Y OBJETIVOS DEL TRABAJO**

#### **1.3.1. Hipótesis**

La menor intensidad de defoliación (mayor altura remanente) aumentará el consumo de materia seca y producción de leche a través de un comportamiento ingestivo más selectivo que permitirá mejor valor nutritivo de lo consumido.

#### **1.3.2. Objetivo general**

Verificar la influencia de la altura post pastoreo en el consumo de materia seca y producción de leche y entender las estrategias comportamentales adoptadas por los animales para lograrlos.

#### **1.3.3. Objetivos específicos**

- Evaluar el comportamiento temporal de vacas lecheras sometidas a tres intensidades de defoliación en la pastura;
- Evaluar el efecto de distintas alturas de manejo del pasto, en el desplazamiento y en la forma de utilización de las estaciones alimentarias y parches por vacas en lactancia;
- Describir las relaciones entre el desplazamiento y el comportamiento temporal e ingestivo de las vacas, en función de la estructura de la pastura generada en cada tratamiento;
- Identificar la altura de manejo que posibilite lograr mejor performance animal.

## **2. EFFECT OF POST-GRAZING SWARD HEIGHT ON INGESTIVE BEHAVIOR, DRY MATTER INTAKE AND MILK PRODUCTION OF HOLSTEIN DAIRY COWS<sup>1</sup>**

### **2.1. SUMMARY**

An experiment was carried out at Paysandú - Uruguay, in spring, to evaluate the effect of three defoliation intensities on a Fescue (*Festuca arundinacea*) based pasture on the behavior, dry matter intake and milk production of dairy cows. Three post-grazing sward height were tested: 15 (TL), 12 (TM) and 9 (TC) cm. Thirty-six autumn-calving Holstein cows were blocked by parity ( $2.6 \pm 0.8$ ), body weight ( $618 \pm 48$  kg) and body condition score ( $2.8 \pm 0.2$ ), and randomized to the treatments. Grazing method was rotational and the pasture accessed from 0800 to 1400 h and 1700 to 0300 h. Cows were milked twice a day (at 0400 and 1500 h). The grazing and ruminating behavior times data were collected with automatic recorders. The number of feeding stations and patches visited and search steps were recorded. Dry matter intake was estimated by alkane method. Response variables were analyzed with PROC GLIMMIX of SAS and mean values were declared different when Tukey test  $<0.05$ . Daily grazing time averaged  $508 \pm 15$  min and was not affected by the treatment. The reduction of post-grazing sward height increased the length of the first grazing session in the morning and in the afternoon. The number of grazing sessions was greater on TL than on TM, with no difference in TC. The number of feeding stations visited was greater on TL than on TC, and neither of them differed from TM. Dry matter intake was greater on TL and TM than on TC (17.8 vs. 14.7 kg DM). Milk production during the paddock occupation period was 13.1, 16.2 and 18.7 kg/day for TC, TM and TL, respectively. The milk concentration did not differ between treatments. The cows on the lower defoliation intensity achieved a selective behavior resulting in high digestible dry matter intake and consequently higher milk production. The cows on TM adopted a compensation mechanism which allowed them to achieve the same dry matter intake

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<sup>1</sup> El artículo se envió para publicación en la revista Journal of Dairy Science.



than cows on TL, but lower milk production. The height and structure of the pasture on TC imposed the cows for a lower intake rate, although they were less selective, probably trying to compensate the low ingestion.

**Keywords:** animal-plant interaction, selectivity, forage harvest

## 2.2. INTRODUCTION

Pasture-based dairy farms systems are receiving special attention around the world because they may provide benefits as to several environmental aspects (Aguirre-Villegas et al., 2017), may present profitability advantages (O'Brien et al., 2015) and are well regarded by consumers in terms of animal welfare (Cardoso et al., 2019). Uruguayan pasture-based dairy farm systems have potential for production growth and should achieve it to maintain international competitiveness (Fariña and Chilbroste, 2019). The maximized utilization of grazed pasture to reduce the costs associated with feeding the dairy cow is a common goal on these dairy production systems (Finneran et al., 2010).

Grazing is a complex process that involves a set of decisions at different scales being the bite the basic unit. Hence, daily pasture dry matter intake by grazing animals is largely dependent on the mass of each bite, which is affected by the sward height (Gibb et al., 1997) and herbage mass (Laca et al., 1992) available to animals. The daily grazing pattern adopted by animals will depend mainly on intrinsic behavior of specie and photoperiod (Linnane et al., 2001), sward characteristics (Chapman et al., 2007), internal state (Chilbroste et al., 2005), spatial memory (Bailey et al., 1996) and anthropogenic management (Chilbroste et al., 2015).

In addition, grazing management strategies have direct effects on pasture growth, where low post-grazing sward heights or without control have an unfavorable impact on pasture regrowth rate and in the total forage production (Chapman, 2016). In Uruguay, some research has pointed out that increasing post-grazing sward height presents benefits for both pasture and animal performances (Mattiauda et al., 2009; Zibil et al., 2016). Despite that, studies that try to understand the relationships between daily dry matter intake and behavior and milk production of dairy cows at different

residual sward heights managements are scarce. The potential to increase milk production by increasing post-grazing sward height, and seeking an optimal point in the plant-animal interface, is still unknown. An experiment was conducted to study the influence of post-grazing sward height on dry matter intake and milk production, as well as the behavioral pattern used by cows to adapt to the different scenarios. The hypothesis was that higher post-grazing sward height increases dry matter intake and milk production through more selective ingestive behavior allowing better nutritional values in the diet.

### **2.3. MATERIALS AND METHODS**

The experiment was carried out at the Experimental Research Station ‘‘Dr. M.A. Cassinoni’’ (EEMAC) of the Faculty of Agronomy (Paysandú, Uruguay, 32°S, 58°W), during late October early November 2017. A pasture of Tall fescue (*Festuca arundinacea* cv. INIA Fortuna) was sown during May 2016 with 10 kg/ha of seed. At the time of sowing it was fertilized with 18 kg of N and 46 kg of P/ha and, on the second year, 180 kg of N/ha were applied divided in 4 applications during June, August, September and October. In February 2017 the sward was homogenized by mechanically mowing at 6 cm and the grazing treatments were installed in April.

#### **2.3.1. Treatments and experimental design**

Three post-grazing sward height were tested in a randomized complete block design with four spatial blocks of 3 paddocks of 0.2 ha each one. Each paddock was grazed by the same three milking cows along the year. The criteria to start grazing in each treatment was when three new extended leaves were developed (Fulkerson and Donaghy, 2001). During April to July the post-grazing sward height were 6 (Control treatment; **TC**), 9 (Medium treatment; **TM**) and 12 cm (Lax treatment; **TL**) and were performed three grazing cycles on TM and TL and two on TC. During August and September were performed two grazing cycles where the treatments were managed with a common post-grazing sward height of 5 cm grazed with a high stock of no-

experimental dairy heifers to prevent elongation of flowering stems (Jáuregui et al., 2017). In late October early November was the evaluation period where it took place all the pasture and animal measurements. The treatments managed from April to July with 6, 9 and 12 cm post-grazing sward height were now managed with 9 (TC), 12 (TM) and 15 cm (TL), respectively. The different post-grazing sward heights were generated maintaining cows on the paddock until the sward reach the targeted height.

### **2.3.2. Animals and management**

In early April, thirty-six autumn-calving Holstein cows were blocked by BW, BCS, parity number and DIM, and randomized to the treatments. During the periods when cows were not grazing in the experimental paddocks they remained in a common herd with similar management. Previous to the evaluation period (late October/early November) cows were with  $618 \pm 48$  kg BW,  $2.8 \pm 0.2$  BCS,  $2.6 \pm 0.8$  parity number and  $224 \pm 7$  DIM.

Before grazing in the treatment paddocks, all the experimental cows grazed in an adjacent paddock with similar herbage allowance. At this moment the milk production was equal between treatments ( $16.7 \pm 0.89$ ;  $P = 0.963$ ). Cows were not supplemented and were only removed from the paddock twice a day to be milked (0400 and 1500 h).

### **2.3.3. Measurements and calculations**

#### **2.3.3.1. Sward features**

A weekly monitoring of pasture's physiological state was performed to determine the start of grazing (Fulkerson and Donaghy, 2001). Sward height was measured pre and post-grazing, and every day during the paddock occupation period, determining the point of maximal leaf density using a graduated ruler at a minimum of 70 random sites in each paddock. The sward mass was estimated pre and post grazing with a rising plate meter (Ashgrove Co., Palmerston North, New Zealand)

using an adaptation of the double sampling technique of Haydock and Shaw (1975). A characterization of pre-grazing sward structure was carried out in  $\approx 700$  points per paddock (16 transects) with the rising plate meter one day before grazing. The points were then classified according to their height in high (**HS**; greater than 30 cm), medium (**MS**; between 15 and 30 cm) and low structure (**LS**; less than 15 cm). Two representative points were chosen in each parcel for each sward structure type (HS, MS and LS), and the leaf sheath height was measured on 5 tillers of fescue in a 0.1 m<sup>2</sup> (30 cm  $\times$  34 cm).

#### **2.3.3.2. Grazing and ruminating behavior**

The grazing behavior data was collected from 27 cows (3 complete blocks) during 24h at two moments: at the beginning (**BM**; day 3) and at the end (**EM**; day 5 for treatments TM and TL and day 6 for TC) of the occupation period. The cows were equipped with behavioral recorders (IGER; Rutter et al. 1997) to measure grazing, ruminating and idling times, as well as the number and length of grazing and ruminating bouts. The raw data was processed using the Graze software (Rutter, 2000) following the definitions described by Gibb et al. (1999).

#### **2.3.3.3. Displacement and feeding station and patch use**

The number of feeding stations visited and the number of steps between feeding stations were registered by visual tests from 0800 to 1130 h and from 1700 to 1930 h, at the beginning and at the end of paddock occupation period. The observations were performed by one observer every 3 cows and the evaluation was sequential during 15 minutes for each individual cow. A feeding station was represented by the hypothetical semi-circle in front of an animal from which the bites were taken without moving the front forefeet (Ruyle and Dwyer, 1985). A new patch was counted when the cow did at least two consecutive searching steps (Gregorini et al., 2007).

#### **2.3.3.4. Animal**

Individual milk yield was measured twice a day at 4:00 and 15:00 h. On the third day of occupation period, an individual milk sample was collected in both the morning and afternoon milking to determine milk fat, protein and lactose concentration by midinfrared spectrophotometry (Milko-Scan, Foss Electric, Hillerød, Denmark).

#### **2.3.3.5. Dosage of C32 and C36-alkane and collection of feces and forage**

Individual herbage dry matter intake and digestibility was determined in 27 cows (3 complete blocks) using alkanes technique (Dove and Mayes, 2006), with hentriacontane (C<sub>31</sub>) as an internal marker, dotriacontane (C<sub>32</sub>) as an external marker and hexatriacontane (C<sub>36</sub>) to estimate fecal output. During 10 consecutive days the cows were dosed, after morning and evening milking, with a cellulose pellet containing 344 mg of C<sub>32</sub> and 145 mg of C<sub>36</sub>. From day 5 to 10 feces samples were collected per rectum, after morning and evening milking, and immediately stored frozen at -20°C. Note that the sample feces collection was performed the last days of occupation period. To achieve this, the alkane dosification started 3 days before grazing in the treatment paddocks, in an adjacent paddock with similar herbage allowance. The feces' samples were dried until constant weight in a 60°C air forced oven and then milled through a 1-mm screen. Herbage samples were collected on day 2 and 4 of feces collection period by hand clipping (Coates and Penning, 2000), while following individual cows during one hour in the first morning and evening grazing session. They were dried until constant weight in a 60°C air forced oven and then milled through a 1-mm screen and stored until they were analyzed for concentration of alkanes and chemical composition.

#### **2.3.3.6. Analytical methods**

The determination of alkanes present in the forage and feces followed the protocol proposed by Dove and Mayes (2006). The identification and quantification of alkanes were made by gas chromatography using a Shimadzu GC-2010 gas

chromatograph equipped with a flame ionization detector, a spool auto-sampler AOC-20S and an injector auto-injector AOC20i. The extracted n-alkanes were injected (1  $\mu$ L) into a column Restek Rtx<sup>®</sup>-5 (30 m 0.25 mm  $\times$  0.25  $\mu$ m, absorbent composed by 5% diphenyl and 95% polysiloxane dimethyl). The carrier gas was N<sub>2</sub> at a constant flow of 30 mL/min. Temperature gradients were controlled for injector (270°C) and column (170°C by 1 min; 30°C/min until 215°C waiting 1 min and 6°C/min for 300°C; 21 min). The flame ionization detector temperature was maintained at 340°C. The gas chromatography procedure was calibrated with a standard external solution containing a mixture of synthetic C<sub>7</sub> to C<sub>40</sub> alkanes (>99% purity, Sigma-AldrichCorp., St. Louis, MO, USA) at concentrations similar to those found in the extract. The areas of the chromatographic peaks corresponding to each alkane were determined using the software Shimadzu GC Solution, where the identification of alkane chain length between C<sub>20</sub> to C<sub>35</sub> was based on comparison with an external standard, by the retention time average of alkane in each column. The peaks identified were converted into amounts of alkanes taking the internal standard C<sub>34</sub> as reference and calculated in mg/kg DM of feces and herbage.

Herbage samples were also analyzed by ash and N according to AOAC (1990) methods number 942.05 and 984.13, respectively. NDF and ADF were measured sequentially (Van Soest et al., 1991; without sodium sulfite in the neutral detergent solution) using an ANKOM200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). NDF assayed with a heat stable amylase. Fiber contents were expressed corrected by ash.

### 2.3.3.7. Calculations

The mean, median, mode, range, variance and standard deviation of sward height during two days of behavioral evaluation was calculated with PROC MEANS of SAS System program (SAS® University Edition, SAS Institute Inc., Cary, NC, USA).

Intake rate was obtained by the ratio between daily dry matter intake and daily grazing time. And ruminating index by the ratio between daily ruminating time and daily NDF intake.

From the number of feeding stations and patches visited, and the number of steps between feeding stations/patches, the following variables were determined to describe the process of grazing in the paddock: i) number of feeding stations per minute: given by the quotient between the total number of feeding stations and the duration, in minutes, of visual test; ii) steps between feeding stations: quotient between the total number of steps by the number of feeding stations visited during the evaluation; iii) displacement rate, in steps per minute: total number of steps divided by the duration in minutes by visual test; iv) time per feeding station: quotient between the duration of the test in seconds and the total number of feeding stations visited; v) feeding stations per patch: given by the quotient between the total number of feeding stations and the total number of patches visited during the visual test; vi) patches visited per hour: quotient between the total number of patches and the duration, in hours, of visual test; vii) time per patch: quotient between the duration of the test in minutes and the total number of patches visited, and viii) steps between patches: quotient between the total number of steps by the number of patches visited during the evaluation (Ruyle and Dwyer, 1985; Gonçalves et al., 2009; Gregorini et al., 2009b).

Energy corrected milk was calculated according to Tyrrell and Reid (1965):

$$\text{Energy corrected milk (kg/cow per day)} = \text{milk yield kg} \times (376 \times \text{fat\%} + 209 \times \text{protein\%} + 948) / 3138 .$$

The dry matter intake (kg DM/cow per day) was estimated from the ratio of n-alkane concentrations found naturally in the forage and dosed alkane (C<sub>31</sub>:C<sub>32</sub>) in

forage and feces samples, according to the equation proposed by (De-Stefani Aguiar et al., 2013):

$$\text{Dry matter intake} = ((\text{concentration of C31 in feces} / (\text{concentration of C32 in feces} - \text{concentration of C32 in forage}) \times \text{daily dose of C32}) / \text{concentration of C31 in forage}) / 1000.$$

The digestibility was calculated using the equation (Dove and Mayes, 2006):

$$\text{Digestibility} = (\text{dry matter intake} - \text{faecal output}) / \text{dry matter intake},$$

where faecal output is the total faecal output estimated using C<sub>36</sub> alkane by the equation:

$$\text{Faecal output} = \text{daily dose of C36} / \text{concentration of C36 in feces}.$$

#### **2.3.3.8. Statistical analyses**

Data were analyzed as a complete randomized block design using the SAS System program (SAS® University Edition, SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed on all variables to identify outliers and inconsistencies, and to verify normality of residuals. Most of the variables were normally-distributed, except feeding stations per minute (fitted with a Poisson distribution), steps between feeding stations, displacement rate, time per feeding station, feeding stations per patch, patches per hour, time per patch, steps between patches and leaf sheath height which were fitted with Log-normal distribution. For both Poisson and Log-normal distribution a log transformation was specified in a model.

Sward height depletion rate was analyzed using the NLIN procedure. The mean, median, mode, range, variance and standard deviation of sward height as well as



grazing and ruminating behavior were analyzed as repeated measures using the GLIMMIX procedure with a mixed model that included treatment, moment and their interaction as fixed effects, and block as random effect. To analyze first grazing meal length as well as displacement, feeding station and patch use, the variables time of the day (morning and evening) and their interactions were added to the model as fixed effects. The dry matter intake, milk production, depletion rate and herbage chemical composition variables were analyzed using the GLIMMIX procedure with a mixed model that included treatment as fixed effect and block as random effect. The proportion of sward structure type and leaf sheath height variables were analyzed with a mixed model that included treatment, sward structure type and their interaction as fixed effect, and block as random effect.

Least square means were separated using Tukey–Kramer tests and significant difference was accepted if  $P < 0.05$  and tendencies to significance was accepted if  $0.05 < P \leq 0.10$ .

## **2.4. RESULTS**

### **2.4.1. Herbage mass, height and depletion dynamics**

Six days of grazing were needed on TM and TL, and 7 days on TC treatment to achieve the targeted post-grazing sward height. The pre-grazing herbage mass ( $2684 \pm 132$  kg/ha), pre-grazing sward height ( $18.8 \pm 0.8$  cm) and post-grazing herbage mass ( $1741 \pm 111$  kg/ha) were not different between treatments ( $P = 0.086$ ). Post-grazing sward height were  $9.0 \pm 0.23$  cm,  $12.0 \pm 0.23$  cm and  $13.5 \pm 0.27$  cm ( $P < 0.001$ ) for target heights of 9 cm, 12 cm and 15 cm, respectively.

The characterization of sward the day before grazing is presented in Table 1. The proportion of LS was similar between treatments, while MS was lower on TL and HS was greater on TL (interaction sward structure type  $\times$  treatment;  $P < 0.001$ ). The leaf sheath height increased from LS to HS and from TC to TL treatment, presenting significance on all tested effects ( $P < 0.001$ ).

Dynamic of sward height depletion throughout the grazing down is presented in figure 1. An exponential decay was fitted for each treatment with a fractional rate of - 0.052, - 0.096 and - 0.116 cm/day for TL, TM and TC, respectively. The depletion rate was lower on TL than TM and TC ( $P < 0.001$ ), with a trend ( $P < 0.055$ ) between the last two.

Table 1. Proportion of low structure (LS), medium structure (MS) and high structure (HS) and leaf sheath height (cm) one day before the grazing according to the treatments.

		Treatments <sup>1</sup>			SEM
		TC	TM	TL	
Proportion of sward structure type	LS	0.08 <sup>B</sup>	0.08 <sup>B</sup>	0.05 <sup>B</sup>	0.054
	MS	0.64 <sup>aA</sup>	0.66 <sup>aA</sup>	0.28 <sup>bB</sup>	0.054
	HS	0.28 <sup>bB</sup>	0.26 <sup>bB</sup>	0.67 <sup>aA</sup>	0.054
Leaf sheath height	LS	2.4 <sup>B</sup>	2.67 <sup>C</sup>	2.8 <sup>C</sup>	0.18
	MS	3.6 <sup>bA</sup>	4.4 <sup>abB</sup>	5.10 <sup>aB</sup>	0.33
	HS	4.4 <sup>bA</sup>	5.8 <sup>aA</sup>	8.2 <sup>aA</sup>	0.86

<sup>a-b</sup>Mean within a row with different lower-case superscripts letters differ ( $P < 0.05$ ).

<sup>A-C</sup>Mean within a column with different capital superscripts letters differ ( $P < 0.05$ ).

<sup>1</sup>Treatments: TC = Control Treatment; TM = Medium Treatment; TL = Lax Treatment.

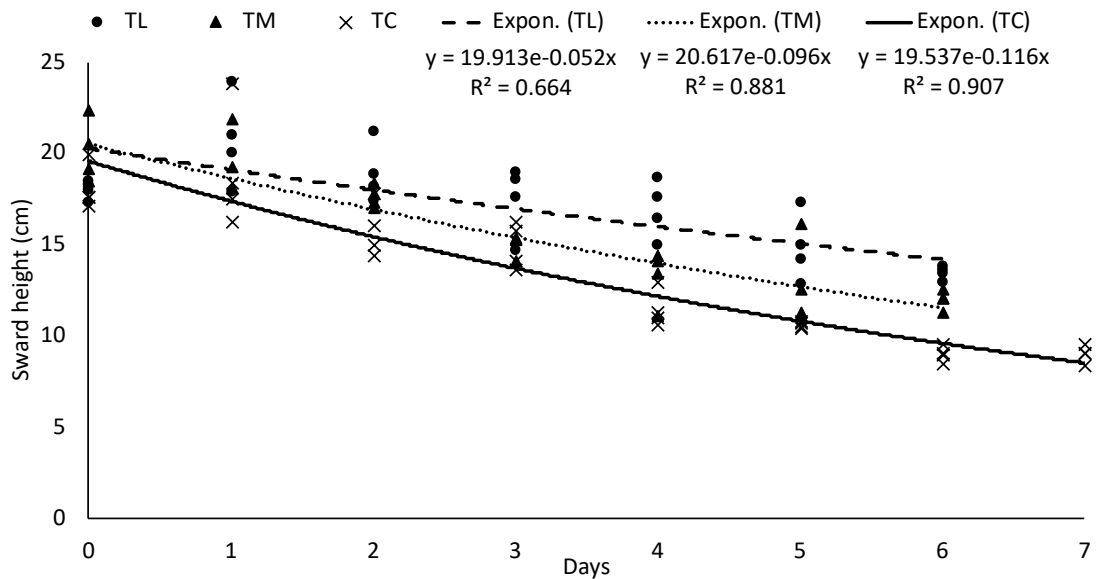


Figure 1. Depletion dynamics of height throughout the grazing down by cows managed with post-grazing sward height of 9 (TC), 12 (TM) or 15 cm (TL). Fitted exponential equations for each treatment are shown within the figures under the series identification.

The sward height distribution during two days of behavioral evaluation is presented in table 2. All the variables presented effect of treatment and moment. The sward height median and mode remained lower than the sward height mean on TC and TM and above on TL. The range, variance and standard deviation increased from TC to TL and in general decreased from the beginning to the end of occupation period. Despite not being significant, on TC the variation seems to be greater between the beginning and the end of the occupation period.

Table 2. Effect of post-grazing sward height (T) and moment of occupation period (M) on sward height distribution

	Treatments <sup>1</sup> and moments of occupation period <sup>2</sup>						SEM	P-value		
	TC		TM		TL			T	M	T × M
	BM	EM	BM	EM	BM	EM				
Mean	15.8	10.6	17.7	13.2	18.9	16.9	0.63	<0.001	<0.001	0.060
Median	15.3	10.1	16.6	12.3	19.1	16.9	0.68	<0.001	<0.001	0.097
Mode	14.8	8.7	16.2	10.0	21.5	13.5	1.30	<0.001	<0.001	0.709
Range	19.8	11.8	18.8	19.1	22.1	18.9	1.71	0.037	0.018	0.075
Variance	24.8	7.2	22.0	20.5	34.3	24.3	4.19	0.034	0.019	0.242
SD	4.9	2.7	4.7	4.4	5.8	4.9	0.45	0.009	<0.008	0.112

<sup>1</sup>Treatments: TC= Control Treatment; TM= Medium Treatment; TL = Lax Treatment.

<sup>2</sup>Moments of occupation period: BM = Beginning Moment; EM = End Moment.

#### **2.4.2. Ingestive and ruminating behavior**

Daily grazing time was unaffected neither by treatment nor by moment, but it presented a treatment by moment interaction (Table 3). The cows on TM spent more time grazing at the beginning than at the end of occupation period (551 vs. 489 min), while TL tended to have a shorter grazing time than TM at the beginning moment (481 vs 551 min). The morning's first grazing meal duration was greater at the end than at the beginning of the occupation period (169 vs 147 min). The TC and TM kept the morning's first grazing meal duration across the occupation period while TL increased at the end of the occupation period (58 vs. 126 min for the beginning and the end, respectively). The number of grazing meals were greater on TL than on TM, with not differences with TC. The number of grazing meals were greater at the beginning (6.3) than at the end moment (4.8) for TL. Moreover, at the beginning TL cows grazed in 6.3 meals while both TM and TC did it in 4.6 meals. The mean grazing meal duration was greater on TM than on TL, and tended to be greater than TC ( $P = 0.090$ ). The time spent ruminating was greater on TC and TL than on TM, and lower at the end than at the beginning (480 vs. 521 min). Nevertheless, number of ruminating bouts were greater on TL and TM than on TC. On the other hand, mean ruminating bout length was greater on TC than on other ones.

Table 3. Effect of post-grazing sward height (T) and moment of occupation period (M) on ingestive and ruminating behavior by lactation cows

	Treatments <sup>1</sup>			SEM	P-value		
	TC	TM	TL		T	M	T × M
<b>Grazing</b>							
Grazing time (min/day)	506	520	497	15	0.504	0.674	0.009
First morning grazing meal length (min)	196 <sup>a</sup>	186 <sup>a</sup>	92 <sup>b</sup>	16	<0.001	0.049	0.001
First evening grazing meal length (min)	184 <sup>a</sup>	170 <sup>ab</sup>	167 <sup>b</sup>	4	0.022	0.12	0.216
Number of grazing meals	5.2 <sup>ab</sup>	4.8 <sup>b</sup>	5.5 <sup>a</sup>	0.38	0.014	0.900	0.003
Mean grazing meal length (min)	104 <sup>ab</sup>	123 <sup>a</sup>	101 <sup>b</sup>	10	0.025	0.617	0.182
<b>Ruminating</b>							
Ruminating time (min/day)	533 <sup>a</sup>	464 <sup>b</sup>	505 <sup>a</sup>	11	<0.001	0.001	0.564
Number of ruminating bouts	9.9 <sup>b</sup>	12.3 <sup>a</sup>	13.0 <sup>a</sup>	0.77	0.012	0.886	0.098
Mean ruminating bout length (min)	51 <sup>a</sup>	37 <sup>b</sup>	35 <sup>b</sup>	3	<0.001	0.895	0.931

<sup>a-b</sup>Mean within a row with different lower-case superscripts letters differ (P < 0.05).

<sup>1</sup>Treatments: TC= Control Treatment; TM= Medium Treatment; TL= Lax Treatment.

### **2.4.3. Displacement pattern and feeding station and patch exploration**

The displacement pattern, feeding station and patch use are shown in Table 4. The number of feeding stations per minute was greater on TL than on TC, and both did not differ from TM. The cows explored greater number of feeding stations at the beginning than at the end of the occupation period ( $4.8 \pm 0.10$  vs  $4.3 \pm 0.10$ ), as well as in the evening grazing than in the morning one ( $4.7 \pm 0.09$  vs  $4.4 \pm 0.09$ ). The time that cows explored each feeding station was greater on TC and decreased at higher post-grazing sward height, being affected by moment of occupation period ( $12.8 \pm 0.31$  vs  $14.2 \pm 0.35$  for the beginning and the end, respectively) as well as the time of the day ( $14.0 \pm 0.31$  vs  $13.0 \pm 0.30$  for morning and evening grazing, respectively). The steps between feeding stations and displacement rate were not affected by treatment, moment of occupation period, or the time of the day. The number of steps between feeding stations in the morning grazing was higher on TC ( $0.36 \pm 0.06$ ) than on TL ( $0.21 \pm 0.04$ ). The cows grazing on TC, at the beginning of occupation period, did more steps between feeding stations ( $0.44 \pm 0.09$  vs  $0.21 \pm 0.04$ ) and per minute ( $1.86 \pm 0.39$  vs  $0.91 \pm 0.20$ ) in the morning grazing than in the evening one. Interaction treatment  $\times$  moment was not significant for all the variables tested.

The number of feeding stations per patch was higher on TL than on TC and TM, and in the morning grazing the difference between them was highlighted ( $18.2 \pm 2.58$  vs  $10.7 \pm 1.48$  and  $11.2 \pm 1.55$  for TL, TC and TM, respectively). At the beginning of the occupation period, TC increased the number of feeding stations explored from morning to evening grazing ( $8.3 \pm 1.35$  vs  $16.0 \pm 2.68$ ). The patches explored per hour were higher on TM than on TL, and in the evening grazing ( $23.3 \pm 2.99$ ) than in the morning one ( $20.1 \pm 2.57$ ). In the morning grazing sessions, TL cows explored fewer patches per hour ( $15.0 \pm 2.23$ ) than TC ( $23.4 \pm 3.41$ ) and TM ones ( $23.3 \pm 3.4$ ), and fewer than in the evening grazing ( $24.9 \pm 3.7$ ). At the beginning, TC explored the double of patches per hour in the morning grazing ( $31.1 \pm 5.28$ ) than in the evening one ( $15.00 \pm 2.58$ ), which differed from TM ( $25.6 \pm 4.51$ ) at the same moment. On the other hand, TL cows at the end explored the double of patches per hour in the evening grazing ( $25.8 \pm 4.48$ ) than in the morning one ( $13.3 \pm 2.21$ ). The residence time per

patch was greater on TL than on TM without differing from TC; however, it had an interaction with the effect of the time of the day ( $3.0 \pm 0.38$  vs  $2.6 \pm 0.33$  for morning and evening grazing, respectively) and in the morning grazing the TL ( $4.09 \pm 0.6$ ) and TC ( $2.6 \pm 0.38$ ) differed from each other. At the beginning moment the TC had a greater time per patch in the evening ( $4.1 \pm 0.7$ ) than in the morning grazing ( $1.9 \pm 0.33$ ), while at the end the TL had a greater time per patch in morning grazing ( $4.7 \pm 0.8$  vs  $2.3 \pm 0.41$ ). The variable steps between patches was not significant for all the tested effects.



Table 4. Effect of post-grazing sward height (T), moment of occupation period (M) and time of the day (TD) on displacement pattern and exploration of feeding stations and patches by lactating cows

	Treatments <sup>1</sup>				P-value					
	TC	TM	TL	SEM	T	M	TD	T × M	T × TD	T × M × TD
Feeding stations per minute (n)	4.3 <sup>b</sup>	4.5 <sup>ab</sup>	4.9 <sup>a</sup>	0.14	0.009	<0.001	0.004	0.829	0.521	0.972
Time per feeding station (sec)	14.4 <sup>a</sup>	13.5 <sup>ab</sup>	12.7 <sup>b</sup>	0.43	0.012	0.002	0.004	0.794	0.564	0.948
Steps between feeding station	0.3	0.3	0.2	0.05	0.196	0.905	0.795	0.712	0.010	0.060
Displacement rate (steps/minute)	1.3	1.3	1.2	0.22	0.857	0.125	0.645	0.408	0.008	0.044
Feeding stations per patch	11.6 <sup>b</sup>	10.7 <sup>b</sup>	14.8 <sup>a</sup>	1.9	0.004	0.516	0.102	0.679	0.001	<0.001
Patches per hour (n)	21.3 <sup>ab</sup>	24.8 <sup>a</sup>	19.3 <sup>b</sup>	3.36	0.038	0.597	0.026	0.853	<0.001	<0.001
Time per patch (min)	2.9 <sup>ab</sup>	2.5 <sup>b</sup>	3.2 <sup>a</sup>	0.43	0.037	0.597	0.026	0.853	<0.001	<0.001
Steps between patches	3.2	3.2	3.1	0.26	0.906	0.528	0.321	0.736	0.151	0.201

<sup>a-b</sup>Mean within a row with different lower-case superscripts letters differ (P < 0.05).

<sup>1</sup>Treatments: TC= Control Treatment; TM= Medium Treatment; TL= Lax Treatment.

#### 2.4.4. Dry matter intake, ingestive process and selected diet

The dry matter intake was greater on TL and TM than on TC. Intake rate was greater on TL than on TC (Table 5). Ruminating index was greater on TC than on TL and on TM. The digestibility of the diet was greater on TL than on TM and TC. The selected diet by cows presented greater crude protein and neutral detergent fiber content on TL than on TM and TC. The acid detergent fiber tended ( $P = 0.066$ ) to be greater on TC than on the other ones.

Table 5. Effect of post-grazing sward height on dry matter intake, ingestive process and nutritional characteristics of selected diet by lactation cows.

	Treatments <sup>1</sup>				P-value
	TC	TM	TL	SEM	
Dry matter intake (kg/day)	14.8 <sup>b</sup>	18.1 <sup>a</sup>	17.5 <sup>a</sup>	0.64	<0.001
Intake rate (g/min)	29.4 <sup>b</sup>	34.9 <sup>ab</sup>	36.0 <sup>a</sup>	1.84	0.040
Ruminating index (min/kg NDF)	62 <sup>a</sup>	47 <sup>b</sup>	49 <sup>b</sup>	3.3	0.004
Digestibility (%)	62.1 <sup>b</sup>	63.7 <sup>b</sup>	69.6 <sup>a</sup>	1.32	<0.001
Crude protein (%DM)	11.3 <sup>b</sup>	12.0 <sup>b</sup>	12.7 <sup>a</sup>	0.25	<0.001
Neutral detergent fiber <sup>2</sup> (%OM)	56.1 <sup>b</sup>	55.8 <sup>b</sup>	58.1 <sup>a</sup>	0.52	<0.001
Acid detergent fiber <sup>2</sup> (%OM)	31.7	30.7	30.7	0.34	0.066

<sup>a-b</sup>Mean within a row with different lower-case superscripts letters differ ( $P < 0.05$ ).

<sup>1</sup>Treatments: TC = Control Treatment; TM = Medium Treatment; TL = Lax Treatment.

<sup>2</sup>Neutral and acid detergent fiber are corrected for ash.

#### 2.4.5. Milk production and composition

Milk yield, energy corrected milk yield, energy output, fat, protein and lactose yield increased with post-grazing sward height (Table 6). Fat, protein and lactose concentration did not differ between treatments.

Table 6. Effect of post-grazing sward height on milk yield and milk component yields and concentration by lactation cows

	Treatments <sup>1</sup>			SEM	P-value
	TC	TM	TL		
Milk yield (kg/day)	13.1 <sup>c</sup>	16.2 <sup>b</sup>	18.7 <sup>a</sup>	0.56	<0.001
Energy corrected milk yield (kg/day)	13.0 <sup>c</sup>	15.8 <sup>b</sup>	18.4 <sup>a</sup>	0.84	<0.001
Fat concentration (%)	3.9	4.0	3.8	0.30	0.805
Fat yield (kg/day)	0.5 <sup>b</sup>	0.6 <sup>ab</sup>	0.7 <sup>a</sup>	0.05	0.017
Protein concentration (%)	3.5	3.3	3.3	0.12	0.453
Protein yield (kg/day)	0.4 <sup>c</sup>	0.5 <sup>b</sup>	0.6 <sup>a</sup>	0.02	<0.001
Lactose concentration (%)	4.5	4.6	4.7	0.07	0.175
Lactose yield (kg/day)	0.6 <sup>c</sup>	0.7 <sup>b</sup>	0.9 <sup>a</sup>	0.03	<0.001

<sup>a-c</sup>Mean within a row with different lower-case superscripts letters differ ( $P < 0.05$ ).

<sup>1</sup>Treatments: TC = Control Treatment; TM = Medium Treatment; TL = Lax Treatment.

## 2.5. DISCUSSION

We have found that the establishment and maintenance of treatments for 8 months prior to the period of measurement generated spatial heterogeneity and a distinctive canopy vertical structure in each treatment that affected the behavioral pattern of the animals. The grazing started with equal sward height and herbage mass between treatments, but the different depletion rate during grazing down resulted in a different post-grazing sward height, although no differences in herbage mass, probably because the different vertical structure of the canopy between treatments (sheath height) and spatial arrangement of the height (proportion of HS, MS and LS) changed the density of the residual strata. Therefore, as suggested by Gibb et al., (1997), the productive and behavioral performance of the animals should be analyzed in a context of complex environment, because mean values of biomass or sward surface height would be poor predictors of cattle foraging behavioral responses (Laca et al., 1992).

For a better correlation of sward characteristics and animal behavior, an analysis of sward height distribution during the beginning and the end of the occupation period was performed. The heterogeneity increased from TC to TL, giving that it had an increase on range, variance and standard deviation of sward height distribution. Although heterogeneity is a complex, rich and multidimensional concept (Laca, 2008), we refer to it as the variation on sward surface height across the paddock and days (space and time scale). When a large variance is found, it means that both good and poor bites are relatively abundant; in the opposite, when a small variance is found, most of the bites are similar (Laca, 2008). The variance was almost double on TL than on TC (29.3 vs 16.1) and, in spite of no effect of treatment by moment interaction, the variance decreased 71% from the beginning to the end of the occupation period on TC, whereas the same reduction was about 7% on TM and 29% on TL. The heterogeneity can affect intake through effects on bite formation and dimensions, as well as selectivity, which will be beneficial when searching costs is not increase because it (Utsumi et al., 2009).

The daily grazing time did not present effect of the treatments, but rather due to the effect of the interaction with time of day and moment of occupation period, it can be argued that each treatment adopted a different behavioral strategy along the grazing down process as a result of the sward structure they found. The daily grazing time is defined by Gibb et al., (1998) as a sum of all grazing meals. At post-grazing sward height of 12 cm (TM) the cows increased grazing time by  $\approx 20$  min, although this was not statistically significant, is highlighted by the lower ruminating time found in this treatment when compared with TC and TL (69 and 41 min lower, respectively). The time spent on ruminating and grazing could have had a compensatory relationship, that is, when the grazing time is longer, the ruminating time tends to decrease (Dado and Allen, 1994), given that the total chewing time is less variable (Beauchemin, 2018). The number of grazing meals found on this study is in accordance with the expected for dairy cattle (Gregorini, 2012), presenting maximum value on TL at the beginning moment (6.3), where forage was abundant. The length of each grazing meal is highly dependent of rumen fill (Chilibroste et al., 1997), although it has been related to a multi-factorial response (Gregorini, 2012; Chilibroste et al., 2015). Normally, between

two consecutive grazing meals, the animal places the ruminating (Gregorini, 2012) to release soluble nutrients from feeds (Chilibroste et al., 2007). On TC, the ruminating bout length was greater than other treatments and, according to Amaral et al. (2013), by decreasing post-grazing sward height the animal is forced to explore strata with a predominance of stems and senescent material, increasing the intervals between meals.

In the literature there are cumulative evidences that the dusk grazing event is the longest and most intense (Gibb et al., 1998; Gregorini, 2012; Chilibroste et al., 2015); however, on this experiment only the cows managed on TL presented this classical grazing pattern. Perhaps because with less favorable grazing conditions on both TC and TM, the cows increased both morning and evening grazing meal length to compensate for low intake rate, giving up diet selectivity. On both TC and TM the compensation was limited by requirements of ruminating and idling time (Chilibroste et al., 2015), but on TC the low intake rate and lower digestibility of the diet resulted on a limited dry matter intake and consequently milk production. On TM, the sward structure allowed a more efficient compensation mechanism, that resulted on intermediary milk production achieved through the same dry matter intake than TL, but with lower digestibility of the diet.

The chemical composition of sward change along the day through photosynthates accumulation, with the maximum levels of dry matter and non-structural carbohydrate concentrations in the evening (Griggs et al., 2005) and in the upper layers of the sward (Delagarde et al., 2000). Therefore, analyzing the two most important grazing events (first morning and evening meal; Gibb et al., 1998), the TL cows grazed in the evening the double than in the morning, while on TC and TM was equal across the day. In addition, the higher intake rate achieved on TL did that the larger proportion of the daily dry matter intake was consumed at the time with the best potential nutritional value of the sward (Abrahamse et al., 2008). The theoretically higher non-structural carbohydrate concentrations in the evening, associated with the highest crude protein level found in herbage samples on TL, probably achieved a better ruminal environment in this treatment, potentializing the digestion of the diet (Linnane et al., 2001) and consequently dry matter intake and milk production. According to Filho et al. (2012) the pattern of ruminal fermentation clearly depends on the changes

that occur as different sward layers are grazed. Changes in ingestive behavior with a reduction in sward height have been associated to short-term adaptations, such as bite mass and intake rate reductions (Gregorini et al., 2009a).

The exploration of feeding stations enhanced the adaptation of the behavioral patterns of cows to adapt to different scenarios. According to Gregorini et al. (2007), feeding station behavior is partially controlled by ruminal fill and their interaction with external factors such as sward characteristics. The departure decisions and residence time per feeding station require the decision of the profitability of each bite to motivate the animal to move to a new feeding station, which is highly connected to the perception of resource availability (Roguet et al., 1998a). We found that cows on TL achieved a higher number of feeding stations explored, associated with lesser time of residence in each feeding station, probably because the cost of travel was lesser than the benefit (Utsumi et al., 2009).

Utsumi et al. (2009) with an “artificial arrangement” of patches of fescue and alfalfa found that the cows increased their patch residence time by increasing the distance between patches, which also increased the travel speed between patches. The authors suggest that animal adopt a strategy linearly related to maximization of intake rate. On this study, the cows on TC remained more time in each feeding station and did more steps between feeding stations than TL cows. According to Utsumi et al. (2009), foragers maximizing intake rate should respond to an increase of travel time between patches by increasing the residence time in each patch. We have not found an effect on travel time between patches, but the residence time was lower on TM if it is compared with TL, resulting in greater number of patches explored by TM cows. On the other hand, the feeding stations explored per patch was greater on TL. These results suggest that TL achieve the selectivity at feeding station and bite scale while TM cows did it at patch scale. The patch can be defined as a spatial aggregation of feeding stations (and bites, consequently) over which intake or movement rate remains relatively constant (Bailey et al., 1996). Therefore, considering that the number of patches per hour was lower on TL than on TM, and analyzing the concept of patch, the TL cows had a more constant intake rate.

If departure decisions at patch scale are influenced by trade-offs between maximizing intake rate and food quality (Searle et al., 2005), we can think that TL patches were more attractive to the cows, because on this treatment the animals remained more time at each patch having the greater diet digestibility. On the other hand, TM cows explored more patches achieving the same dry matter intake than TL cows, but the selected diet was inferior in terms of quality and, consequently, the milk production lower as well.

The general rule is that large herbivores tend to spend more time in feeding stations as forage biomass increases (Searle et al., 2005), however, on this case, moreover the mean herbage mass did not differ between treatments, the cows on TL grazed more “high structures” (Giles et al., 2018) than on TM and on TC. Similar results were reported by Faber (2012) with the same pasture specie. In a short-term study on natural grasslands, Gonçalves et al. (2009) found the opposite, the animals increased the residence time and decreased the number of feeding stations visited when sward height increased. In accordance with our results, Mezzalana et al., (2013) observed that under low sward heights, animals explored more each feeding station, and they argue that probably grazing patterns were affected by spatial memory, giving that it was a large study (more than 4 months). On the same line, according to Roguet et al. (1998a) and Searle et al. (2005), grazing herbivores tend to utilize feeding stations in such a way as to remove more digestible forage before moving on; however, the animal can prioritize intake rate rather than selection when the transmission of signals to the brain in response to low amounts of digesta in the rumen occurs (Gregorini et al., 2009b). The latter probably occurred on TC and on TM, but with a different trade-off, in response to the different canopy structure they found.

Studying the effect of manipulated ruminal fill on beef heifers, Gregorini et al. (2007) observed that the “hungrier” (fewer ruminal fill) the animals were, the greater the time invested in each feeding station was, and the faster they searched. This can help explain why TC cows explored for a longer time each feeding station, since dry matter intake on this treatment remained below potential. This is in agreement with the assumption that cattle can reduce forage manipulation time during feeding to increase intake rate (Parsons et al., 1994). In the opposite, TL cows with greater

ruminal fill and a large bite mass (Short-term measurement; Oborsky et al., 2019), probably spent more time manipulating each bite while search for a new feeding station to explore. This leads us to believe that the difference in time spent ruminating per kg of NDF (15 min per kg of NDF less on TL than on TC) can be influenced by differences on processing during eating.

The information about feeding station exploration is widely variable between studies (Roguet et al., 1998b; Searle et al., 2005; Gregorini et al., 2007, 2009b, 2011; Gonçalves et al., 2009; Da Trindade et al., 2012; Amaral et al., 2013; Mezzalira et al., 2013, 2014), but there is a consensus that it is highly dependent of sward vertical structure and spatial distribution, herbage mass and internal state of the animals, resulting in a trade-off between quantity and quality and determining the behavior pattern adopted along the grazing down throughout the day and between the days.

## **2.6. CONCLUSIONS**

The lax management (TL) of the grass allowed high digestible dry matter intake and therefore better productive performance, achieved through behavioral adaptations throughout the grazing down process expressing greater selectivity. The TM cows adopted a behavioral strategy different from the others, achieving the same dry matter intake as the TL cows, but without success in terms of milk production. The height and structure of the pasture on TC imposed the cows for a lower intake rate, although they were less selective, probably trying to compensate the low ingestion. The response to heterogeneity is not the adoption of a uniformed pattern throughout the grazing down, but changing throughout the occupation period and throughout the day, demonstrating that the dairy cow, in grazing, has a great plasticity in its behavior and does it at different scales.



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### 3. RESULTADOS Y DISCUSIÓN GENERALES

Las alturas logradas postpastoreo están acorde a lo planteado como objetivo y si bien el TL tuvo solamente 1,5 cm más que TM, la estructura vertical del canopeo, representada por la altura de vaina, y la distribución horizontal del recurso, que se generaron a lo largo de 8 meses de manejo previo diferencial entre tratamientos llevó a que los animales tuviesen una respuesta comportamental distinta según cada tratamiento, más allá de la altura media postpastoreo per se. No obstante, se encontraron diferencias en el comportamiento de los animales ya en el inicio del período de ocupación (Figura 2), en el cual tanto la altura como la biomasa eran similares. Sin embargo, el TL presentó mayor dispersión de alturas que TM y TC.



Figura 2. Patrón de actividades de pastoreo y rumia durante 24 horas al inicio del período de ocupación según el tratamiento (TL = Tratamiento Laxo; TM = Tratamiento Medio; TC = Tratamiento Control).



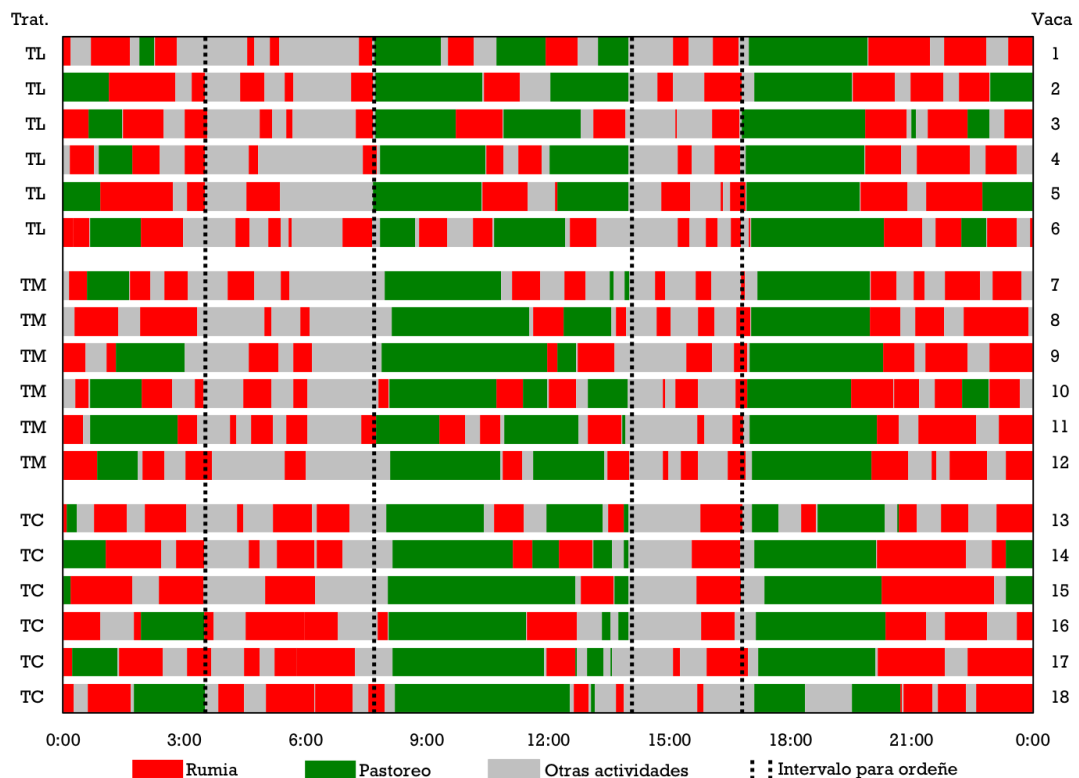


Figura 3. Patrón de actividades de pastoreo y rumia durante 24 horas al final del período de ocupación según el tratamiento (TL = Tratamiento Laxo; TM = Tratamiento Medio; TC = Tratamiento Control).

Las vacas del TM parecieron adoptar una estrategia comportamental distinta, ya que pastorearon por más tiempo al inicio (Figura 2) del período de ocupación que al final (Figura 3), así como las vacas del TL lograron consumir sus requerimientos en un menor tiempo al inicio del período de ocupación, mostrándose más selectivas que las demás ya que hicieron sesiones de pastoreo más cortas y en mayor número, comportamiento éste característico de ambientes con alta oferta de forraje (Carvalho, 2005).

Las mayores presiones de pastoreo obligan al animal a explorar horizontes de pastoreo, donde la relación hoja/vaina se reduce, disminuyendo la tasa de consumo (Amaral et al., 2013), como lo ocurrido con los tratamientos TM y TC. Las diferencias en consumo de materia seca encontradas en ese ensayo son esperables. El consumo de materia seca del TM no se vio reflejado en la producción de leche, como lo fue en el

TL, eso se debe a que el costo de mantener el mismo consumo fue pérdida de calidad en la dieta consumida (menor selectividad), afectando la respuesta en leche.

#### **4. CONCLUSIONES**

El manejo laxo del pasto (TL) permitió alto consumo de materia seca y por consiguiente mejor performance productiva, lograda a través de adaptaciones comportamentales a lo largo de los días de pastoreo expresando mayor selectividad.

Las vacas del TM adoptaron una estrategia comportamental diferente a las demás, logrando el mismo consumo de materia seca que las vacas del TL, pero sin éxito en términos de producción de leche.

Las vacas del TC no lograron compensar la menor tasa de consumo impuesta por la altura y estructura de la pastura, aunque fueron menos selectivas, probablemente con ese propósito.

La respuesta a la heterogeneidad no es la adopción de un patrón uniforme durante el pastoreo sino cambios a lo largo del período de ocupación y durante el día, lo que demuestra que la vaca lechera en pastoreo tiene una gran plasticidad en el comportamiento y lo hace a diferentes escalas.

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