**UNIVERSIDAD DE LA REPÚBLICA**

**FACULTAD DE AGRONOMÍA**

# SUPLEMENTACION ENERGÉTICO-PROTEICA Y DINÁMICA DE PASTOREO DE GANADO DE CARNE EN CRECIMIENTO PASTOREANDO DIFERIMIENTOS INVERNALES DE CAMPO NATURAL

**por**

**Fiorella Carla CAZZULI ALBA**

TESIS presentada como uno de los requisitos para obtener el título de Doctora en Ciencias Agrarias, opción Ciencias Animales

MONTEVIDEO

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Tesis aprobada por el tribunal integrado por el Ing. Agr. (MSc, PhD) Carlos Nabinger, el DMV (MSc, PhD) Eduardo Bohrer de Azevedo y el Ing. Agr. (MSc, PhD) Pablo Boggiano el 13 de junio de 2023. Autora: Ing. Agr. (MSc) Fiorella Cazzuli. Directora: Dra. (PhD) Carolina Bremm. Codirector: Ing. Agr. (PhD) Martín Durante.

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TABLA DE CONTENIDOS

RESUMEN

La pérdida de peso durante el invierno del ganado en crecimiento sobre campo natural puede ser revertida mediante la suplementación con energía y proteína. Para que esta práctica sea rentable, es necesario maximizar la eficiencia en el uso del suplemento —EUS—, diferencia en la ganancia media diaria —GMD—, entre animales suplementados —S— y testigo —T—, RespSup —respuesta a la suplementación— por kilo de materia seca —MS. Se compilaron 25 ensayos realizados en Uruguay entre 1993 y 2018. La EUS fue de 0,21 ± 0,076 kg RespSup/kg MS, promediando una RespSup de 0,38 ± 0,180 kg/an/día. No se encontró asociación entre EUS y tasa de suplementación ni tipo de suplemento ($p < 0,05$), pero la asignación de forraje la afectó negativamente, mientras que la disponibilidad la afectó positivamente, si bien en menor magnitud. Las condiciones del clima afectaron la EUS ($p < 0,05$), con mayores EUS en inviernos de temperaturas más bajas y con más heladas. Otro análisis conjunto se realizó con 15 de los 25 ensayos originales para estimar las tasas de sustitución (TS) y determinar la existencia de distintas fases en la evolución de la RespSup a partir de sus patrones de evolución. Las TS fueron de 0,3-1,1 kg/kg y fueron asociadas negativamente con EUS. Tres tipos de RespSup fueron identificadas: linear, cuadrática y Weibull. Las cuadráticas estuvieron asociadas con la disponibilidad y tasas de sustitución, mientras que las Weibull estuvieron asociadas con las heladas. Finalmente, un experimento de tipo *grazing-down* fue realizado comparando animales S y T en su dinámica de defoliación. No se encontraron diferencias entre la dinámica horizontal, y cuando el horizonte superior fue consumido en > 80 %, el horizonte subsecuente había sido consumido en un 50 %. La suplementación invernal sobre campo natural es una tecnología altamente confiable que mejora el desempeño del ganado de carne en crecimiento.

**Palabras clave**: suplementación energético-proteica, campo natural, ganado de carne, dinámica de pastoreo

SUMMARY

ENERGY-PROTEIN SUPPLEMENTATION AND GRAZING DYNAMICS OF GROWING BEEF CATTLE GRAZING STOCKPILED NATIVE GRASSLANDS IN WINTER

The weight loss of growing beef cattle grazing native grasslands in winter may be reverted by supplementing with energy and protein. To render this practice profitable, its efficiency -SFE- difference in average daily gain- ADG - between supplemented - S - and control - C animals- ADGchng - supplement response - per kg of dry matter -DM intake- must be controlled. Twenty-five trials carried out in Uruguay between 1993 and 2018 were gathered. The average SFE was 0.21 ± 0.076 kg/ADGchng/kg DM, from an average ADGchng of 0.38 ± 0.180 kg/animal/day. No association was found between SFE and supplementation rate nor type ($p < 0.05$), but forage allowance negatively affected it while herbage mass positively affected it, yet in a smaller magnitude. Weather conditions during trials affected SFE ($p < 0.05$), with greater SFE in winters with lower temperatures and more frosts. Another collated analysis using only 15 of the original 25 trials was performed to estimate supplement substitution rates (sSbR) and to assess the existence of different phases and ADGchng evolution patterns. sSbR were 0.3-1.1 kg/kg and were negatively associated with SFE. Three responses were identified (linear, quadratic and Weibull). Quadratic patterns were closely associated with sward biomass and substitution rates, while Weibull were associated with frosts. Finally, a grazing-down experiment was performed comparing S and C animals in their grazing dynamics. No differences were found between the horizontal dynamics, and when the upper grazing horizon was depleted by > 80 %, the subsequent horizon was being depleted by 50 %. Supplementation is a highly reliable technology for improving the performance of growing beef cattle.

**Key words**: energy-protein supplementation, native grasslands, beef cattle, grazing dynamics

# 1. INTRODUCCIÓN

## 1.1. CONTEXTO

La ganadería de carne basada en un uso bajo o controlado de insumos externos de los pastizales del Río de la Plata, dentro del bioma Pampa enfrenta varios desafíos de intensificación sostenible, como aumentar su productividad, detener la conversión del uso de suelo hacia otros rubros (agricultura o forestación), proteger la provisión de servicios ecosistémicos y continuar siendo una fuente de ingresos sustentable para las personas vinculadas al rubro. Jaurena et al. (2021) plantean opciones de intensificación para enfrentar dichos desafíos, posicionando al campo natural como la base forrajera principal, pero amortiguando a su vez sus principales limitantes inherentes: marcada estacionalidad productiva y valor nutricional muchas veces insuficiente para estar a la altura de los objetivos productivos de las empresas agropecuarias.

Entre dichas opciones de intensificación, estos autores mencionan varias tecnologías: diferimiento de forraje, control de la estructura de la pastura, entre otras (figura 1). En las siguientes secciones se describirán brevemente cada una de ellas.

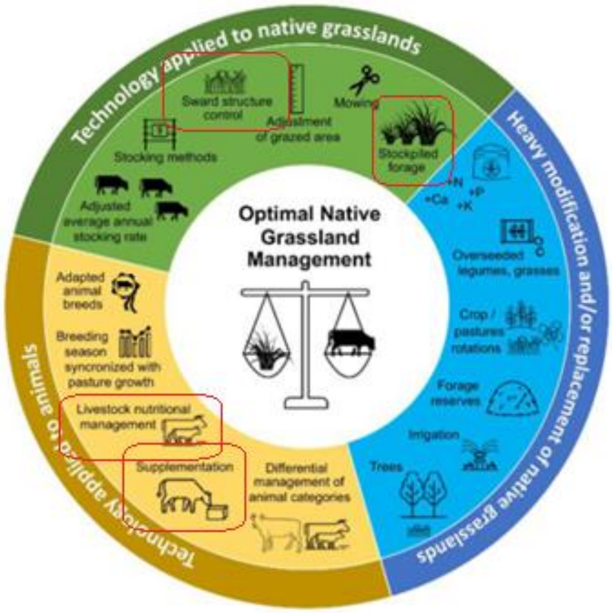


**Figura 1.** Tecnologías propuestas por Jaurena et al. (2021) para contribuir a levantar las limitantes que ofrece el campo natural, pero manteniéndolo como base forrajera principal.

Nota: en rojo se resaltan las tecnologías particularmente importantes para el presente trabajo.

### 1.1.1. Diferimiento de forraje

Según la terminología sugerida por Allen et al. (2011), un forraje al que se le permite acumularse para ser pastoreado más adelante en el tiempo es un forraje acumulado, que también puede referirse como forraje diferido (o diferimiento de forraje). Sollenberger et al. (2012) definen a los diferimientos como la acumulación de forraje en ausencia de defoliación, para su posterior uso cuando la pastura presenta tasas de crecimiento limitadas. Su principal ventaja es que es una tecnología simple y de bajo costo. La contracara es que se genera un compromiso entre la cantidad de forraje acumulado y su calidad. A medida que se acumula biomasa, los componentes asociados con el valor nutritivo (como el porcentaje de proteína) disminuyen proporcionalmente, lo cual se conoce como efecto dilución (Lemaire y Belanger, 2020) (figura 2).

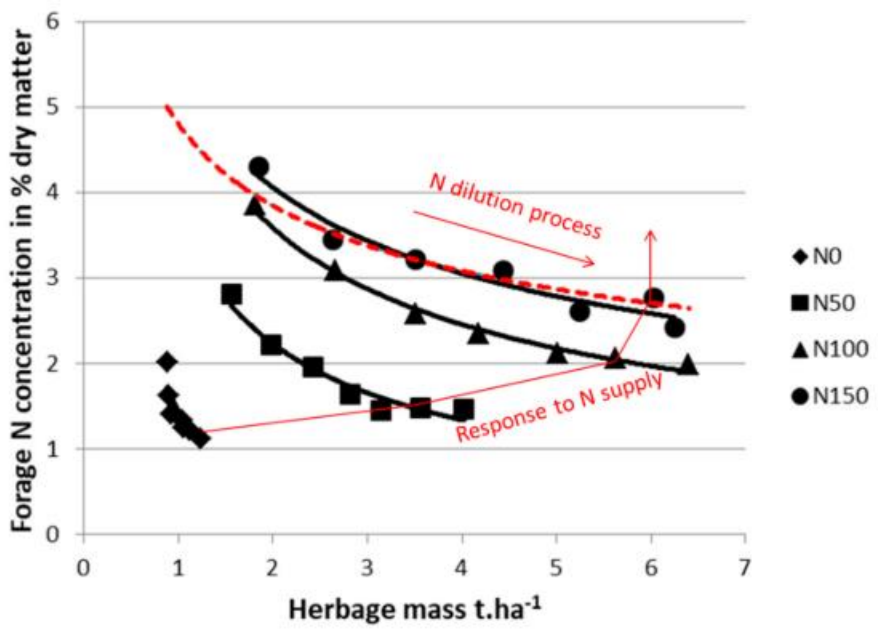


**Figura 2.** Variaciones de concentración de N en relación con la biomasa de forraje durante el crecimiento primaveral de festuca bajo diferentes dosis de N aplicado.

Fuente: Lemaire y Belanger (2020), adaptado de Lemaire y Denoix (1987).

El efecto de dilución ha estado estudiado mayoritariamente en pasturas cultivadas antes que, en campos naturales, específicamente de los pastizales del Río de la Plata, si bien existen algunos antecedentes (Ayala y Carámbula, 1994). En los sistemas productivos, como las praderas de Norteamérica, el diferimiento (*grassbancking* o *stockpiling*) puede usarse como medida de manejo adaptativo a la sequías (Derner y Augustine, 2016), pero no únicamente. En el caso del bioma pampa, uno de los diferimientos de forraje más comunes es el generado en otoño para ser consumido en el invierno, cuando el crecimiento del campo se ve limitado por las bajas temperaturas. De manera de lograr un mejor aprovechamiento del diferimiento, Jaurena et al. (2021) proponen utilizar categorías animales de menores requerimientos y seleccionar áreas más aptas para la acumulación de forraje de calidad (Lemaire y Belanger, 2020).

Lograr un balance adecuado entre cantidad total de biomasa acumulada y su valor nutritivo es clave para poder hacer una óptima utilización de una pastura diferida, ya sea que se le agregue suplementación energético-proteica o no. En el presente trabajo, se hace foco en el uso de campos naturales diferidos desde el otoño hacia el invierno, combinados con suplementación estratégica energético-proteica.

#### 1.1.2. Suplementación invernal energético-proteica

La suplementación es una herramienta de manejo que permite mejorar la respuesta productiva de los animales. Según Quintans (2014), el incremento de los niveles nutricionales en momentos de demandas fisiológicas clave puede llevarse adelante de diversos modos, particularmente en casos de limitantes de disponibilidad de forraje o cuando exista un inadecuado balance nutricional. Esto ocurre para el campo natural durante los meses invernales, particularmente aquellos dominados por especies de gramíneas perennes estivales de baja productividad o valor nutritivo invernal.

En una modelación de la evolución esperable del peso vivo de ganado de carne que compara esquemas de campo natural «tradicional» con otros esquemas más intensificados (Montossi et al., 2014), se observa que, durante el primer invierno, la suplementación revierte el descenso esperado del peso vivo de los animales, ante una alternativa de continuar sobre campo natural (figura 3).

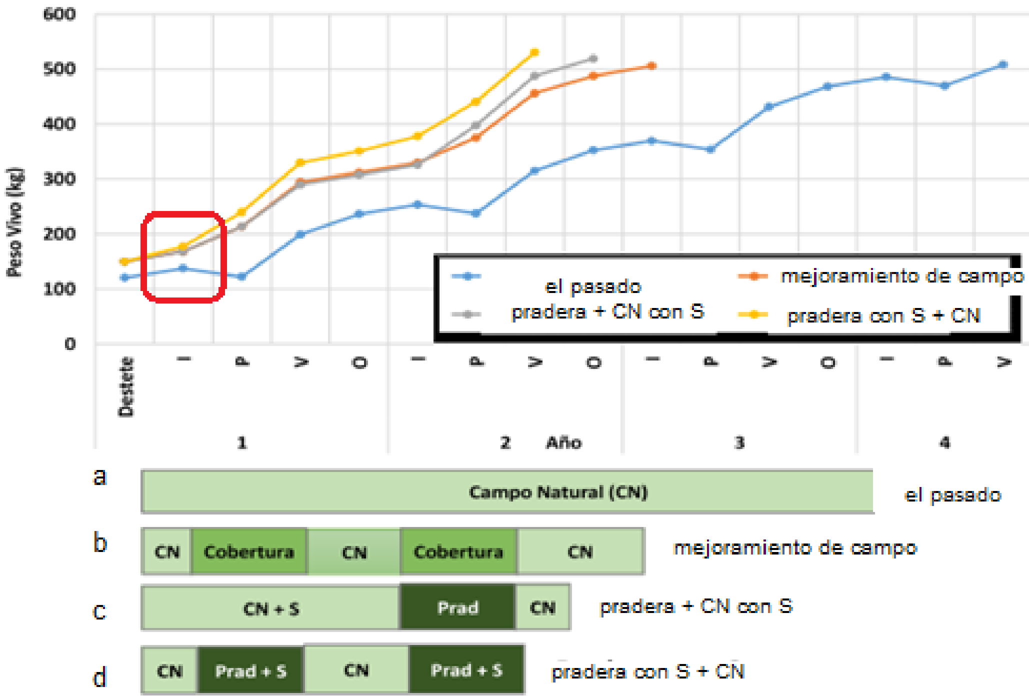


**Figura 3.** Impacto de la intensificación de la invernada sobre el crecimiento de los animales y su edad de faena (adaptado de Montossi et al., 2014).

Nota: el recuadro rojo representa la evolución positiva de peso vivo en el escenario *c*, con respecto a la evolución negativa del escenario *a*, durante el primer invierno de los animales.

La fuente utilizada para suplementar no es tan importante como el hecho de ofrecer un aumento comparativo de nutrientes mediante un suplemento. Cazzuli (2017) concluyó en su revisión bibliográfica que la suplementación invernal sobre campo natural en vacunos presentó respuesta positiva a la suplementación, independientemente del tipo de suplemento utilizado y la presencia o no de forraje diferido. Por ejemplo, utilizando el silo grano húmedo de sorgo, la respuesta animal osciló entre 0,188 y 0,632 kg/an/día, si bien se registraron casos puntuales de ausencia de respuesta. Al utilizar afrechillo de arroz, siempre se registraron respuestas positivas en la ganancia de peso vivo (PV), si bien la magnitud de esta dependió de la acumulación previa de forraje, ya que, con diferimiento de forraje, las ganancias logradas fueron superiores que sin acumulación previa otoñal.

Existe una interacción entre el forraje ofrecido y la suplementación; esto determina que puedan darse fenómenos de sustitución de forraje por suplemento o de descenso del consumo de forraje de animales suplementados con respecto a los no suplementados. Moore et al. (1999) afirman que los desvíos entre el desempeño animal esperado y el observado generalmente se explican por los efectos asociativos de la suplementación sobre el consumo voluntario y la concentración total de energía de la dieta. Esto significa que se pueden presentar fenómenos de sustitución de forraje por suplemento (Stockdale, 2000) y que la magnitud de estos efectos puede tener consecuencias relevantes por el impacto en el beneficio económico marginal de la suplementación, ya que los suplementos son más caros que el forraje.

En cuanto a la eficiencia de uso del suplemento (EUS) —entendida como la cantidad de suplemento necesaria para generar un kilo extra de peso vivo—, en los trabajos experimentales de Cazzuli (2017) se constató que es posible controlar este parámetro de eficiencia mediante la restricción de la tasa de suplementación, en comparación con regímenes *ad libitum*. De todos modos, ni en este trabajo experimental ni en ninguno de la revisión bibliográfica que lo antecede se mencionan las asociaciones posibles entre las características de la pastura y la EUS ni las variaciones que podrían darse a lo largo de todo el período de suplementación (diferentes fases) en campo natural.

### 1.1.3. Estructura de la pastura y dinámica de defoliación

Según Marriott y Carrère (1998), la estructura de la pastura es el arreglo tridimensional de los componentes de su biomasa. Específicamente, estos autores, adaptando un trabajo de Noy-Meir (1995), señalan que la estructura vertical tiene especial impacto sobre procesos de pastoreo en pequeña escala y corto plazo, mientras que la estructura horizontal es importante en todas las escalas de tiempo y espacio. Coincidentemente, Cangiano et al. (2002) sostienen que tanto la distribución vertical como la horizontal son importantes para comprender las interacciones entre el tapiz y los animales. A su vez, Török et al. (2018) sugieren que la propia actividad de pastoreo altera la estructura y el funcionamiento de los tapices naturales, lo cual demuestra la elevada complejidad inherente a los sistemas pastoriles, especialmente basados en campo natural.

La estructura de cualquier pastura es importante, ya que está fuertemente asociada al consumo de forraje de los animales. Entre la estructura del pasto que afecta el consumo se encuentra la proporción relativa de tejidos verdes y la altura del tapiz. Específicamente en pasturas con predominio de especies C4, (si bien también ocurre en pasturas con predominancia de especies C3 (Hodgson et al., 1977), cuanto mayor sea la proporción de hojas verdes y de biomasa verde en general, mayor es el consumo animal (Burns y Sollenberger, 2002). Por otro lado, Boval et al. (2007) encontraron que la altura de una pastura dominada por una especie C4 estaba positivamente correlacionada con la profundidad, el tamaño y la tasa del bocado. Da Trindade et al. (2016) trabajaron sobre campo natural del bioma Pampa (pastizales del Río de la Plata) y reportaron que alturas de 11-13 cm optimizan el consumo de forraje de ganado de carne en crecimiento.

Por otro lado, Delagarde et al. (2000) reportaron importantes variaciones a lo largo del perfil de raigrás bajo pastoreo rotativo en términos de composición química, lo que demuestra que, incluso en pasturas monoespecíficas, la estructura interna de los tapices puede ser muy heterogénea. En pasturas pluriespecíficas y por lo tanto, mucho más heterogéneas, como puede ser una comunidad de campo natural, se espera observar variaciones intraestructura de gran magnitud.

En cuanto a la dinámica de defoliación observable en procesos de pastoreo, existen varios trabajos que describen este fenómeno, especialmente los que se basan en ensayos de tipo *grazing-down*. Un ejemplo es el publicado por Ison et al. (2019) trabajando con vacas lecheras pastoreando alfalfa. Los animales pastoreaban el estrato superior del tapiz y solo luego de cubrir el 80 % del área de pastoreo volvían a pastorear otras áreas del potrero. Además, el descenso del consumo animal era explicado por la transición del horizonte superior de pastoreo al resto de los horizontes subyacentes. Por otro lado, Benvenutti et al. (2015), trabajando con pasturas tropicales (*Axonopus catarinensis,* Valls) y ganado de carne, también encontraron que los animales solo pastoreaban los estratos más bajos del tapiz una vez que el estrato superior ya había sido fuertemente consumido.

Quedó referenciada, entonces, la importancia que tiene la estructura de la pastura sobre el consumo animal, así como las relaciones tan complejas que existen entre el animal, la pastura y la dinámica de pastoreo. En ninguno de los casos mencionados en esta sección se agregó la complejidad extra que implicaría una suplementación energético-proteica.

## 1.2. VACÍO DE INFORMACIÓN

Si partimos de la base de la importancia que tiene la estructura de la pastura sobre el consumo animal, y sobre eso agregamos un proceso de suplementación, se generan relaciones todavía más complejas en el comportamiento ingestivo (Da Silva et al., 2009). Entre estas complejidades se pueden citar selectividades diferenciales entre animales suplementados y no suplementados. Por ejemplo, Pötter et al. (2010) encontraron que animales suplementados fueron más selectivos y consumieron más hojas que los no suplementados, lo que evidencia parte de esta complejidad. A su vez, Pérez-Prieto et al. (2011) reportaron que el valor nutritivo de la pastura seleccionada mejoraba con mayores asignaciones de forraje y el agregado de suplementación. No obstante, el efecto de la suplementación sobre la calidad del forraje ingerido no está del todo demostrado. French et al. (2001) reportaron que, al ofrecer suplementos concentrados en condiciones de asignaciones forrajeras medias y altas, no variaba la digestibilidad de la materia orgánica de la dieta, a pesar de poder seleccionar forraje de mejor digestibilidad que sus compañeros no suplementados, dada la tasa de sustitución de forraje por suplemento. Según Barbero et al. (2015), menores alturas del tapiz de pasturas con *Brachiaria bizantina* (Hochst ex A. Rich, Stapf) en situaciones de suplementación son la clave para mejorar la eficiencia del sistema.

También existe una interacción entre la estructura de la pastura y la suplementación. Barbero et al. (2015) demostraron que diferentes combinaciones de alturas y niveles de suplementación modifican la ingesta de MS de forraje de vacunos de sobreaño pastoreando *Brachiaria sp*. Newman et al. (2002) encontraron que la altura del tapiz de una pastura de *Hemarthria sp.* afectó la AF y parcialmente la GMD de vaquillonas con y sin suplementación proteica.

Si bien existen estudios sobre el comportamiento ingestivo en campo natural del bioma Pampa (Da Trindade et al., 2012, 2016) (pastizales del Río de la Plata), hasta donde sabemos no existe literatura que caracterice cómo es el comportamiento ingestivo de bovinos sobre campo natural en términos de estratos de pastoreo ni cómo interacciona este al introducir la suplementación como parte de la dieta de los animales.

El presente trabajo tiene como propósito realizar una contribución adicional al conocimiento general y de los mecanismos subyacentes de todas estas tecnologías, de manera de poder mejorar su diseño, metodología y eventual aplicación.

## 1.3. HIPÓTESIS

Factores —como la estructura de la pastura, valor nutritivo del forraje y del suplemento— afectan la eficiencia del uso del suplemento energético-proteico del ganado en esquemas de suplementación sobre campo natural diferido. Además, la suplementación afecta la dinámica de pastoreo de los animales suplementados en comparación con animales testigo.

## 1.4. OBJETIVOS

### 1.4.1. Objetivo general

Evaluar la interacción planta-animal-suplemento (energético-proteico) en vacunos de carne en fase de crecimiento durante el invierno, pastoreando campo natural diferido desde el otoño.

### 1.4.2. Objetivos específicos

Se plantean los siguientes objetivos específicos:

a) Cuantificar la magnitud y variación de la EUS y analizar las posibles asociaciones de la EUS con características del forraje, animales, suplementos y clima.
b) Analizar la evolución de la EUS a lo largo del período de suplementación.
c) Caracterizar la dinámica de desaparición de forraje del campo natural diferido con animales con y sin suplementación.

## 1.5. ESTRUCTURA GENERAL DE LA TESIS

El capítulo 1 desarrolla la introducción al tema, las hipótesis, los objetivos y el esquema general de la tesis.

Los capítulos 2, 3 y 4 se corresponden con los objetivos a, b y c respectivamente y constituyen el cuerpo principal del trabajo.

Específicamente, el capítulo 2 trata del análisis de una recopilación de los trabajos de suplementación invernal de vacunos en crecimiento sobre campo natural en Uruguay, centrando el análisis en la eficiencia de uso del suplemento promedio. Este artículo fue publicado en el *Translational Animal Science Journal*, de la American Society of Animal Science.

El capítulo 3 es la continuación del anterior —trabajo con bases de datos de ensayos previos—, esta vez centrado en la estimación de las tasas de sustitución y de las diferentes fases identificables en el proceso de suplementación. Este artículo publicado en el *Grasses Journal*, del Multidisciplinary Digital Publishing Institute (MDPI).

El capítulo 4 trata los resultados de un ensayo de campo en donde el objetivo fue aportar más información en temas relacionados a la dinámica de pastoreo y su interacción con la suplementación. Este artículo fue publicado en el *Grass and Forage Science Journal*, de la British Grassland Society y la European Grassland Federation.

El capítulo 5 presenta la discusión general de todo trabajo, integrando los resultados para generar una síntesis conjunta, y el capítulo 6 plantea someramente las principales conclusiones. Por último, el capítulo 7 presenta la bibliografía correspondiente a los capítulos 1, 5 y 6.

## 2. SUPPLEMENT FEED EFFICIENCY OF GROWING BEEF CATTLE GRAZING NATIVE CAMPOS GRASSLANDAS DURING WINTER: A COLLATED ANALYSIS

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## 2.1. LAY SUMMARY

Beef cattle are reared on native grasslands worldwide. In the native *Campos* –the subtropical humid grasslands part of the Pampa biome in southern South America– animals often lose weight during winter due to insufficient quantity or quality of available forage. Therefore, supplementation with concentrates is advocated. Notwithstanding its productive impact, this practice is unprofitable when supplement feed efficiency is low. We collated data from 25 trials carried out from 1993 to 2018 in Uruguay to better understand how and why supplement feed efficiency varies in growing cattle grazing stockpiled *Campos*. On average animals gained 0.21 kg of body weight per kg of consumed supplement, but variation was large. Winters with more frosts resulted in greater responses, rendering the practice more efficient. The amount of forage per animal negatively affected efficiency, while overall forage availability positively affected it (yet in a smaller magnitude), suggesting that in order to be more efficient, a balance between them is needed. Protein concentration of supplements was associated with supplement feed efficiency. Nonetheless, the proportion of green biomass in offered herbage did correlate with weight

gain and grazing behaviour of the cattle. The total digestible nutrients-to-protein ratio of Campos winter herbage –halfway between that of rangelands and sown pastures– would explain the observed relatively high supplement feed efficiency.

**Teaser text:** Enhancing the efficiency with which supplements are used on cattle grazing Campos would increase profits, reduce economic risks, and reduce overgrazing of these native grasslands, thus improving the overall sustainability of Uruguayan extensive beef production.

## 2.2. ABSTRACT

Supplementing growing cattle grazing native subtropical *Campos* grasslands during winter improves the low, even negative, average daily weight gain (ADG) typical of extensive animal production systems in Uruguay. Nonetheless, to render the practice profitable it is crucial to control supplement feed efficiency (SFE), that is, the difference in ADG between supplemented and control animals (ADGchng) per unit of supplement dry matter (DM) intake. Little has been studied specifically on how SFE varies in these systems. The objective of this study was to quantify the magnitude and variation in SFE of growing beef cattle grazing stockpiled native *Campos* grasslands during winter and assess putative associations with herbage, animals, supplements, and climatic variables. We compiled data from supplementation trials carried out in Uruguay between 1993 and 2018, each evaluating between one and six supplementation treatments. The average ADG of unsupplemented and supplemented animals were 0.13±0.174 and 0.49±0.220 kg/animal/day, respectively. In both cases, ADG decreased linearly as the proportion of green herbage in the grazed grassland was lower, but the ADG of unsupplemented animals was further reduced when winter frosts were numerous. Estimated SFE were moderately high, with an average of 0.21±0.076 ADGchng/kg DM, resulting from average ADGchng of 0.38±0.180 kg/animal/day in response to an average supplementation rate of 1.84±0.68 kg supplement DM intake/animal/day (0.86±0.27 %body weight). No association was found between SFE and supplementation rate nor type (protein vs. energy-based; $p>0.05$), but forage allowance negatively affected it, and herbage mass positively affected it, yet in a smaller magnitude, suggesting that a balance is needed between the two to maximize SFE. Weather conditions during trials affected SFE ($p<0.05$), with greater SFE in winters with lower temperatures and more frosts. Daytime grazing time was consistently lower in supplemented animals compared to their unsupplemented counterparts, whereas ruminating time during the day was similar, increasing as the proportion of green herbage

decreased. Herbage intake estimated from energy balance suggested the existence of some substitution effect. This agrees with the moderately high SFE and with the total digestible nutrients-to-protein ratio of these subtropical humid grasslands being higher than in semiarid rangelands and dry-season tropical pastures but lower than in sown pastures.

**Abbreviations:** ADG, average daily gain; SFE, supplement feed efficiency; ADGchng, change in ADG (supplemented – control); DM, dry matter; TDN, total digestible nutrients; CP, crude protein; DDGS, dry distillers grains with solubles; BW, body weight; FA, forage allowance; gFA, green forage allowance, NDF, neutral detergent fibre, ADF, acid detergent fibre, DMD, dry matter digestibility; ME, metabolisable energy; cADG, control animals´ ADG; sADG, supplemented animal´s ADG; HDMI, herbage dry matter intake; DOM, digestible organic matter.

**Key words:** concentrate supplementation, growing cattle, native grasslands, nutritive value, protein, supplement feed efficiency.

## 2.3. INTRODUCTION

Native grasslands are the foundation of extensive animal production agroecosystems worldwide (Jaurena et al. 2021). However, the nutrients they provide are not always sufficient to meet desired animal performance goals. This is often the case for growing ruminants during winter or the dry season, when available herbage is either insufficient or of limited nutritive value (Hall et al., 1998; Williams et al., 2018; Orcasberro et al., 2021).

Complementing the diet of grazing animals with energy, protein and mineral supplementation, can help overcome such constraints and enhance production, but it also increases economic and financial risks whenever profits are sensitive to changes in costs, as is often the case for extensive systems (DelCurto et al. 2000; De Figueiredo et al., 2007; Bowen & Chudleigh, 2020). Therefore, it is crucial to anticipate the economic efficiency of supplementation, which is in part determined by the supplement feed efficiency (SFE). Usually, SFE is expressed as the difference in average daily liveweight gain (ADG) between supplemented and control (unsupplemented) animals (ADGchng) per unit of supplement intake, the latter typically expressed on a dry matter (DM) basis.

For animals grazing sown pastures, variation in SFE is often attributed to variation of substitution effects, that is, the decrease in intake of grazed herbage observed in supplemented animals (Grainger & Mathews, 1989; Moore et al. 1999; Clariget et al. 2021a). Therefore, SFE often correlates with herbage offer, allowance, and/or nutritive value because these modulate substitution effects. Conversely, for animals grazing native grasslands, analyses of what drives SFE are scarcer. In a comprehensive review, Moore et al. (1999) observed that supplementation consistently increased herbage intake of animals grazing native *prairies* of North America (n=55), presumably improving SFE, whereas it decreased herbage intake in animals grazing sown pastures (n=132). This differential response between these two forage bases was associated with the ratio of total digestible nutrients-to-protein (TDN:CP) of 7 in the grazed herbage (Moore et al., 1999), and therefore to protein deficiency (DelCurto et al., 2000). The conclusion that the primary limiting nutrient is crude protein (CP) and substitution effects are small was also reached for cattle grazing Australian rangelands or tropical pastures during the dry season (Poppi et al., 2018).

The *Campos* are subtropical humid highly diverse native grasslands that foster extensive sheep and cattle production across the Pampa biome in Uruguay, southern Brazil, and center-eastern Argentina. These systems are currently facing the challenge of both increasing profitability and at the same time maintaining native grasslands as their main feed source in order to keep low system-wide costs (Jaurena et al., 2021), be resilient against extreme climatic events (Briske 2017), and preserve the various valuable ecosystem services they provide (Modernel et al., 2016; Tittonell, 2021).

*Campo*s grasslands benefit from relatively mild temperatures and no dry season, but during winter, dominant C4 grasses show rapid decreases in growth rate and loss of nutritive value, frequently resulting in reduction or loss of animal performance (Beretta et al., 2000) and well-being. Therefore, winter supplementation is advocated to ensure both adequate reproductive function of females and defined growth paths and slaughter ages of males (Luzardo et al., 2014ab; Simeone et al., 2010; Cazzuli et al., 2018). Yet, little is known about the magnitude and causes of variation in SFE in these systems. This increases uncertainty in assessing productive and economic impact of supplementation, impairing farmers´ decision-making.

One source of variation in SFE might be type of supplement. A wide range of subproducts are locally available for animal feed, such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*) bran, maize (*Zea mays*) and sorghum (*Sorghum bicolor*) grain, dry distillers grains with solubles (DDGS), as well as high-protein concentrates (Montossi et al., 2014). Additionally, SFE might depend on the quantity and quality of herbage on offer, which depends on management of autumn stockpiling and winter stocking rate (Fedrigo et al. 2022; DoCarmo et al. 2018). Furthermore, in contrast to rangelands in other climates, *Campos* grasslands retain variable amounts of green biomass over winter, depending on the proportion of C3 species present in the sward and the number and intensity of frosts, which may affect the protein concentration of available herbage (Nuñez et al. 2022).

The aims of this study were i) to quantify SFE and its variability in growing cattle grazing native *Campos* grasslands during winter, and ii) to assess whether SFE is associated with pasture, animal, supplement, or climatic variables to infer putative causes of its variation. To this end, we collated and analysed a largely unpublished dataset of 25 trials of late autumn-winter concentrate supplementation of growing beef cattle grazing *Campos* grasslands carried out in Uruguay over the last 30 years.

## 2.4. MATERIALS AND METHODS

### 2.4.1. Database compilation

Data were gathered from 25 supplementation trials carried out between 1993 and 2018 in which growing beef cattle grazing native *Campos* grasslands in Uruguay were supplemented during late autumn and winter. These trials represent the totality of experiments carried out in Uruguay with those characteristics and within the mentioned period (1993-2018), and only cases using lick blocks as supplements were excluded, as well as validation experiences (no experimental control). All trials included an unsupplemented control treatment. Experimental units consisted of a group of animals grazing an independent paddock. On average, trials had seven animals per paddock, but variation was large, with a minimum of 4 and a maximum of 30. Most trials had several supplemented treatments, depending on their specific objective (comparison of supplement type, supplementation frequency, method, rate, etc.) and were carried out with seven-to-nine-month calves (male or female), but six trials used 18-month steers. Cattle breeds were Hereford, Aberdeen Angus, their cross, or Braford.

Sixteen trials had two replicates spatially arranged in a completely randomized design, whereas nine trials had only one paddock per treatment (Table 1). The collated database comprised a total of 108 comparisons between an unsupplemented (control) and a supplemented treatment. Only two of these datasets were published as refereed articles. For the remaining cases, the responsible researchers were contacted to access the experimental protocol and original dataset. Annex 1 briefly summarizes each trial.

**Table 1.** Summary of collated trials on supplementation of cattle grazing native rangelands carried out in Uruguay between 1993 and 2018. All trials included one unsupplemented control treatment.

| Trial ID | Location, Year | Supplementation treatments* | Breed and category | Duration (days) | Replicates | Animals/trial | *n* SFE** | Source |
|---|---|---|---|---|---|---|---|---|
| 1* | La Magnolia, 2013 | TMR with fibre | Braford male calves | 97 | 2 | 40 | 6 | Cazzuli et al. (2018) |
| 2* | Glencoe, 2013 | TMR with fibre | Hereford male calves | 120 | 2 | 40 | 6 | Cazzuli et al. (2018) |
| 3* | La Magnolia, 2014 | RB (ground and pelleted) | Braford male calves | 68 | 2 | 40 | 8 | Montossi et al. (2017) |
| 4* | Glencoe, 2014 | RB (ground and pelleted) | Hereford male calves | 108 | 2 | 50 | 8 | Montossi et al. (2017) |
| 5* | Glencoe, 2015 | Various (maize, expellers and RB) | Hereford male calves | 141 | 2 | 50 | 8 | Montossi et al. (2017) |
| 6* | Glencoe, 2009 | RB | Hereford male and female calves | 113 | 2 | 48 | 6 | Luzardo et al. (2014a) |
| 7* | Glencoe, 2010 | RB | Hereford male calves | 111 | 2 | 48 | 6 | Luzardo et al. (2014a) |
| 8* | Glencoe, 2011 | RB | Hereford male calves | 119 | 2 | 48 | 6 | Luzardo et al. (2014a) |
| 9 | Palo a Pique, 2009 | HMSGS | British crossbred male calves | 99 | 1 | 56 | 3 | Rovira and Velazco (2014) |
| 11 | Palo a Pique, 2009 | HMSGS | British crossbred male calves | 84 | 1 | 54 | 4 | Rovira et al. (2014) |
| 12* | Palo a Pique, 2013 | HMSGS | British crossbred steers | 84 | 2 | 32 | 6 | Rovira (2014a) |
| 13 | Salsipuedes, 2009 | TMR | British crossbred female calves | 84 | 1 | 90 | 2 | Blasina et al. (2010) |
| 14* | Ptas del Chuy, 2011 | TMR | British crossbred male calves | 81 | 2 | 48 | 4 | Esteves et al. (2013) |
| 15* | Palo a Pique, 2014 | HMSGS | British crossbred steers | 55 | 2 | 32 | 6 | Rovira (2014b) |
| 16* | Glencoe, 2007 | RB | British crossbred male calves | 98 | 2 | 24 | 2 | Luzardo et al. (2014b) |
| 17 | Palo a Pique, 2012 | TMR with fibre | British crossbred male calves | 77 | 1 | 12 | 1 | Rovira (2014c) |
| 20 | Palo a Pique, 2000 | RB and TMR | British crossbred female calves | 87 | 1 | 30 | 2 | Campos and Terra (2002) |
| 21 | Glencoe, 2005 | Various (maize, expeller and RB) | British crossbred male calves | 96 | 1 | 40 | 4 | Pittaluga et al. (2005) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22 | Glencoe, 2011 | RB | Hereford steers | 97 | 1 | 18 | 2 | Brito et al. (2011) |
| 24* | Glencoe, 2004 | Various (RB and expeller) | British crossbred steers | 42 | 2 | 70 | 4 | Arrieta et al. (2008) |
| 25* | Glencoe, 2004 | Various (RB and expeller) | British crossbred steers | 78 | 2 | 70 | 8 | Arrieta et al. (2008) |
| 26 | Palo a Pique, 1992 | RB | Hereford female calves | 89 | 1 | 80 | 3 | Quintans et al. (1993) |
| 27 | Palo a Pique, 2008 | TMR | British crossbred male calves | 77 | 1 | 56 | 3 | Rovira and Velazco (2012) |
| 28* | Cañada del Pueblo, 2008 | DDGS | Hereford female calves | 89 | 2 | 40 | 2 | Berretta et al. (2019) |
| 29* | Tomás Gomensoro, 2008 | DDGS | British crossbred male calves | 84 | 2 | 40 | 2 | Berretta et al. (2019) |
| Total | | | | | | 1156 | 108 | |

TMR: total mixed ration; RB: rice bran; HMSGS: high-moisture sorghum grain silage (combined with protein supplements); * Trial with 2 replicates; ** Number of calculated SFE values.

In all trials, paddocks were continuously stocked at a fixed stocking rate for the duration of the trial (stocking period). The trials´ stocking period extended from June/July to September, i.e. the local winter, lasting 42 to 141 days (except for one that started late in May, and two that ended in mid-October). Herbage intake was always voluntary, by direct grazing of herbage stockpiled prior to the stocking period. Stockpiled herbage accumulated over variable periods of time, following either a mechanical cut or short-term heavy grazing in late summer/early autumn. Therefore, the amount and proportion of green mass present at the start of the stocking period varied greatly between trials. Concentrate supplements were fed either manually in their troughs (daily or every two to four days) or using self-feeders, at a rate of between 0.5 and 1.7 % of body weight (BW).

Diverse concentrate supplements were used in each trial, including rice bran, DDGS, soy (Glycine max) and sunflower (Helianthus annuus) expellers, maize and high-moisture sorghum grain silage with or without added nitrogen. Supplements had similar metabolic energy concentration but varied widely in CP concentration. Consequently, the energy-to-protein ratio of supplements varied greatly between trials. Supplements were categorized according to their CP concentration: above 20% (Harris, 1980) supplements were considered protein based. In all trials, supplemented animals were gradually acclimated to supplements over a 7 to 10 d period prior to the stocking period.

Animal behavior was registered in 14 trials, with observers using binoculars throughout daylight hours (0700-1830 h, approximately), registering the activity of all animals of each plot every 15 minutes and classifying them into grazing and ruminating, among other activities.

#### 2.4.2. Response and auxiliary variables

Animals were weighed individually at the beginning and at the end of the stocking period. In most cases, shrunk weight was determined, using 12-16 h of fasting (Meyer et al., 1960; Watson et al., 2013), but in some cases only unshrunk weight was available and a 6% adjustment was applied to make all data more comparable following  Clariget et al. (2021b). Then ADG was calculated as final minus initial average BW of all animals in each paddock (experimental unit), divided by the stocking period (days). The response to supplementation was estimated as the difference in ADG between supplemented and unsupplemented animals (ADGchng).

Supplement intake of the group of animals in each experimental unit was measured on a DM basis as the total amount of supplement DM offered minus supplement DM refusal in the trough, which was collected and weighed. All trials had negligible amounts of supplement refusal.

Average sward height and herbage mass were measured at the beginning of each trial. In some cases, these assessments were repeated during or at the end of the trial. For nine trials individual sward height records were available, and the frequency of heights were estimated for five strata (0-4, 4-8, 8-12, 12-16, >16 cm). Forage allowance was estimated as kg of DM per kg of BW on total (FA) and green mass basis (gFA) (Table 2).

**Table 2.** Secondary calculations and estimations divided into categories according to what they describe (Type) using parameters from original datasets, protocols and publications from experiments on supplementation on native rangelands in Uruguay (1993-2018).

| Type | variable | units | Observations |
|---|---|---|---|
| Conditions | stocking period | days | - |
| Pasture | 0-4 cm | frequency | frequency calculation |
| Pasture | 4-8 cm | frequency | frequency calculation |
| Pasture | 8-12 cm | frequency | frequency calculation |
| Pasture | 12-16 cm | frequency | frequency calculation |
| Pasture | 16+ cm | frequency | frequency calculation |
| Pasture | ME | Mcal/kg | 4.4 × 0.82 × DMD / 100 (ARC, 1980) |
| Pasture | DMD | % | 88.9 – (0.779×ADF%) (Osítis et al., 2003) |
| Animal | ADG | kg BW/animal/d | (BW f - BW i)/(date f - date i) |
| Animal | ProdHa | kg BW/ha | (BW f - BW i)/area |
| Animal | ProdHa/day | kg BW/ha/d | (BW f - BW i)/area/day |
| Animal | Stocking rate BW | kg/ha | BW/area |
| Animal | Stocking rateLU | LU/ha | BW/380/area |
| Animal/Supplement | SFE | | kg DM/ADGchng |
| Animal/Supplement | SuppRate | | kg sDMI/ kg BW |
| Animal/Pasture | FA | kg/kg | kg DM/ kg BW |
| Animal/Pasture | gFA | kg/kg | kg green DM/ kg BW |
| Animal/Pasture | | gFA/FA | - |

Conditions: experimental conditions; Animal: animal related variables; Pasture: pasture related variables; Supplement: supplement related variables; i: initial; f: final; ProdHa: BW production/hectare; LU: livestock unit (380 kg BW); SuppRate: supplementation rate (%BW); sF: supplemented´s HDMI; cF: control´s HDMI

Chemical composition was determined in herbage samples taken at different moments throughout the stocking period, and in a sample of the concentrate supplement at the beginning of the stocking period. In the few cases where supplement chemical composition data were unavailable, average values from local tables were assumed (Mieres et al., 2004). All samples were oven-dried at 60°C for 72 h. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined according to Van Soest et al. (1991), ash included, whereas DM content and CP concentrations were determined according to AOAC (1990). Dry matter digestibility (DMD, %) was estimated following Osítis et al. (2003) as 88.9 - 0.779 * ADF%, and the concentration of metabolisable energy (ME, MJ/kg DM) was estimated as (4.4 x 0.82 x DMD% / 100) x 4.184 (Agricultural Research Council, 1980). In addition, the ME/CP ratio of supplements was calculated (units: 100 MJ/kgCP).

Meteorological data –daily rainfall, minimum, maximum, and average daily temperature, and the number of ground frosts– was obtained from the meteorological station nearest to each trial.

#### 2.4.3. Statistical analyses

The SFE data were collated across the 25 trials, rendering 65 independent comparisons. Simple average, and the 20, 50 (median) and 80% percentile were estimated. Then, a logistic function was fitted to the distribution of SFE frequency (TBLCurve v2.0, Sigma).

To determine the relationship between variables, Spearman correlations between SFE and auxiliary variables were explored, and then simple and multiple regressions models fitted to estimate quantitative responses to putative determinants of SFE and the ADG of control and supplemented animals.

A mixed model was developed with SFE as the response variable, using trial as a random effect and all variables with Spearman coefficients between them and SFE which presented values above +0.10 or below -0.10, as fixed effects (see below Spearman correlations). The best model was selected using the AIC criterion.

Statistical analyses were performed with the base package of R software (R Core Team, version 4.0.3, 2020) in combination with Infostat (Di Rienzo et al., 2015). The threshold for statistical significance was $p<0.05$.

## 2.5. RESULTS

### 2.5.1. Sward, animal and supplement type and intake

There was little or no difference between control and supplemented treatments in initial animal BW, nor in the amount and nutritive value of herbage offered, within each trial (Figure 1), yet there was ample variation between trials. Thus, sward height ranged from 2.5 to 19.0 cm and herbage mass, from 460 to 6160 kg DM/ha, whereas herbage CP concentration varied between 4.3 and 16.6 %, and DMD, between 43 and 70% (Table 3). The proportion of green herbage in the standing biomass also varied widely, between 15 and 87% (Figure 1), and was negatively associated with total herbage mass and sward height ($r = -0.46$; $p<0.01$) and positively with CP concentration ($r=0.64$; $p<0.01$) (data not shown in tables).

**Table 3.** Descriptive statistics (mean), standard deviation (SD), coefficient of variation (CV), minimum (min) and maximum (max) related to animals and pastures of a dataset of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

| Variable | mean | SD | CV | min | max |
|---|---|---|---|---|---|
| cADG (kg/animal/day) | 0.13 | 0.17 | 130 | -0.19 | 0.58 |
| sADG (kg/animal/day) | 0.49 | 0.22 | 45 | 0.05 | 1.24 |
| ADGchng (kg/animal/day) | 0.38 | 0.18 | 48 | -0.10 | 1.02 |
| Shrunk average BW (kg) | 201 | 51 | 25 | 123 | 371 |
| Stocking rate (kgBW/ha) | 429 | 119 | 28 | 217 | 755 |
| Forage allowance (FA) (kgDM/kgBW) | 5.2 | 3.0 | 57 | 1.1 | 19.1 |
| Green forage allowance (kg green DM/kgBW) | 2.1 | 1.2 | 57 | 0.7 | 6.5 |
| Green FA/FA | 0.4 | 0.2 | 37 | 0.2 | 0.9 |
| Sward height (cm) | 7.4 | 3.9 | 52 | 2.5 | 19.0 |
| Herbage mass (kgDM/ha) | 2079 | 969 | 47 | 461 | 6163 |
| Herbage DM content (%) | 0.5 | 0.1 | 21 | 0.2 | 0.7 |
| Green Herbage mass (%) | 0.4 | 0.2 | 37 | 0.2 | 0.9 |
| Herbage CP (%) | 8.4 | 2.1 | 25 | 4.3 | 16.6 |
| Herbage ADF (%) | 43.7 | 7.3 | 17 | 23.7 | 59.1 |
| Herbage NDF (%) | 65.3 | 8.3 | 13 | 30.7 | 81.1 |
| Herbage energy (MJ ME/kg DM) | 8.5 | 1.1 | 12.9 | 6.5 | 12.1 |
| Herbage DM digestibility (%) | 55.0 | 5.8 | 10 | 42.9 | 70.5 |
| Forage ME/CP (100MJ/kgCP) | 1.0 | 0.2 | 19 | 0.6 | 1.6 |
| Supplementation rate (%BW) | 0.9 | 0.3 | 31 | 0.3 | 2.0 |
| Supplement DM intake (kg/an/day) | 1.8 | 0.7 | 37 | 0.4 | 3.9 |
| SFE (ADGchng/kg DM) | 0.21 | 0.08 | 41 | 0.07 | 0.40 |
| SFE (ADGchng/kg CP) | 1.23 | 0.58 | 47 | 0.36 | 2.68 |
| SFE (ADGchng/MJ) | 0.02 | 0.01 | 39 | 0.01 | 0.03 |
| Supplement ME content (MJ/kg) | 11.7 | 0.7 | 6 | 10.1 | 13.4 |
| Supplement CP content (%) | 18.4 | 8.3 | 45 | 7.1 | 43.9 |
| Supplement ME/CP (100MJ/kgCP) | 0.7 | 0.3 | 35 | 0.3 | 1.8 |
| Stocking period (days) | 92.5 | 24.7 | 27 | 42.0 | 141.0 |

Green: green DM proportion

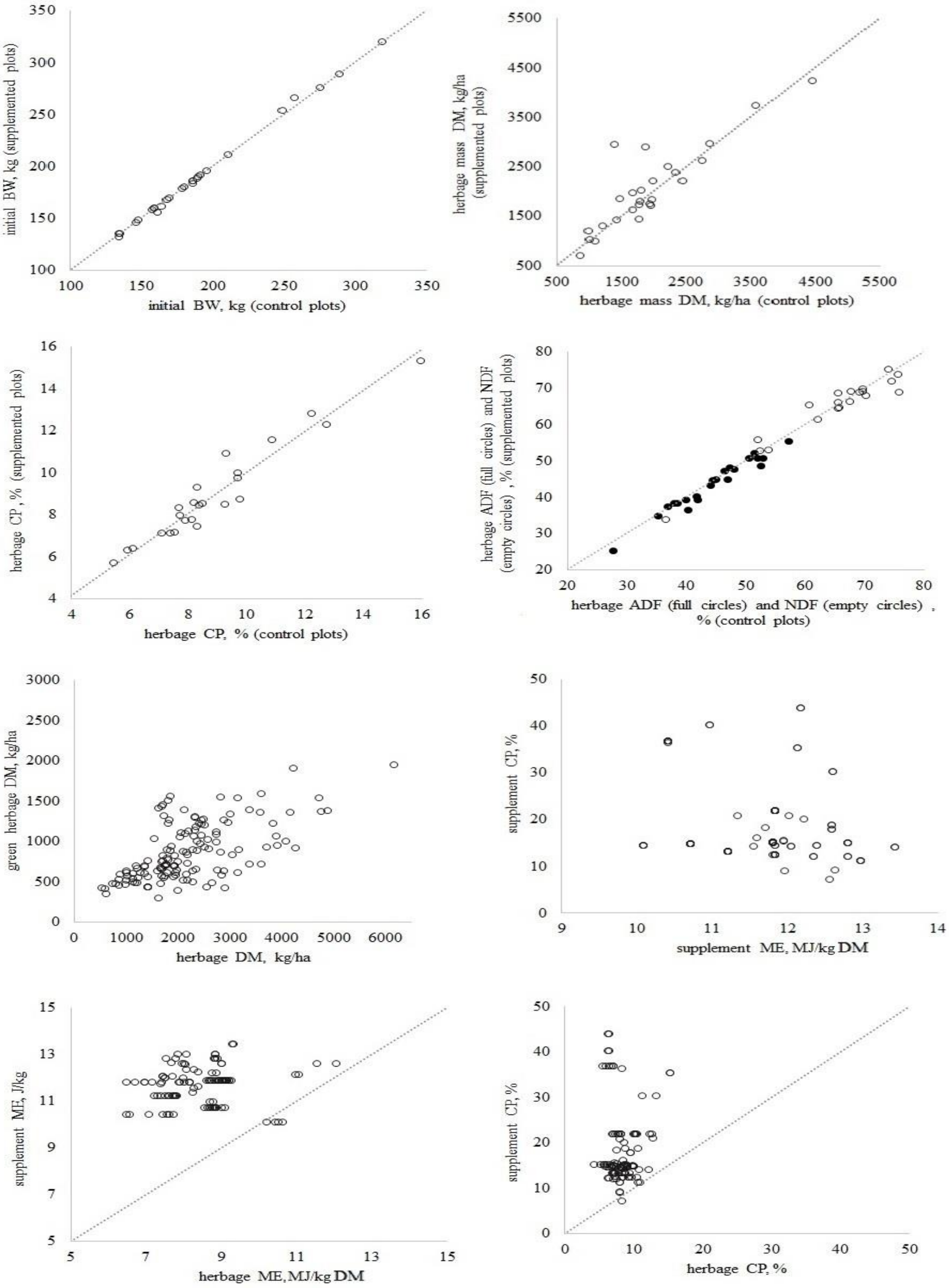


**Figure 1.** Main descriptive characteristics from sward, animal, and supplements of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

BW, body weight; DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; ME, metabolisable energy

Supplementation rate (0.86±0.26%; min 0.3, max 2.0 % of BW) and supplement DM intake (1.84±0.68 kg/animal/d; min 0.4, max 3.8 kg/animal/d) presented substantial variation among trials (Table 3). Supplements varied little in ME concentration (min. 10.1, max. 13.4 MJ/kg DM) but widely in CP concentration (min. 7.1, max. 43.9%), and therefore their ME/CP ratio was variable (Figure 1, Table 3). Considering a 20% threshold CP concentration (Harris, 1980), supplements categorized as "non-protein based" (i.e., energy-based) had mean ME and CP concentrations of 11.7±0.807 MJ/kg DM and 14.1±2.043%, respectively (data not shown), whereas protein supplements had on average almost the same ME concentration (11.5±0.682 MJ/kg DM) but twice as great CP concentration (28.7±8.170%).

2.5.2. ADG of supplemented and control animals

The average ADG of unsupplemented animals (cADG) was positive yet low at 0.13±0.174 kg/animal/d, ranging from -0.19 to 0.58 kg/animal/d. The average ADG of supplemented animals (sADG) was considerably greater at 0.49±0.220 kg/animal/d, ranging from 0.05 to a maximum value of 1.24 kg/animal/d (Table 3, Figure 2). This resulted in ample variation in ADGchng, between -0.10 and 1.02 kg/animal/d, with an average of 0.38±0.167 kg/animal/d.

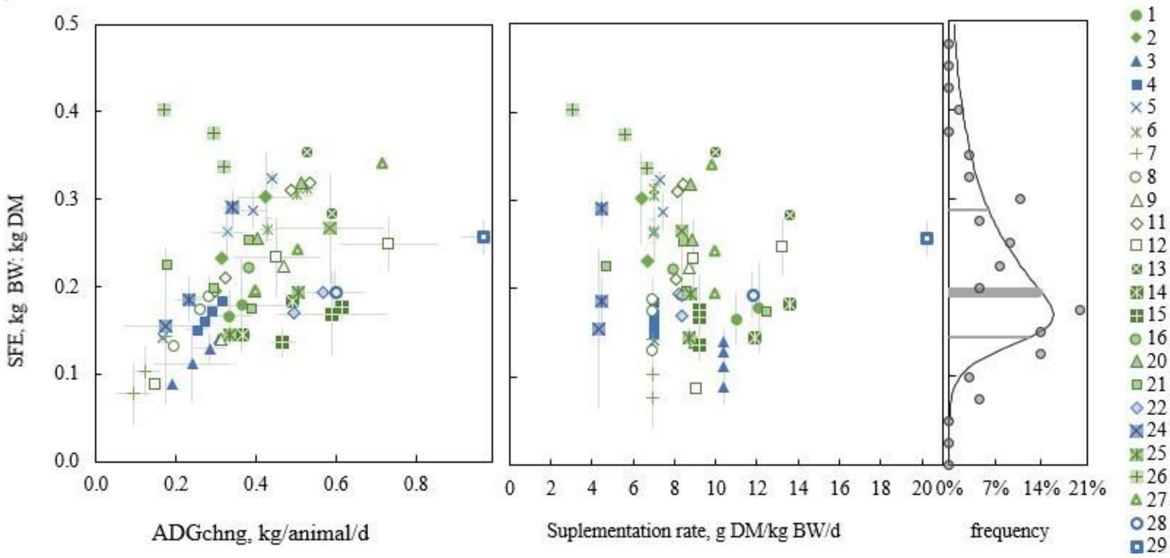


**Figure 2.** Supplement feed efficiency (SFE) by average daily gain change (ADGchng) and supplementation rate, and SFE values frequencies of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

BW, body weight; DM, dry matter. References on the right hand of the figure correspond to each trial´s ID, as in Table 1.

### 2.5.3. SFE and its relationship with ADGchng and supplement intake

The average SFE was 0.21±0.076 ADGchng/kg DM, ranging between 0.07 and 0.40 ADGchng/kg DM. Expressed on a CP basis, SFE varied between 0.36 and 2.68 ADGchng/kg CP, and expressed as ME it ranged between 0.01 and 0.03 ADGchng/MJ (Table 3).

The distribution of SFE frequencies was asymmetrical, with few values below 0.10 ADGchng/kg DM, an average of 0.21 and a median of 0.19 ADGchng/kg DM. Three out of five values ranged between 0.15 and 0.29 ADGchng/kg DM (*i.e.*, the 20 and 80% percentiles, respectively, Figure 2). When analysing the association between SFE and its two components, variation in SFE appeared to be more closely associated with changes in ADGchng (and sADG and cADG) than in supplement intake or supplementation rate (Tables 4 and 5).

Discriminating SFE between protein and “non-protein based” (energy-based) supplements yielded no distinct pattern in these relationships (Figure 3).

**Table 4.** Multiple regressions with SFE as the response variable and supplement intake and ADGchng of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

| Regressor1 | Estimate | P-value | Regressor2 | Estimate | P-value | Regressor3 | Estimate | P-value | R2 | AIC |
|---|---|---|---|---|---|---|---|---|---|---|
| Supp Intake | -0.07 | <0.0001 | ADGchng | 0.47 | <0.0001 | - | - | - | 0.73 | -383 |
| Supp Intake | -0.16 | <0.0001 | $(\text{Supp Intake})^2$ | 0.01 | <0.0001 | ADGchng | 0.55 | <0.0001 | 0.85 | -451 |
| Supp Intake | -0.06 | <0.0001 | ADGchng | 0.61 | <0.0001 | $(\text{ADGchng})^2$ | -0.17 | 0.0132 | 0.74 | -388 |
| Supp Intake | -0.01 | <0.0001 | - | - | - | - | - | - | 0.06 | -252 |
| ADGchng | 0.13 | 0.0002 | - | - | - | - | - | - | 0.12 | -255 |
| Supp Intake | - | 0.2144 | $(\text{Supp Intake})^2$ | - | 0.5741 | - | - | - | 0.06 | -250 |
| ADGchng | -0.72 | <0.0001 | $(\text{ADGchng})^2$ | -0.59 | <0.0001 | - | - | - | 0.37 | -290 |

*Supp Intake = kg DM per animal per day; ADGchg = kg DM/kg BW change per animal per da*

**Table 5.** Spearman´s correlation coefficients between supplement feed efficiency and crude protein and metabolisable energy contents in forage and supplements and their intake both separately and of the whole diet of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

| **SFE** | **r** | **P-value** | **SFE (ME)** | **r** | **P-value** | **SFE (CP)** | **r** | **P-value** |
|---|---|---|---|---|---|---|---|---|
| SFE MJ | 0,91 | 0,000 | | | | | | |
| SFE kgCP | 0,57 | 0,000 | | | | | | |
| ME f | -0,12 | 0,214 | ME f | -0,19 | 0,054 | ME f | -0,24 | 0,012 |
| ME s | -0,11 | 0,300 | ME s | -0,19 | 0,058 | ME s | 0,07 | 0,517 |
| ME s intake | -0,18 | 0,065 | ME s intake | -0,22 | 0,020 | ME s intake | 0,07 | 0,472 |
| CP f | -0,18 | 0,067 | CP f | -0,28 | 0,004 | CP f | -0,05 | 0,646 |
| CP s | 0,26 | 0,006 | CP s | 0,26 | 0,006 | CP s | -0,53 | 0,000 |
| CP s intake | 0,04 | 0,650 | CP s intake | 0,06 | 0,571 | CP s intake | -0,49 | 0,000 |
| s DM intake | -0,17 | 0,072 | s DM intake | -0,21 | 0,034 | s DM intake | 0,06 | 0,558 |
| cADG | -0,34 | 0,006 | cADG | -0,31 | 0,011 | cADG | -0,39 | 0,001 |
| sADG | 0,21 | 0,027 | sADG | 0,17 | 0,078 | sADG | 0,17 | 0,079 |
| ADGchng | 0,58 | 0,000 | ADGchng | 0,51 | 0,000 | ADGchng | 0,45 | 0,000 |
| Supp rate (%) | -0,17 | 0,077 | Supp rate (%) | -0,21 | 0,028 | Supp rate (%) | -0,08 | 0,403 |

*f: forage; s: supplement; SuppRate: supplementation rate (%BW).*

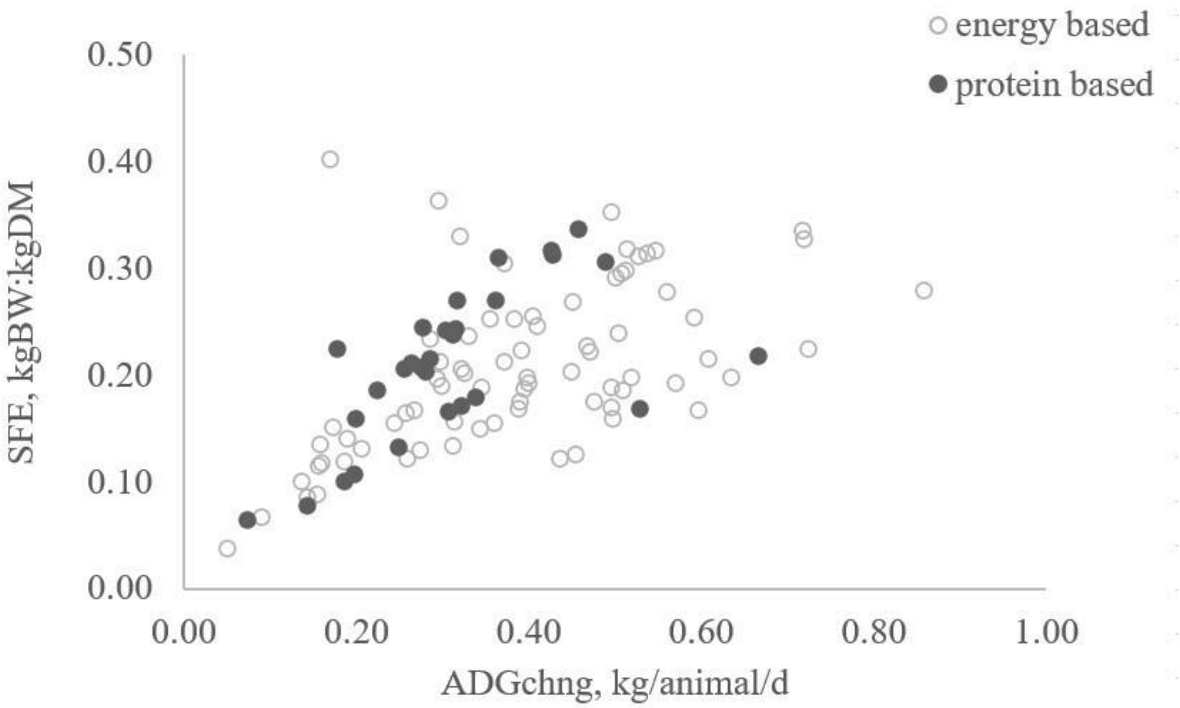


**Figure 3.** Supplement feed efficiency (SFE) by average daily gain change (ADGchng) by type of supplement of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

BW, body weight; DM, dry matter; energy-based supplement: <20% crude protein (CP, %) content; protein-based supplement: >20% CP content

2.5.4. <u>Relationship between SFE and auxiliary variables</u>

The mixed model´s fixed effects explained 78% of its variability and it resulted in the following equation: SFE = 0.0435 + 0.00017 *HerbageMass – 0.0684*ForageAllowance ($p<0.05$).

Neither of the variables describing the nutritive value of herbage were associated with SFE (Table 6). Opposite to what was expected, sADG was negatively associated with sward height, herbage mass and allowance, and cADG was negatively associated with initial herbage mass. Interestingly, the proportion of the paddock with sward heights below 4 cm was positively associated with both sADG and cADG. An association emerged between the proportion of green herbage and sADG, cADg and ADGchng, which in turn was dependent on climate (Figure 4).

**Table 6.** Spearman´s correlation coefficients between supplement feed efficiency and other variables of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

| Variable(1) | Variable(2) | Spearman | P-value |
|---|---|---|---|
| SFE | Sward height | 0.17 | 0.082 |
| SFE | Herbage mass | 0.11 | 0.270 |
| SFE | FA | 0.14 | 0.153 |
| SFE | forageCP | -0.18 | 0.070 |
| SFE | tmin 90p | 0.32 | 0.004 |
| SFE | tmin 60p | 0.29 | 0.011 |
| SFE | F 90 p | -0.28 | 0.019 |
| cADG | F Trial | -0.65 | 0.000 |
| cADG | tmin Trial | 0.60 | 0.000 |
| cADG | F 30p | -0.50 | 0.001 |
| cADG | PP 30p | 0.41 | 0.002 |
| cADG | F 90 p | 0.45 | 0.002 |
| cADG | %Grazing | -0.47 | 0.008 |
| cADG | 4-8 cm | -0.49 | 0.013 |
| cADG | PP Trial | -0.33 | 0.014 |
| cADG | PP 90p | 0.31 | 0.023 |
| cADG | greenFA/FA | 0.28 | 0.028 |
| cADG | 0-4 cm | 0.41 | 0.040 |
| cADG | iHerbage mass | -0.28 | 0.046 |
| sADG | Green herbage mass | 0.58 | 0.000 |
| sADG | greenFA/FA | 0.57 | 0.000 |
| sADG | Avg herbage mass | -0.38 | 0.000 |
| sADG | Supplementation rate | 0.36 | 0.000 |
| sADG | Sward height | -0.36 | 0.000 |
| sADG | PP Trial | -0.36 | 0.001 |
| sADG | F 90 p | 0.35 | 0.003 |
| sADG | fHerbage mass | -0.34 | 0.003 |
| sADG | iHerbage mass | -0.31 | 0.005 |
| sADG | PP 90p | 0.30 | 0.005 |
| sADG | FA | -0.26 | 0.007 |
| sADG | PP Trial | 0.28 | 0.010 |

| | | | |
|---|---|---|---|
| sADG | NDF | -0.28 | 0.010 |
| sADG | tmin Trial | 0.29 | 0.011 |
| sADG | P Supl | 0.27 | 0.012 |
| sADG | F 60p | 0.29 | 0.013 |
| sADG | 0-4 cm | 0.36 | 0.018 |
| ADGchng | F 30p | 0.44 | 0.000 |
| ADGchng | F Trial | 0.36 | 0.002 |
| ADGchng | PP 60p | -0.32 | 0.003 |
| ADGchng | Bite rate | 0.37 | 0.008 |
| ADGchng | Green Herbage mass | 0.25 | 0.015 |
| ADGchng | F 60p | 0.26 | 0.027 |
| ADGchng | greenFA/FA | 0.22 | 0.029 |

T min: minimum temperature (°C); PP: precipitations (mm); F: number of frosts; 30-60-90p: 30, 60, 90 days prior to beginning of trial; Green: green DM proportion; SH: sward height; i: initial

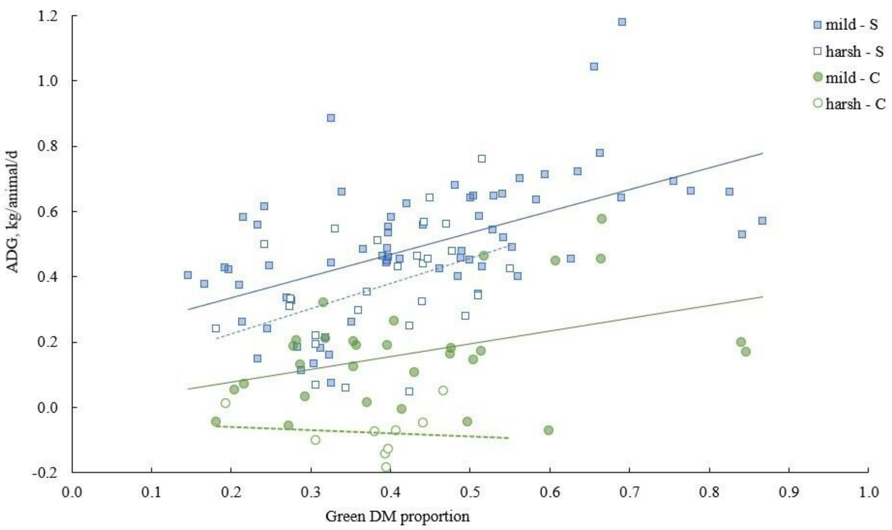


**Figure 4.** Average daily gain change (ADGchng) by green herbage dry matter (DM) proportion and type of weather of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

S, supplemented plots; C, control plots; mild weather: less than 20 frosts during trial; harsh weather: more than 20 frosts during trial

Weather variables were associated with some of the relevant parameters explaining SFE (Table 6). Control ADG was negatively associated with the number of frosts that occurred 30 days before the beginning of the trials. On the other hand, the number of frosts that occurred 60 and 90 days prior to the trials were all positively associated with sADG. Additionally, ADGchng was positively associated with frosts occurring both 60 and 30 days before the trials began.

During the stocking period, in winter, both cADG and sADG were positively associated with minimum average temperatures and negatively associated with rainfall, yet only cADG was negatively affected by the number of winter frosts. Consequently, the number of frosts during the stocking period positively affected ADGchng.

#### 2.5.5. Animal behaviour

In all trials grazing time during the hours of daylight was consistently lower for supplemented animals than their control counterparts. Unsupplemented animals grazed for 8 to 9 h, while supplemented animals grazed 8 h or less and as little as 2 to 4 h in some cases (Figure 5). The magnitude of the difference was variable and not related to the nutritive value of offered herbage nor the climatic conditions (although maximal grazing time in control animals appeared to occur at intermediate values of the proportion of green biomass). Conversely, no difference in rumination time during the hours of daylight was observed between supplemented and unsupplemented animals, and both groups increased their rumination time from less than 0.5 h when the proportion of green herbage in the standing biomass was high, to more than 1.5 h when it decreased to less than 0.3 (Figure 5).

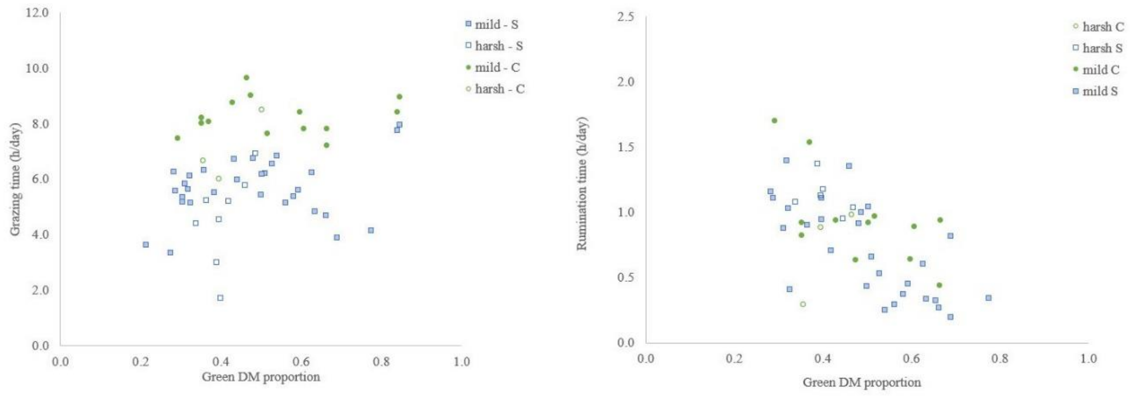


**Figure 5.** Grazing time (a) and rumination time (b) by dry green herbage dry matter (DM) proportion of a database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

S, supplemented plots; C, control plots; mild weather: less than 20 frosts during trial; harsh weather: more than 20 frosts during trial

## 2.6. DISCUSSION

Overall positive responses to supplementation were observed. The average SFE of the database was 0.21±0.076 ADGchng/kg DM, but SFE was quite variable, ranging from 0.05 to 0.40 ADGchng/kg DM. Variation between- and within-trials was more closely associated with variation in ADGchng than in supplement intake or supplementation rate (despite these being naturally associated with SFE because they are part of its calculation). Forage allowance negatively affected SFE, while herbage mass affected it in a positive yet smaller manner. On the other hand, when analysing the Spearman correlations, it can be observed that neither sward height nor chemical composition of the herbage mass were directly associated with variation in SFE. Unlike reports from semiarid rangelands and tropical pastures with a dry season (Moore et al 1999; DelCurto et al., 2000; Poppi et al. 2018), little evidence was found for protein concentration playing a major role in determining SFE for growing cattle grazing native *Campos* grasslands during winter. Weather variables, on the other hand, influenced SFE, with greater values observed in colder winters and milder autumns.

The response in animal performance relative to the cost of the additional nutrients provided is a key economic consideration when assessing the efficacy of supplementation in extensive animal production systems (McLennan et al., 2017). Since costs are in part associated with the amount of offered supplement, improving SFE is desirable (Wilkinson, 2011). For animals grazing sown pastures, negative association effects (substitution) are often observed, at least for supplementation rates above a certain threshold (Moore et al., 1999). For instance, Bowman & Sanson (1996) suggest that energy supplementation above 0.5% BW increases substitution and hence worsens SFE. In the present study, SFE and supplement DM intake were not associated or were less relevant than ADGchng. This was not due to the variation of supplement energy concentration which was relatively small. Within a wide range of supplementation rates (0.30-2.03 %BW), the largest SFE values were observed at intermediate levels and without a distinct pattern, which means that the substitution effect mentioned in the literature (Bowman & Sanson, 1996) would not be operating through any particular supplementation rate threshold in our data. Thus, the substitution mechanism would not seem to be related to the supplementation rate, although considering that unsupplemented animals spent more time grazing than their supplemented counterparts, some amount of substitution may be assumed, nonetheless.

The ADG of unsupplemented animals was negatively affected by weather conditions, specifically by harsher conditions both before (autumn) and during the stocking period (winter). Kuinchtner et al. (2018) point out that limitations to young cattle performance during the cool season in native grasslands may be due to low forage quality that is a consequence of lower temperatures and frosts that inhibit growth of C4 grasses. Furthermore, our results suggest that frosts actually kill the accumulated biomass of C4 species. Indeed, the proportion of green herbage in the standing biomass was never above 50% in harsh winters. Even though not all pasture related variables were associated with cADG, the fact that the green proportion of the forage allowance (greenFA/FA) was positively associated with this may point in the same direction. In fact, with stockpiled native grasslands, Fedrigo et al. (2021) observed that as sward height increased from 5 to 10 cm, NDF and ADF increased while CP concentration decreased, meaning that unsupplemented animals would benefit from shorter, greener swards, compared to a taller pasture but with low green DM content. Additionally, other than the negative effect that low environmental temperature may have on digestibility (Christopherson & Kennedy, 1983), Sarker & Holmes (1974) concluded that frost formation on vegetation was the cause of a decreased daily grazing time, and this could also explain what was observed with our control animals, whose only nutrient source came from the pasture and always dedicated more time to grazing activities than their supplemented counterparts (Figure 5). Even though ADGchng also correlated with harsh winter conditions, these associations were weaker than what was observed with control animals, but also the number of frosts that occurred two months before the trial positively affected both ADGchng and sADG (Table 6). Wheeler et al. (2002) found that during one month after the first killing frost, beef cows did not respond to supplementation, but later in the winter, supplementation improved the utilization of stockpiled bermudagrass forage. This could mean that even if frosts affected supplemented animals, the impact could have been of a relatively short term, or that it may vary throughout the supplementation period.

Our understanding of the relationship between supplementation and animal performance for rangelands is limited (Bohnert and Stephenson, 2016). In our research, very few associations could be established between SFE and most pasture-related variables, despite their great variability. Forage allowance was one notable exception, negatively affecting SFE, suggesting that more available forage, than what animals need, would go unutilized at some point, thus rendering inefficiencies. However, as herbage mass was the other exception, positively affecting SFA - even though its importance is less than that of FA- it could be thought that some balance is needed between allowance and mass, at least up to a certain point of herbage mass. Given that substitution is related to the herbage DM intake (HDMI) of unsupplemented animals (Stockdale, 2000), this would suggest some substitution existed in our database, which would explain inefficiencies. As McCollum & Horn (1990) stated in a protein supplementation review, a SFE of 0.33 may be considered a benchmark standard in growing cattle, which is higher than what was observed in our database, meaning that inefficiencies occurred, probably spurred by substitution effects. Furthermore, if the greater the HDMI, the greater the substitution rate, the lower the supplement response or ADGchng (Hills et al., 2015) SFE would be expected to decline.

To assess, at least to some extent this possibility, HDMI was estimated *via* energy balance (CSIRO Cattle Explorer, 2012), assuming a DMD of 65% of consumed herbage. We focused on the magnitude of the difference in HDMI between supplemented and unsupplemented animals and its relationship to the herbage total digestible nutrients-to-protein ratio (TDN:CP, Figure 6). There is an apparent concordance in the lack of large substitution or additive effects observed in our database with the fact that the ratio of TDN:CP of the *Campos* grasslands lies in-between that of sown pastures (large substitution effects) and native *prairies* (large additive effects).

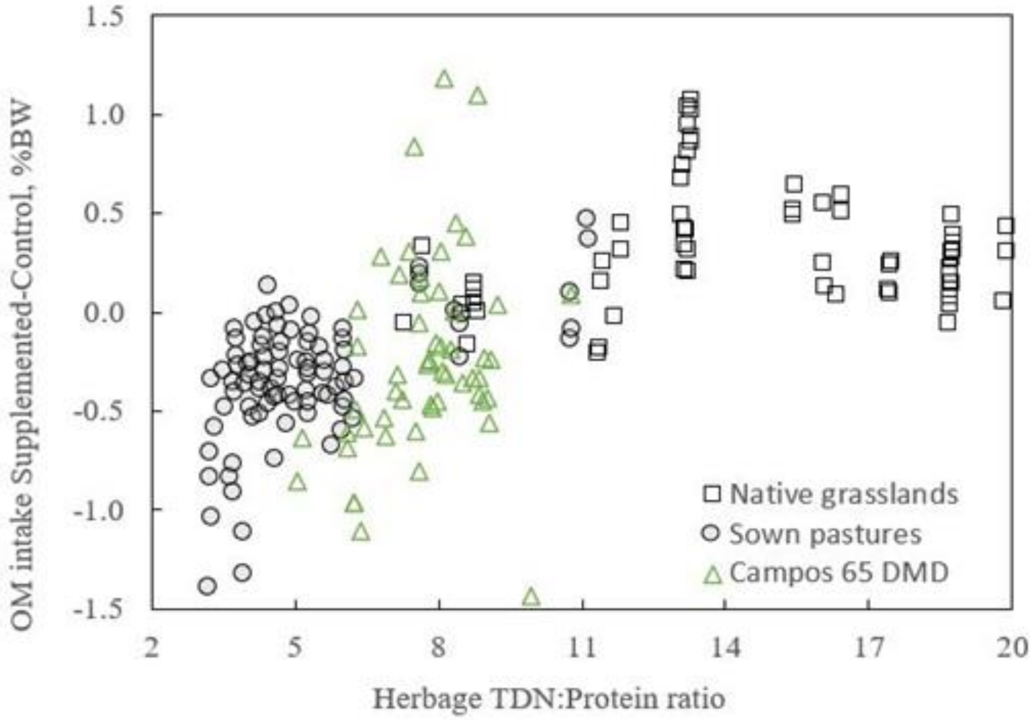


**Figure 6.** Difference between supplemented and control animals in organic matter (OM) intake by herbage TDN:CP by source of estimation

TDN, total digestible nutrients; CP, crude protein; "Native grasslands and sown pastures" from Moore et al. (1999); "Campos 65 DMD", estimation from database of supplementation experiments for young beef cattle on native grasslands in Uruguay (1993-2018).

Supplement response variability can be explained by substitution effects, due to the similarities or differences between the nutritive value of forage and supplement (Tonello et al., 2011), specifically by both pasture and supplement CP concentration and it may decrease when herbage CP increases (Detmann et al., 2014). In the review of Moore et al. (1999), the lowest response to concentrate supplementation was observed when molasses alone or with very low nitrogen addition were given to animals grazing native grasslands, whereas the greatest responses were observed on improved forages using balanced concentrates. Our data shows that ADGchng was relevant in determining SFE more than supplement DMI; therefore, the interaction between the nutrients offered from pasture and supplement could explain part of the variability. Additionally, the database presented enough variability on native grasslands´ nutrient content, as well as on supplement CP, yet not so much on ME concentration. If we consider the CP concentration of sown pastures and their relation to SFE on the one hand (CP = 13-15%, SFE 0.11-0.10, (Clariget et al., 2021a) and on the other hand we consider other native grasslands of the world, such as mid-late winter of dormant native tallgrass prairie (CP = less than 6% in, Bodine & Purvis, 2003), or Australian tropical rangelands (CP= around 6%, White et al., 2010), we could place our *Campos* grasslands somewhat in the middle. Should this be the case, it could be assumed that native grasslands are in the CP deficit threshold, with some cases above and others below it.

Poppi et al. (2018) suggest that supplementation response is based on achieving the best combinations to increase metabolizable protein and metabolisable energy supply when animals are grazing low CP concentration forage. The protein concentration of herbage in native *Campos* grasslands during winter varies, but 8.4% is commonly observed (Cazzuli et al., 2019; Orcasberro et al., 2021; Fedrigo et al., 2021), above what McLennan et al. (2017) consider “low CP forages” (<7% CP). This CP concentration is about one third that of sown temperate pastures in winter (20±6%, Mieres et al., 2004), but not as low as values reported for tropical grasslands during the dry season (e.g. less than 3% in Australian rangelands, Bowen et al., 2017). Again, this database appears to be somewhat in the middle of all other forage bases in terms of CP concentration. In addition, the actual consumed CP by the animals would be even greater since animal selection improves diet quality on this type of rangelands (Piaggio et al., 1995). When SFE and ADGchng were classified into protein and “non-protein based” (energy-based) supplementation treatments, no distinct pattern could be observed; if forage CP had been the most important limiting factor, some kind of distinction in either SFE or ADGchng should have been observed. When protein is limiting growth, protein deposition is linearly increased by protein supply (Schroeder and Titgemeyer, 2008). In our dataset - comprising growing cattle- no differential response could be found using high protein concentration supplements, nor a significant correlation between SFE and herbage CP. According to Poppi & McLennan (1995), protein intake is expected to be the main limiting nutrient for cattle in a growing phase, at least in tropical environments, something we did not find in our analysis. Additionally, McCollum & Horn (1990) affirmed that SFE lower than 0.33 suggest no N deficiency, in which case our data would match this criterion, at least on average. In the case of Bowman et al. (2004), HDMI and NDF and CP intakes decreased linearly with increasing non-structural carbohydrate supplementation of beef heifers on low quality forage-based diets (5.5 %CP, 49.0 ADF%, 71.3 NDF%), in which both forage and supplement digestible organic matter (DOM):CP seemed to be superior predictors of response to supplementation compared with forage CP levels alone. Actually, Lima et al. (1999) observed that DOM:CP was as important as CP concentration for beef heifers grazing a C4 grass in explaining ADG variations. All this suggests that protein may not be the most important limiting factor of these native grasslands production systems, not even for young growing cattle.

## 2.7. CONCLUSIONS

Positive responses to supplementation occurred in the 25 collated trials. In general, SFE were relatively high, with 80% of SFE above 0.15 ADGchng/kg DM. The average SFE was 0.21±0.08 ADGchng/kg DM. Considering the variables directly related with SFE, a greater variation was observed with ADGchng than with supplementation rate. Forage allowance affected SFE in a negative way, while herbage mass positively affected yet it in a smaller magnitude, suggesting a balance between these two variables is needed to maximize SFE.

Weather conditions during the stocking period was the only variable that showed a significant effect on SFE, through its detrimental impact on the performance of unsupplemented animals in harsh winters that led to a greater supplement response (ADGchng) and thus greater SFE.

Little evidence was found on the existence of a major overriding role for protein deficiency on native *Campos* grasslands of the Pampa biome as the main factor limiting animal growth during winter. This is probably because the nutritive value of herbage of *Campos* grasslands seems to be higher than in other grasslands, such as tropical rangelands or North American prairies, because *Campos* retain a variable, and potentially high, proportion of green leaves over winter.

## 2.8. ACKNOWLEDGMENTS

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## 2.9. CONTRIBUTIONS

FC and FL conceived the research work as a whole. FC gathered the datasets, with the help of PR, VB, AS, XL, SL and GB. The analysis design was discussed between FC, FL, JSánchez, AH, PR, VB, AS, MJ, MD, JSavian, JIV and XL. JSánchez ran the initial meta-analyses and meta-regressions, together with FC. The rest of the analyses were run by FC, FL, AH and XL. The discussion of the results was carried out by FC, FL, AH, JSánchez, PR, VB, AS, MJ, MD, JSavian, DP, FM, XL, SL, GB, JIV and CB. Finally, FC and

FL led the manuscript writing, and all authors contributed critically to the drafts and gave their final approval for publication.

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## 3. BEEF CATTLE GRAZING NATIVE GRASSLANDS MAY FOLLOW THREE DIFFERENT SUPPLEMENT REPONSE PATTERNS

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## 3.1. ABSTRACT

Previous studies on winter supplementation of growing cattle grazing stockpiled native Campos grasslands suggest that forage allowance (FA), herbage mass, and weather conditions before and during the supplementation period could all affect supplement feed efficiency (SFE) – that is, the difference or change in average daily gain (ADG) between supplemented (S) and control (C) animals (ADGchng, kg) per unit (kg) of supplement dry matter (DM) intake. In this study, we analyse data from fifteen collated winter supplementation trials carried out in Uruguay between 2004 and 2018. The working hypotheses of this research paper were: (i) that average substitution rates are positive, and (ii) that ADGchng is not constant throughout the supplementation period and that its variation may be attributed to sward, animal or weather variables. There were two main objectives: (i) to estimate the average supplement substitution rate (sSbR, kg forage, f, dry matter, DM intake reduction: kg supplement DM intake) and potential herbage intake substitution rate (hSbR, kg fDM intake reduction: kg fDM intake of control animals), and its association with SFE, and, (ii) to assess the existence of different phases and supplementation response patterns and its association with other relevant variables. Estimated substitution rates were always positive (sSbR = 0.3–1.1 kg/kg; hSbR = 0.1–0.3 kg/kg) and were negatively and moderately associated with SFE. Supplementation proved to be a dynamic process where three possible supplementation responses over the supplementation period were identified (linear, quadratic and Weibull). While linear patterns did not appear distinctly associated with any particular set of variables, quadratic models were mostly associated with herbage biomass and substitution rates, whereas Weibull models were the clearest in their association with frosts. Regardless of the response pattern, at the beginning of the trials it was the animals' body weight and supplement quality that most influenced supplement response, whereas towards the end, supplementation intake, supplemented animals´ ADG and forage quality played a more relevant role. The estimated parameters and response patterns are expected to be used as inputs in decision support systems for livestock farmers in the future.

**Key words**: concentrate supplementation, supplement feed efficiency, substitution rate, supplementation response pattern.

## 3.2. INTRODUCTION

The relevance of winter supplementation on extensive/semi-extensive beef production systems on native grasslands of the Pampa Biome has been largely documented [1–3]. When supplements are used to avoid food scarcity, beef production levels can be sustained even in years with low forage production, but with higher production costs [4]. Therefore, for this practice to be economically feasible, supplement response and supplement feed efficiency (SFE) must be controlled to some extent [5]. This SFE is defined as the difference or change in average daily gain (ADG) between supplemented (S) and control (C) animals (ADGchng, kg) per unit (kg) of supplement dry matter (DM) intake. In the preceding paper of this same series [6], the authors found an average SFE of 0.21 ± 0.1 ADGchng/kg supplement DM intake, from growing beef cattle grazing native grasslands in winter.

These same authors observed a greater variation in ADGchng than in the supplementation rate, which means that understanding how ADGchng varies throughout the supplementation period could shed some light on the supplementation process. Additionally, these authors found that ADG decreased linearly as the proportion of green herbage decreased, and that the ADG of unsupplemented animals was further reduced when winters were harsher. These two findings—the relevance of ADGchng and the incidence of sward traits influenced by the weather on SFE—pose new research questions as to how these variables behave within the supplementation period.

Initial sward conditions in terms of quantity and/or nutritive quality of the forage may differ from final conditions, as reported by Gekara et al. [7], especially on a continuously grazed pasture and fixed stocking rate, thus affecting SFE. This would imply that SFE could vary within the same supplementation period, due to altered sward conditions. Many studies have been carried out in which, either the influence of supplementation is analysed against the grazing behaviour of cattle [8,9], the study focuses on the efficiency of N utilisation and/or physiology-related variables [10–12], or the authors review its influence on herbage DM intake (HDMI), digestibility or ruminal pH [13]. However, these studies tend to focus more on the average result rather than on the SFE phases. According to Wang et al. [14], the minimum evaluation duration for estimating SFE is 35 days. This constrains any supplementation field experiment in a single season aiming to determine SFE phases directly, with no more than 2–3 SFE values per plot per period. Under this situation, an indirect study, such as analysing ADGchng throughout the supplementation period, may be more suitable.

To accurately assess dietary adequacy and predict performance of grazing livestock receiving supplements, it is necessary to identify the conditions under which substitution is likely to occur and to quantify its magnitude [15]. As defined by Grainger and Mathews [16], pasture substitution rate is the reduction in pasture intake per kg concentrate eaten on a DM basis. Specifically, the calculation of the forage by supplement substitution rate (sSbR) is the difference in control (c) animals´ herbage dry matter intake (HDMI) and supplemented (s) animals´ HMDI, as a proportion of supplement DM intake (sSbR = (cHDMI-sHDMI): supplement DM intake; difference in kg forage DM/kg supplement DM). Another way to analyse the substitution effect in supplementation schemes would be to assume that the maximum HDMI would be observed in C plots, and thus, the potential HDMI substitution rate is calculated as the difference between cHDMI and sHDMI, as a proportion of the theoretical potential maximum HDMI from C plots (hSbR = (cHDMI - sHDMI) : cHDMI; difference in kg forage DM/kg forage DM from control).

In a review of the use of pasture and supplements for dairy cows in temperate zones, Rogers [17] concluded that both the amount of concentrate supplement DM offered and the basal pasture intake (i.e., control animals´ pasture intake) influenced the substitution rate. Beever and Doyle [18] analysed a theoretical scenario where dairy cows grazing temperate sown pastures were supplemented, and assuming a constant substitution rate of supplement between 0 and 1.0 kg DM pasture/kg DM supplement, they suggested that total diet DM intake is expected to increase, but the increase rate would decline at higher amounts of supplementation. Da Trindade et al. [19] suggested for beef cattle grazing a specific native grassland that both its structure (in terms of herbage mass and sward height) and forage allowance (FA) affect herbage intake. Forage allowance is defined as the kg of total bodyweight (BW) of animals grazing, for every kg of forage DM on offer in the pasture. These two variables must be operating to some degree in determining SFE. In fact, Cazzuli et al. [6] found that, in these native grasslands, a balance is needed between FA and herbage mass to optimise SFE. In addition, these authors suggested the existence of some magnitude of substitution, further supported by the fact that unsupplemented animals spent more time grazing then their supplemented counterparts. Still, the amount of this assumed substitution or its association with SFE remains unclear.

The working hypotheses of this research paper were: (i) that substitution rates are positive with growing cattle grazing on stockpiled native Campos grasslands during winter, at least on average, and (ii) that ADGchng is not constant throughout the supplementation period and that its variation may be attributed to certain sward, animal or weather variables. The aim of this study was to further understand the variability in SFE, specifically: (i) to estimate the average supplement substitution rate (sSbR) and potential herbage intake substitution rate (hSbR), and their association with SFE, and (ii) to assess the existence of different phases and ADGchng patterns over the supplementation period within each supplementation trial and its eventual association with other variables, such as sward and animal characteristics or weather variables.

## 3.3. MATERIALS AND METHODS

### 3.3.1. Database compilation

Data were gathered from 15 supplementation trials carried out between 2004 and 2018 in which growing beef cattle grazing native Campos grasslands in Uruguay were supplemented during late autumn and winter. More details of the database compilation and the response and auxiliary variables of the database can be found in Cazzuli et al. [6]. In this paper, only some of the trials (15) collated by these authors were used, because not all of the original 15 trials presented data in such detail that more than two phases were identifiable (i.e., only initial and final BW were available). Their descriptive statistics are presented in Table 1.

**Table 1.** Descriptive statistics (mean), standard deviation (SD), coefficient of variation (CV), minimum (min), and maximum (max) related to animals and pastures of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

| Variable | Mean | SD | CV | Min | Max |
|---|---|---|---|---|---|
| cADG, kg/animal/day | 0.19 | 0.15 | 79 | -0.02 | 0.52 |
| sADG, kg/animal/day | 0.52 | 0.23 | 45 | 0.07 | 1.24 |
| ADGchng, kg/animal/day | 0.34 | 0.18 | 52 | -0.10 | 1.02 |
| Shrunk average BW, kg | 196 | 36 | 18 | 145 | 282 |
| Stocking rate, kgBW/ha | 428 | 95 | 22 | 239 | 630 |
| Forage allowance (FA), kgDM/kgBW | 5.5 | 3.4 | 63 | 1.1 | 19.1 |
| Green forage allowance, kg green DM/kgBW | 2.2 | 1.4 | 63 | 0.68 | 6.5 |
| Green FA/FA | 0.4 | 0.2 | 42 | 0.2 | 0.9 |
| Sward height, cm | 8.1 | 4.2 | 52 | 2.5 | 19.0 |
| Herbage biomass, kgDM/ha | 2157 | 1062 | 49 | 461 | 6163 |
| Herbage DM, % | 55.1 | 8.7 | 16 | 23.7 | 73.3 |
| Green Herbage, % of DM | 43.0 | 17.4 | 41 | 14.6 | 86.7 |
| Herbage CP, % of DM | 8.3 | 2.4 | 29 | 4.3 | 16.6 |
| Herbage ADF, % of DM | 43.4 | 8.2 | 19 | 23.7 | 59.1 |
| Herbage NDF, % of DM | 64.8 | 9.1 | 14 | 30.7 | 81.1 |
| Herbage energy, MJ ME/kg DM | 8.6 | 1.3 | 15 | 6.5 | 12.1 |
| Herbage DM digestibility, % | 55.3 | 6.5 | 12 | 42.9 | 70.5 |
| Forage ME/CP, 100MJ/kgCP | 1.1 | 0.2 | 19 | 0.7 | 1.6 |
| Supplementation rate, %BW | 0.8 | 0.3 | 35 | 0.4 | 2.0 |
| Supplement DM intake, kg/animal/day | 1.7 | 0.6 | 34 | 1.1 | 3.9 |
| SFE, ADGchng/kg DM of supplement | 0.20 | 0.08 | 40 | -0.06 | 0.35 |
| SFE CP, ADGchng/kg CP of supplement | 1.11 | 0.55 | 50 | -0.26 | 2.40 |
| SFE ME, ADGchng/MJ of supplement | 0.02 | 0.01 | 40 | -0.00 | 0.03 |
| Supplement ME content, MJ/kg of DM | 11.5 | 0.7 | 6 | 10.1 | 12.8 |
| Supplement CP content, % of DM | 20.2 | 9.2 | 46 | 12.1 | 43.9 |
| Supplement ME/CP, 100MJ/kgCP | 0.5 | 0.2 | 33 | 0.2 | 0.8 |
| Stocking period, day | 96.0 | 27.1 | 28 | 42.0 | 141.0 |

cADG: control (C) animal´s average daily gain (ADG), sADG: supplemented (S) animal´s ADG; ADGchng: change in ADG between S and C animals; BW, body weight, DM: dry matter; Green: green DM proportion; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; ME: metabolisable energy; SFE: supplement feed efficiency.

The trials in this paper correspond to trial IDs 1–8, 14, 16, 24–25 and 27–29 (see Appendix A).

Apart from all of the estimations and variables described by Cazzuli et al. [6], additional variables were created to explore the relationship between sward-related traits and the variables of interest (SFE, ADGchng), which were: ratio of supplement crude protein (CP) content (sCP) to supplement metabolisable energy (ME) content (sME) ("sCP:sME"); ratio of sCP to forage (f) CP content (fCP) ("sCP:fCP"); and ratios "sCP:fME" and "sME:fME".

3.3.2. Herbage dry matter intake and estimations of substitution rate

To estimate HDMI, CSIRO´s Cattle Explorer [20] spreadsheet was used, with actual data from the trials, but assuming three possible forage dry matter digestibility values (DMD): 45, 55 and 65% for a theoretical modelling exercise. Even though a few trials presented laboratory DMD values, most of them were calculated as, DMD = 88.9 − (0.779 × ADF% [21]. Therefore, to be able to model more scenarios, three DMD values were chosen as extreme values from the calculated DMD from ADF data from our database.

With the three estimations of HDMI, the ME and CP intakes were estimated in turn, using the original dataset values for forage CP and estimated ME. Finally, substitution rates were estimated using the three DMD scenarios plus a fourth which assumed that S animals harvested higher quality forage compared with C animals (sDMD = 65%; cDMD = 55%). These substitution rates were calculated both considering the amount of forage dry matter (DM) substituted by supplement DM (sSbR) and considering the potential herbal intake, which is assumed to have been consumed by C animals (hSbR).

The sSbR was calculated as follows:

sSbR, kg DM of pasture/kg DM of supplement = (cHDMI − sHDMI)/sDMI

where cHDMI = average herbage DM intake (kg DM) of control animals consuming similar forage (within the same experiment); sHDMI = herbage DM intake of supplemented animals (kg DM); sDMI = supplement DM intake (kg DM).

Finally, the potential herbage intake substitution rate (hSbR) was calculated as follows:

hSbR, kg DM of pasture/kg DM of pasture = (cHDMI − sHDMI)/cHDMI)

For further statistical description of these variables, see Appendix B.

The separate average BW of C and S against time for all of the experiments was plotted, and distinct phases were observed, where slopes (i.e., ADG) behaved differently at certain breakpoints in time.

### 3.3.3. Phases identification and the change of ADGchng vs growing degree days

The first step in the phases´ identification process was the plotting of the average BW of C and S values separately, against time for all the experiments. Once it was clear that phases were identifiable, dates were transformed into growing degree days (GDD, base temperature = 0 °C) to standardise all trials regardless of the year of their occurrence or the differences in dates within winter. Data extracted using the nasapower R package [22]—regarding minimum (min) and maximum (max) air temperatures (T, °C) estimated 2 m above the surface—were obtained for each experiment for all dates from the beginning of the experiment to the end (i.e., the whole supplementation period, excluding the adaptation phase).

Then, the average air temperature (T avg) for each date was calculated as:

T avg = (T min/T max)/2

To translate T avg into an accumulated thermal sum, T avg was added to the initial T avg of each experiment (iT avg), so that each subsequent day was added onto the previous one, thus:

T day n = iT avg + T avg (n)

In this way, the final value of each experiment was the accumulated growing thermal time, measured in GDD. Growing degree days, or heat units, are generally used to estimate the growth and development of certain crops during the growing season. In this way, it was possible to standardise both weather and pasture growth conditions throughout all trials.

To determine what attributes were associated with ADGchng according to the main phases observed for each trial, accumulated ADGchng was calculated for the beginning of the trials (at an arbitrary thermal sum of 300 GDD) and for the last stages of the trials (at an arbitrary thermal sum of 1000 GDD), generating the new variables “ADGcnhg300” and “ADGchng1000”. These two new variables were calculated separately for each trial, according to the best fitted model explained below. Thus, accumulated ADGchng was estimated by replacing GDD by 300 and 1000 in their best fitted equation. The arbitrary values of 300 °Cd and 1000 °Cd were chosen so as to include all trials in this analysis, since with less than 300 °C, some trials were excluded, and the minimum length of the trials was 1000 °Cd.

3.3.4. Statistical analysis

Statistical analyses were performed with the R software (version 4.0.3) [23] in combination with Infostat [24]. The threshold for statistical significance was $p < 0.05$.

Spearman correlation coefficients were estimated between SFE and HDMI and between SFE and sSbR and hSbR, considering 5% of significance level ($p < 0.05$).

To formally prove the existence of different phases, the “segmented” R package was used to fit a regression model with broken-line relationships for each experiment. Segmented or broken-line models are regression models where the relationships between the response and one explanatory variable are piecewise linear, i.e., two or more straight lines connected at unknown values or breakpoints.

An analysis of variance was performed for accumulated ADGchng to test the interaction between experiment and thermal sum, and because this interaction was significant ($p < 0.05$), regression analyses were run separately for each experiment, in which linear and non-linear models were tested: linear ($y = a + bx$), quadratic ($y = a + bx − cx2$), square root ($y = a + exp(b)x$), logarithmic ($y = a + log(b)x$), asymptotic ($y = Asym + (R0 − Asym)*exp(−exp(lrc)x)$) and Weibull ($y = Asym − Drop*exp(−exp(lrc)xpwr)$), where: y was the response variable, x was the GDD, a was the intercept, b was the linear coefficient, c was the quadratic coefficient, Asym was the horizontal asymptote, R0 was the intercept, lrc was the constant rate, Drop was the change from Asym to the y intercept, and pwr was the power to which x was raised. Models were chosen by their Akaike (AIC) criterion for each trial (the smaller AIC value corresponded to the best fitted model). When different experiments were fitted to the same model pattern (e.g., linear), the models were compared by analysis of covariance and contrasts using the “lsmeans” R package, at a 5% significance level, to verify the possibility of using a single model for the experiments.

A principal component analysis (PCA) was performed considering all explanatory variables and their association with the trials and their best fitted models. After that, 20 variables were selected by their contributions to the PCA ($cos > 0.7$) to generate the final analysis. The package “factoextra” from R was used.

Pearson (for variables with normal distribution) or Spearman (for non-normal distribution variables) correlation coefficients were estimated between ADGcnhg300 and ADGcnhg1000 and all of the explanatory variables ($p < 0.05$). After that, the top 10 variables with the greatest correlation values were included in multiple linear regression models for ADGchng300 and ADGchng1000. The models were checked for collinearity (by Variance Inflation Factor < 5) and the final models were selected by the lowest AIC value, since the parameters were significant ($p < 0.05$).

## 3.4. RESULTS

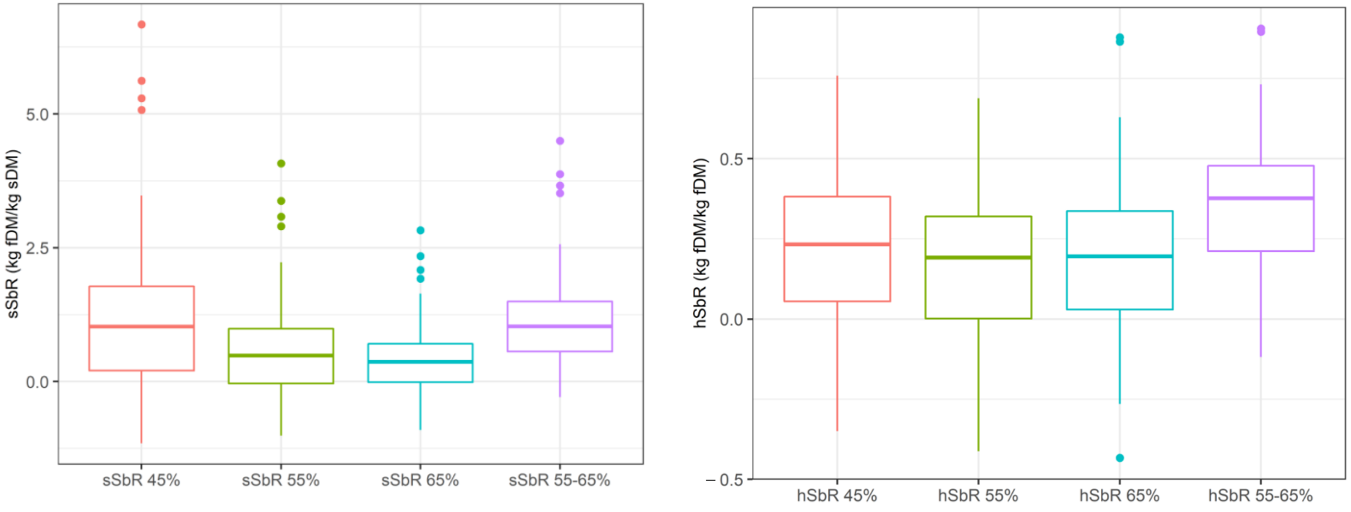
Herbage DM intake estimations were between 6.9 and 4.1 kg DM/animal/day for the lowest (45%) and highest (65%) assumed DMD, respectively, corresponding to 3.2–1.7 %BW intakes, for the lowest and highest assumed DMD, respectively. Herbage DM intake was positively correlated with SFE, presenting coefficients between 0.40 and 0.50 (Table 2). Additionally, the greater the assumed DMD, the greater was the observed coefficient.

**Table 2.** Spearman´s correlation coefficients between supplemental feed efficiency (SFE) and estimated herbage dry matter intake (HDMI) of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

| **Correlation between SFE and** | **Spearman** | **p-value** |
|---|---|---|
| HDMI 45%DMD (kg/animal/d) | 0.44 | < 0.001 |
| HDMI 55%DMD (kg/animal/d) | 0.46 | < 0.001 |
| HDMI 65%DMD (kg/animal/d) | 0.48 | < 0.001 |
| HDMI 45%DMD (%BW) | 0.40 | < 0.001 |
| HDMI 55%DMD (%BW) | 0.46 | < 0.001 |
| HDMI 65%DMD (%BW) | 0.50 | < 0.001 |

%DMD: dry matter digestibility; BW: body weight; SFE as kg BW change/kg supplement DM intake

Substitution rates differed depending on their assumed DMD (Figure 1). Supplement sSbR assuming greater DMD for supplemented plots than their control counterparts (sSbR 55–65 DMD = 1.2 ± 0.8 kg/kg) presented similar average values than assuming minimum DMD (45%, sSbR = 1.2 ± 1.3 kg/kg), yet with less variability. Similarly, hSbR assuming greater DMD for supplemented plots (hSbR = 0.3 ± 0.2 kg/kg) presented the greatest value and the least variability.




(a) (b)

**Figure 1.** a) Estimated substitution rates using three different digestibilities, substituting forage for supplement (sSbR) and, b) potential herbage intake substitution rate (hSbR) related to animals and pastures of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

DM: dry matter; fDM: forage DM; sDM: supplement DM; HDMI: herbage DM intake; sDMI: supplement DM intake; SbR: supplement substitution rate (control HDMI – supplemented HDMI) / sDMI); hSbR: potential forage intake substitution rate (control HDMI – supplemented HDMI) / control HDMI). 45, 55 and 65%: assumed forage dry matter digestibility (DMD) to estimate HDMI using CSIRO´s spreadsheet model; 55-65%: assuming control animals consume a lower forage DMD (55%) than supplemented animals (65%) to estimate HDMI using CSIRO´s spreadsheet model.

All substitution rates, both as supplement (sSbR) and as potential herbage intake substitution rate (hSbR) substitutions, were strongly and negatively correlated with SFE ($p < 0.01$), regardless of the DMD assumed in their estimations (Table 3). Considering that sSbR and SFE are not independent variables (because supplement intake is used to calculate both), these correlations may be misleading. Nonetheless, because hSbR is a completely independent variable from SFE, these strong associations determine that the more supplemented animals decrease their HDMI, the less efficient they will be in converting the consumed supplement. This is particularly the case as the assumed forage DMD increases up to 65% for both supplemented and control plots.

**Table 3.** Spearman´s correlation coefficients between supplement feed efficiency and three different digestibilities, substituting forage for supplement (sSbR) and potential herbage intake substitution (hSbR) related to animals and pastures of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

| Correlation between SFE and | Spearman | p-value |
|---|---|---|
| sSbR 45% | -0.72 | < 0.001 |
| sSbR 55% | -0.76 | < 0.001 |
| sSbR 65% | -0.79 | < 0.001 |
| sSbR 55-65% | -0.72 | < 0.001 |
| hSbR 45% | -0.76 | < 0.001 |
| hSbR 55% | -0.78 | < 0.001 |
| hSbR 65% | -0.79 | < 0.001 |
| hSbR 55-65% | -0.78 | < 0.001 |

sSbR: supplement substitution rate (control herbage dry mater intake, HDMI – supplemented HDMI) / supplement DMintake); hSbR: potential herbage intake substitution rate (control HDMI – supplemented HDMI) / control HDMI). 45, 55 and 65%: assumed forage dry matter digestibility (DMD) to estimate HDMI using CSIRO´s spreadsheet model; 55-65%: assuming control animals consume a lower forage DMD (55%) than supplemented animals (65%) to estimate HDMI using CSIRO´s spreadsheet model.

Even though not all trials presented the same number of phases, almost all of them showed at least two phases where the slope of C and S BW changed over time (Figure 2). Furthermore, many showed similar BW changes between S and C plots during the first phase.

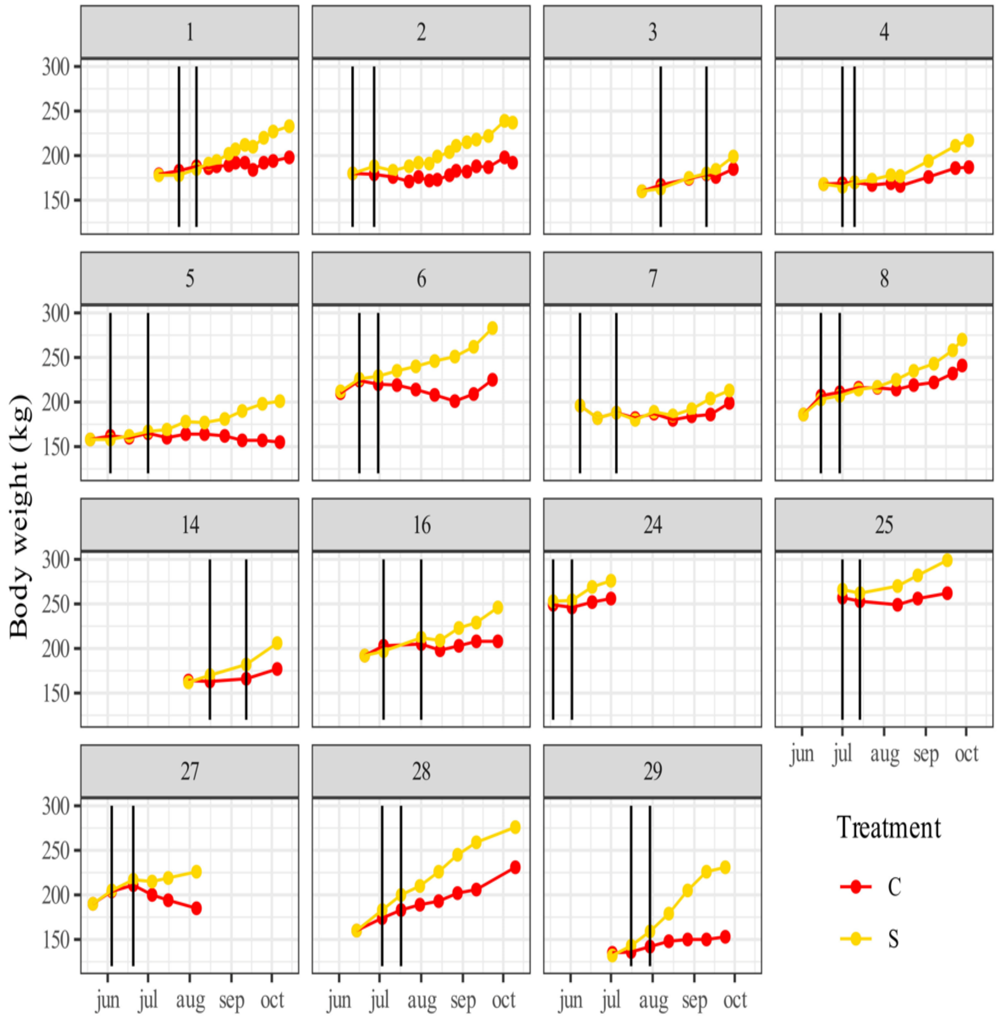



**Figure 2.** Body weight (BW) against time, separated by control (C) or supplemented (S) animal groups and their different phases of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

Control groups (C): red lines; Supplemented groups (S): yellow lines; limits of phases according to the FindPick function (quantmod R®): black vertical lines

Three main supplementation responses (regression models) were identified (Figure 3). The response was either linear, quadratic or with a Weibull distribution, where ‘y’ represents the accumulated ADGchng (i.e., the total BW difference between S and C groups), and ‘x’ is GDD. In the first case (linear response), accumulated ADGchng increased at a regular rate. In the case of the quadratic and Weibull response, the response was slow at the beginning of the trials, and then it accelerated until the end of the period (quadratic) or slowed down towards the end (Weibull).

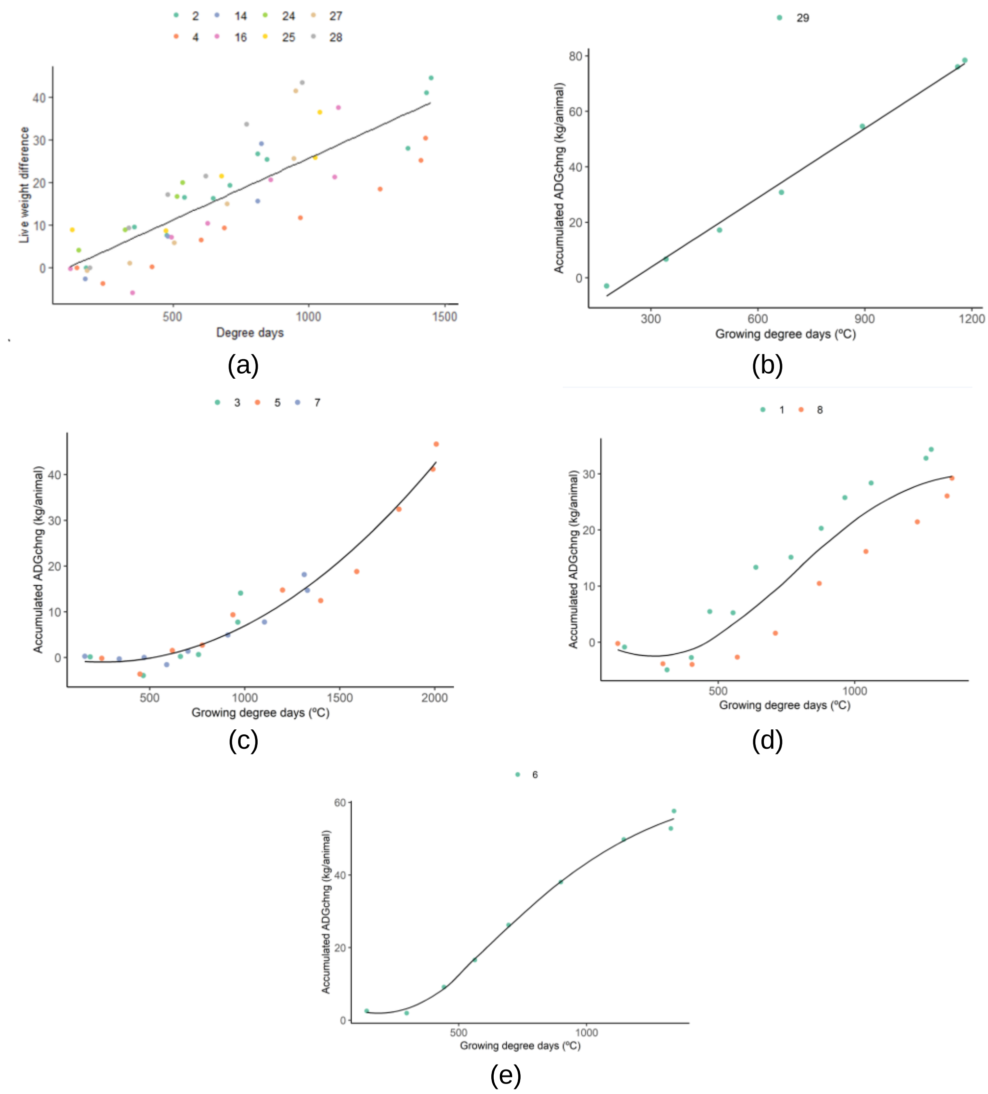



**Figure 3.** Accumulated average daily gain change (ADGchng) between supplemented and control plots, against growing degree days (GDD, base temperature = 0° C) for all experiments of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018). a) Trials 2, 4, 14, 16, 24, 25, 27, 28 (linear); b) Trial 29 (linear); c) Trials 3, 5, 7 (quadratic); d) Trials 1, 8 (Weibull); e) Trial 6 (Weibull)

a) $y = -3.37 + 0.029x$, $R2 = 0.6872$, $RSE = 7.52$, $p < 0.0001$; b) $y = -21.19 + 0.083x$, $R2 = 0.9944$, $RSE = 2.69$, $p < 0.0001$; c) $y = -0.078 – 0.00716x + 0.0000142x2$, $R2 = 0.9531$, $RSE = 3.01$, $p < 0.0001$; d) $y = 29.82 – 32.68*exp(-exp(23.54)x3.46)$, $RSE = 5.1$, $p < 0.0001$; e) $y = 57.72 – 57.19*exp(-exp(17.59)x2.60)$, $RSE = 1.96$, $p < 0.0001$.

Components 1 and 2 of the PCA explained 77.9% of the total data variation. Linear models did not present a distinct association with any particular set of other relevant variables. It is important to highlight that trial 29 (Linear 2) had the highest accumulated ADGchng and was associated with sDMI, sADG, sCP, sPC:sEM, fME and fCP. On the other hand, quadratic models appeared more associated with herbage biomass and substitution rates (both sSbR and hSbR). Weibull models were more closely associated with frosts, yet very distant from all other variables (Figure 4).

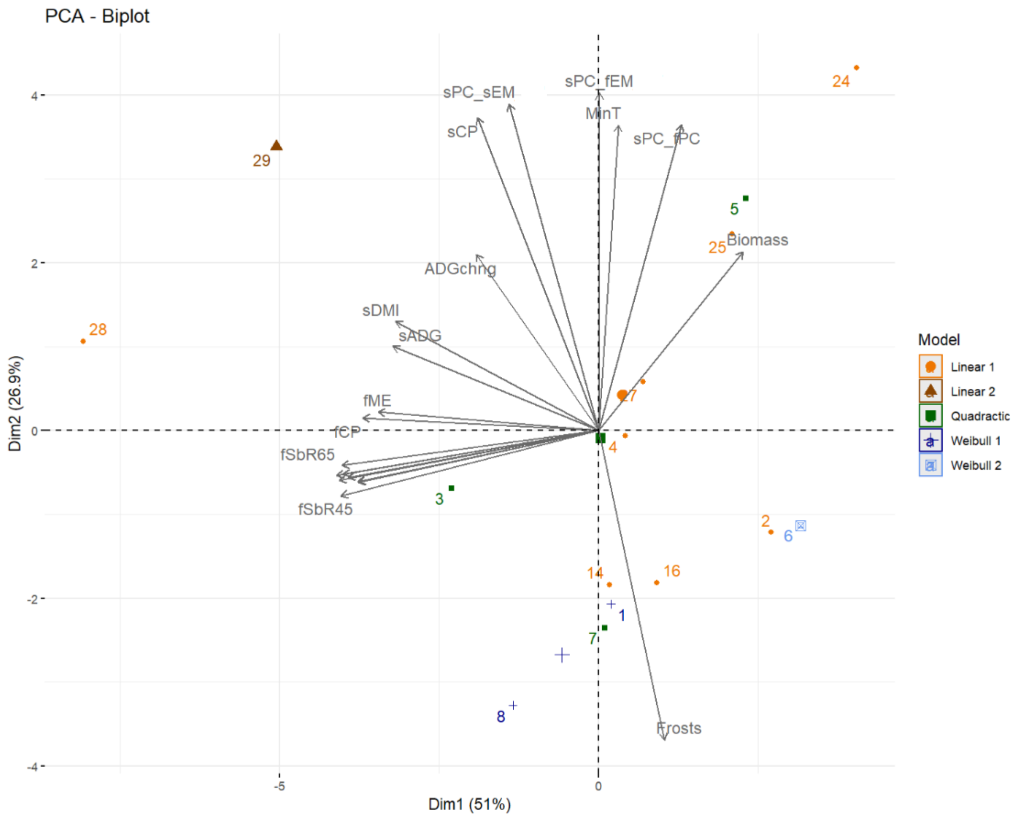



**Figure 4.** Principal components analysis separated into the five possible regression models explaining ADGchng (supplement response) vs GDD (growing degree days, base temperature = 0°C) of all experiments of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

Figure 5 presents the accumulated ADGchng for all trials, plotted against growing degree days and shows where 300 and 1000 GDD may be found for each of them. The animal´s body weight and the supplement CP:ME ratio positively affected the initial phases of the trials (ADGchng300). The last phases of the trials (ADGchng1000) were positively affected by supplement DM intake, supplemented animal´s ADG and forage CP:ME ratio, while being negatively affected by the CP content in the forage (Table 4).

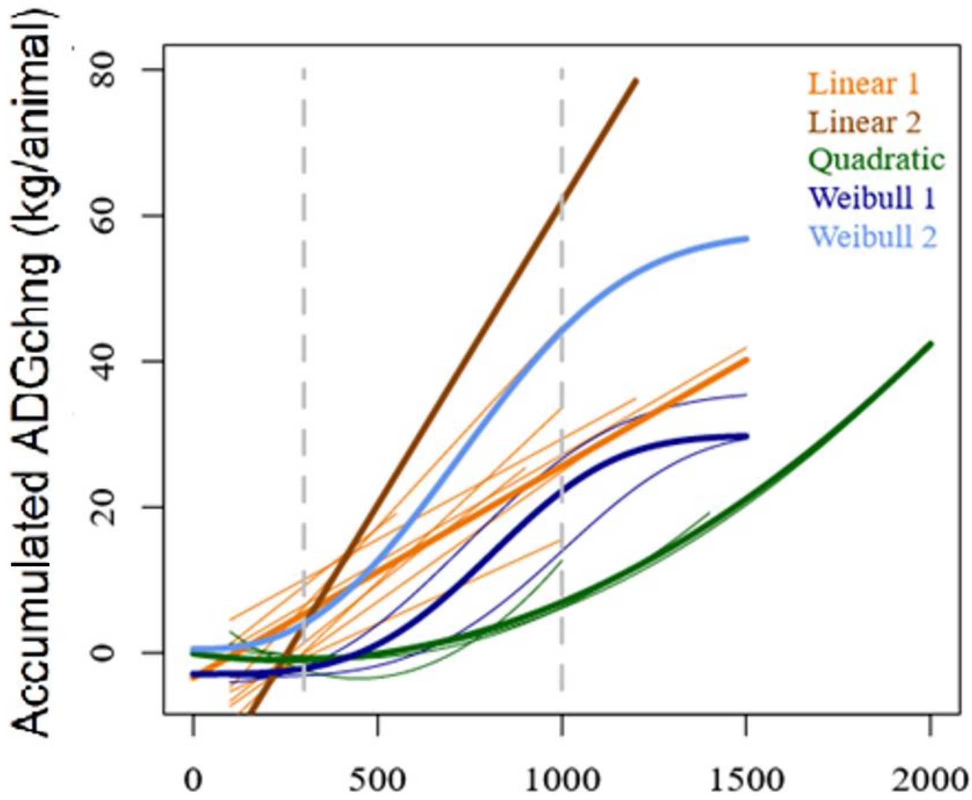



**Figure 5.** Accumulated average daily gain change (ADGchng) between supplemented and control plots, against GDD (growing degree days, base temperature = 0° C), by best fitted models for all experiments of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

**Table 4.** Multiple linear regression models´ equations for ADGchng at 300 and 1000ºCd GDD, of a database of fifteen supplementation experiments for young beef cattle on native grasslands in Uruguay (2004-2018).

| Multiple linear regression equation | R2 | Adj. R2 | p-value |
|---|---|---|---|
| ADGchng300 = -22.21 + 0.092 * BW + 3.66 * sCP:sME | 0.73 | 0.69 | <0.01 |
| ADGchng1000 = 17.90 * sDMintake + 43.51 * sADG + 45.3 * fCP:fME – 7.01 * fCP | 0.85\| | 0.79 | <0.05 |

ADGchng: average daily gain (ADG) change or supplementation response at either 300 growing degree days (GDD, °Cd, base temperature = 0°C) or 1000 GDD, BW: body weight (kg), sCP: supplement crude protein (CP) content, sME: supplement metabolisable energy content, sDMintake: supplement DM intake, sADG: supplemented animals´ ADG, fCP: forage CP content; fME: forage ME.

## 3.5. DISCUSSION

The estimated substitution rates were always positive, regardless of the assumed digestibility, which means that for supplementation schemes on native grasslands of the Pampa biome, some amount of forage will be substituted by supplement. Their values ranged between 0.3–1.1 kg/kg for supplement substitution and 0.1–0.3 kg/kg for potential herbage intake. In addition, substitution rates were negatively and moderately associated with SFE, or in other words, the more animals decreased their potential herbage intake, the less efficient they became in using the offered supplement. Considering that sSbR and SFE are not independent variables (because supplement intake is used to calculate both), these correlations may be misleading. Nonetheless, because hSbR is a completely independent variable from SFE, these strong associations determine that the more supplemented animals decrease their HDMI, the less efficient they will be in converting the consumed supplement, which is in line with Méndez et al. [25], who found that the main factor affecting pasture HDMI is sSbR, at least under no limiting grazing conditions. Using fixed stocking rates—as in all of the evaluated trials—supplementation proved to be a dynamic process, explained by the difference in the change in body weight of control and supplemented animals over time, suggesting the existence of differential response phases. This is in accordance with Palma et al. [10], who found differences in voluntary forage intake throughout the supplementation period. These responses could fit into one of three regression models: linear, quadratic or Weibull. While the linear models did not appear distinctly associated with any particular set of variables, quadratic models were mostly associated with herbage biomass and substitution rates, whereas Weibull models were the clearest in their association with frosts. Regardless of the response pattern, during the first phases of the trials, the supplementation response was positively associated with the animal's body weight and supplement quality, whereas towards the end of the trials, the response was more associated with the supplementation intake, supplemented animals´ ADG and forage quality. This reflects the changing animal and pasture conditions—in terms of sward structure and chemical composition—as the trial progressed through the autumn/winter period.

Animal responses to concentrate feeding depend on both animal and feed factors, and among the major feed factors is the rate of substitution of concentrates for pasture [26]. While HDMI was found to be positively associated with SFE, both supplement DM and herbage estimated substitution rates proved to be negatively associated with SFE (Tables 2 and 3), which means that supplemented animals consuming more forage and substituting less are obviously more efficient, as stated by Méndez et al. [25]. Since there is a positive relationship between substitution rate and FA [16], and the average FA of our dataset can be considered medium-low (5.5 ± 3.4 kg DM/kg BW) if we compare it with Pérez-Prieto and Delegarde [27] for ground-level clipping sown pasture estimations (6.1 ± 0.4 kg DM/kg BW), we may deduce that the observed values (Table 1) can be considered moderate-low. This moderate-low dry matter substitution rate in combination with the CP intake, both from pasture and supplement, allowed acceptable combinations of metabolisable CP and ME such that moderate ADGs were achieved with supplementation (0.52 ± 0.23 kg/animal/day). As Penno et al. [28] state, one of the key factors determining the intake response to supplementary foods is FA, in our case moderate-low. Additionally, Dixon and Stockdale [29] state that when ruminants are consuming low to medium digestibility forages, the substitution of grain for forage is generally lower than with diets based on high-quality forages (i.e., temperate sown pastures), and considering that our production systems present an intermediate forage nutritive value compared to temperate sown pastures on one hand, and on the other hand semiarid rangelands and dry-season tropical pastures [6], the sSbR of our database may be also expected to be somewhat in between these two environments, rendering SFE values in between these two situations.

The average hSbR results suggest that supplemented animals consuming more digestible pastures than their control counterparts through the exercise of their selectivity (hSbR 55–65%) eat considerably less forage than their theoretical maximum potential, compared to what happens when both control and supplemented DMD are the same (Figure 1). Given that among the multiple factors affecting substitution rate, one is the digestibility of the pasture [26], coincidently, the estimation of the actually consumed pasture in our database proved to be associated with the supplementation efficiency (Table 2) in some way through the differential digestibility between control and supplemented plots. In real production system situations, this would imply that, when animals are exposed to a highly heterogenous pasture in terms of digestibility—in such a way that supplemented animals may exert an important amount of selectivity—the first recommendation would be to reduce the forage allowance to control SFE through a lower forage on offer. Nonetheless, given that FA negatively affects SFE, while herbage mass positively affects it [6], an extra complexity emerges, in which we need to find an even finer balance between allowing animals to exert selectivity but at the same time controlling the FA, to prevent SFE from dropping.

In a fixed stocking rate supplementation scheme (heads/ha) as in our database, the sward is expected to change from initial to final conditions in terms of quantity, quality, and spatial arrangement. It was possible to observe different phases over time (Figure 2), in which all experiments presented at least one phase in which the BW change over time of C and S animals behaved oppositely, where S animals were actively gaining BW while C animals were maintaining or losing BW. In a grazing-down experiment, Cazzuli et al. [30] could not find any differences between S and C animals concerning change in green content over time of their swards nor on their plant functional types´ species preferences. This suggests that the differences in BW change between C and S animals in this work—especially of C animals' BW change—may be only partially explained by herbage quality or species composition of the sward throughout the duration of the trials. In fact, Table 4 shows that BW explained accumulated ADGchng in the early phases of all trials, while supplementation intake and forage quality were more closely associated with the final phases of the supplementation schemes. It is possible that a lower forage quality condition (CP content at least) plays a greater role in explaining a greater supplementation response during the latest phases of the supplementation activity, compared to the beginning.

Since many variables were associated with the linear response models, it may be assumed that their response was more dependent on each trial´s characteristic, without a well-defined response pattern (Figure 4). Bonhert et al. [31], who worked with low-quality C3 and C4 species, found that the intake and digestion of these grasses differ and that the physiological response of ruminants to protein supplementation is dependent on forage type. In the case of native Campos grasslands, dominated by a highly heterogeneous mosaic of C3 and C4 species [32], multiple sward quality and structure may be expected, depending on the predominant weather trend of a particular year.

Thus, at least in the case of linear models, which represented over 50% of the trials in our database and in which the supplement response was expected to increase at constant rates, a highly complex arrangement of sward, supplement quality and weather scenarios may explain greater or lower SFE values. Nevertheless, as a general principle, and as discussed above, the key issues probably will be associated with finding a certain balance between FA and forage biomass. In the case of both non-linear models (quadratic and Weibull), ADGchng might take longer to be observed at the beginning of the supplementing period (Figure 3). Quadratic response patterns were mostly associated with herbage biomass and substitution rates. If we consider the importance of sward structure affecting HDMI on Campos grasslands [19,33,34], and the fact that HDMI is positively associated with SFE (Table 2), we could state that, in these quadratic response cases, sward structure, for example, herbage biomass, plays a key role in determining high SFE, as opposed to forage quality or supplement intake. Should this be the case, this would be in agreement with Cunha et al. [35], who claim that forage nutrient content of several forage bases—from sown pastures to native grasslands—explains only a small fraction of HDMI, ADG and CH4 emissions. When analysing the Weibull response models (20% in our trials), the occurrence of frosts seems to be the most important explanatory variable for this response type. Considering that temperature usually has a considerable effect on grass digestibility, mainly through its effect on leaf-to-stem ratio [36], frosts could be indirectly affecting the Weibull-type responses through a lower overall digestibility of the pasture.

At the beginning of all of our trials (300 GDD), regardless of the response model type, the animals´ BW positively influenced ADGchng as well as the supplement´s CP:ME ratio. During a more advanced phase of the trials (1000 GDD), it was a greater supplement intake and sADG, as well as lower forage CP, yet a greater forage CP:ME ratio (Figure 5 and Table 4). In a meta-analysis performed by Detmann et al. [12], there was an average positive response of protein-based supplements on tropical pastures (fCP < 6%) with beef cattle, as the amount of offered supplement increased. Our results during an advanced phase of the trials (1000 GDD) matched the average reported by these authors, where the greater the supplement intake, the greater supplement response was expected. In our database, both energy and protein-based supplements can be found, yet according to the preceding paper of Cazzuli et al. [6], no differences were found between either of these types of supplements on ADGchng, nor between the supplementation rate and SFE. The top leafy stratum of pastures is expected to be preferred among all other pasture components [37], meaning that it would be consumed before the rest of the sward, and therefore, the green leaf content would be expected to decline throughout trials. Even though Cazzuli et al. [30] found an opposite trend—at least at the end of a long stockpiling period and before grazing activities began—Benvenutti et al. [37] found that the fCP content of a C4 species dominated sward was greater in the upper stratum of the sward. Should the latter be the case, during the final phases, the pastures would be offering lower quality forage in terms of fCP, which matches our results because, during this late stage, the lower the fCP, the greater the response. On the other hand, and matching our results, Detmann et al. [12] found that supplement response decreased as the fCP increased, but these results were observed with very low fCP contents, whereas in this collated analysis fCP was above 8% on average. An fCP threshold could exist under which no effect could be expected (early phases, 300 GDD) and above which (later phases, 1000 GDD) it could be observed.

### 3.6. CONCLUSIONS

As stated in our working hypothesis, a positive and low-moderate substitution rate occurred in our dataset, suggesting that there is still room for improvement of supplemental efficiency. Additionally, we concluded that the digestibility of the consumed pasture affects the supplement feed efficiency to some extent. Thus, efficiency is a multi-factorial issue, predominantly associated mostly with forage and supplement intake affecting the animal´s digestive physiology but without excluding the native pasture´s nutritive characteristics and array.

Also accordingly with our initial hypothesis, three different supplementation responses could be identified (one of them being linear, and two of them being non-linear), with a slow beginning and an accelerated phase afterwards. Quadratic response patterns were explained mostly by herbage biomass and substitution rates, while Weibull response patterns were mostly associated with frosts.

Regardless of the response pattern, at the beginning of the trials, it was the animals´ body weight and supplement quality that most influenced supplement response in a positive way, whereas towards the end, both lower supplementation intakes and CP forage contents played a more relevant role in explaining greater responses. This information may be used in commercial supplementation schemes to help predict what to expect in each phase and to introduce modifications accordingly, such as altering supplement type if possible, shifting paddocks, etc.

Studying supplementation phases in detail in future research, especially in terms of sward conditions throughout the supplementation period, could shed further light on the variability of supplement feed efficiency along the period in which supplementation takes place. The estimated parameters and their variability in forage intake and substitution rates could be useful if included in decision support systems for livestock farmers in the future.

## 3.7. AUTHOR CONTRIBUTIONS

Conceptualization, FC, FL, CB and MD; methodology, FC, FL, CB and MD; software, FC, FL, CB and MD; validation, FC, FL, CB and MD; formal analysis, FC, CB, MD, AH and JSanchez, investigation, FC, AH, JSanchez, PR, VB, AS, MJ, MD, JVS, DP, FM, XL, SL, GB, JIV, FL and CB; resources, MJ, FL; data curation, FC, MD; writing- original draft preparation, FC, CB, MD;

writing—review and editing, FC, AH, JSanchez, PR, VB, AS, MJ, MD, JVS, DP, FM, XL, SL, GB, JIV, FL and CB; visualization, FC, CB, MD, FL; supervision, CB and MD; project administration, MJ; funding acquisition, MJ, FL, FC. All authors have read and agreed to the published version of the manuscript.

## 3.8. AKNOWLEDGMENTS

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## 3.9. APPENDIX

### Appendix A

Summary of collated trials on supplementation of cattle grazing native rangelands carried out in Uruguay between 1993 and 2018. All trials included one unsupplemented control treatment.

| Trial ID | Location, Year | Supplementation treatments* | Breed and category | Duration (days) | Reps. | Animals /trial | Source |
|---|---|---|---|---|---|---|---|
| 1* | La Magnolia, 2013 | TMR with fibre | Braford male calves | 97 | 2 | 40 | [2] |
| 2* | Glencoe, 2013 | TMR with fibre | Hereford male calves | 120 | 2 | 40 | [2] |
| 3* | La Magnolia, 2014 | RB (ground and pelleted) | Braford male calves | 68 | 2 | 40 | [38] |
| 4* | Glencoe, 2014 | RB (ground and pelleted) | Hereford male calves | 108 | 2 | 50 | [38] |
| 5* | Glencoe, 2015 | Various (maize, expellers and RB) | Hereford male calves | 141 | 2 | 50 | [38] |
| 6* | Glencoe, 2009 | RB | Hereford male and female calves | 113 | 2 | 48 | [39] |
| 7* | Glencoe, 2010 | RB | Hereford male calves | 111 | 2 | 48 | [39] |
| 8* | Glencoe, 2011 | RB | Hereford male calves | 119 | 2 | 48 | [39] |
| 14* | Ptas del Chuy, 2011 | TMR | British crossbred male calves | 81 | 2 | 48 | [40] |
| 16* | Glencoe, 2007 | RB | British crossbred male calves | 98 | 2 | 24 | [41] |
| 17 | Palo a Pique, 2012 | TMR with fibre | British crossbred male calves | 77 | 1 | 12 | [42] |
| 24* | Glencoe, 2004 | Various (RB and expeller) | British crossbred steers | 42 | 2 | 70 | [43] |
| 25* | Glencoe, 2004 | Various (RB and expeller) | British crossbred steers | 78 | 2 | 70 | [43] |
| 27 | Palo a Pique, 2008 | TMR | British crossbred male calves | 77 | 1 | 56 | [44] |
| 28* | Cañada del Pueblo, 2008 | DDGS | Hereford female calves | 89 | 2 | 40 | [45] |
| 29* | Tomás Gomensoro, 2008 | DDGS | British crossbred male calves | 84 | 2 | 40 | [45] |
| Total | | | | | | 1156 | |

ID: identification number; TMR: total mixed ration; RB: rice bran; HMSGS: high-moisture sorghum grain silage (combined with protein supplements); * Trial with 2 replicates.

## Appendix B

Descriptive statistics of herbage matter intake (HDMI), supplement substitution rate (sSbR) and potential herbage intake substitution (hSbR): (mean), standard deviation (SD), coefficient of variation (CV), minimum (min), and maximum (max) of a dataset of supplementation of cattle grazing native rangelands carried out in Uruguay between 1993 and 2018.

| Variable | Min | Mean | Min | SD | CV |
|---|---|---|---|---|---|
| HDMI 45%DMD (kg/animal/d) | 2.47 | 6.48 | 10.30 | 1.69 | 26 |
| HDMI 55%DMD (kg/animal/d) | 1.88 | 4.94 | 7.85 | 1.21 | 24 |
| HDMI 65%DMD (kg/animal/d) | 1.43 | 3.90 | 6.07 | 0.94 | 24 |
| HDMI 45%DMD (%BW) | 0.00 | 2.90 | 5.39 | 1.04 | 36 |
| HDMI 55%DMD (%BW) | 0.00 | 2.21 | 3.69 | 0.75 | 34 |
| HDMI 65%DMD (%BW) | 0.00 | 1.74 | 2.77 | 0.58 | 34 |
| sSbR 45% | −1.16 | 1.06 | 5.62 | 1.26 | 119 |
| sSbR 55% | −1.01 | 0.50 | 3.38 | 0.81 | 162 |
| sSbR 65% | −0.90 | 0.32 | 2.35 | 0.59 | 185 |
| sSbR 55–65% | −0.29 | 1.09 | 3.88 | 0.76 | 70 |
| hSbR 45% | −0.35 | 0.20 | 0.67 | 0.21 | 108 |
| hSbR 55% | −0.41 | 0.13 | 0.61 | 0.21 | 160 |
| hSbR 65% | −0.43 | 0.12 | 0.60 | 0.22 | 179 |
| hSbR 55–65% | −0.12 | 0.31 | 0.71 | 0.18 | 56 |

%DMD: dry matter digestibility; BW: body weight; sSbR = (control HDMI − supplemented HDMI)/supplement dry matter intake; hSbR = (control HDMI − supplemented HDMI)/control HDMI. 45, 55 and 65%: assumed forage DMD to estimate HDMI using CSIRO´s spreadsheet model; 55−65%: assuming control animals consume a lower forage DMD (55%) than supplemented animals (65%) to estimate HDMI using CSIRO's spreadsheet model.

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# 4. THE DEFOLIATION DYNAMICS OF A STOCKPILED NATIVE GRASSLAND PASTURE FOLLOW SIMILAR PATTERNS BETWEEN SUPPLEMENTED AND UNSUPPLEMENTED BEEF CALVES

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## 4.1. ABSTRACT

It is unclear to what extent and on which variables does supplementing beef cattle on native grasslands affect sward structure, specifically on the dynamics of its grazing horizons. Three hypotheses were tested: i) during a grazing down process under similar forage allowance, supplemented animals take longer to finish each grazing stratum, than their unsupplemented counterparts, ii) in both cases, the upper stratum will be heavily depleted before the subsequent strata are grazed, iii) some species and/or forage fractions are consumed faster than others, regardless of the animals being supplemented (corn dried distillers grains with solubles, DDGS, at 0.7% of their body weight, BW, on a dry matter, DM, basis) or not. Three blocks of stockpiled native grasslands were used and split into two treatments plots (n=6), on which either supplemented (S) or control (C) heifers of 10.6±0.6 months of age and an initial BW of 143±9 kg, were used. A 2.5 x 0.5 m observation grid was installed on the sward, generating 384 observation points on each plot. On these observation plots, sward height (SH) and visually assessed green forage mass percentage (%G) were registered every other day for 12 consecutive days. No differences were found between the horizontal grazing dynamics between supplemented and control animals in terms of how they switched from the upper grazing horizon to the successive ones. In both cases, when the upper grazing horizon was heavily depleted, the subsequent horizon was being depleted by its half. Differences of preference for C3 species over C4 were observed for both treatments, but this effect was more meaningful for control animals. Grazing time never fully compensated for the decline in intake rate during depletion throughout the grazing horizons. Pasture intake declined when the animals transitioned from grazing the top grazing horizons to the lower horizons, irrespective of the level of supplementation. Managing the sward structure in terms of sward height will be beneficial to maximising individual animal performance, for both C and S animals. Native grasslands paddocks with a greater C3 gasses predominance will always be preferred to C4 dominated paddocks, regardless of an eventual supplementation practice.

**Key words**: stockpiled native grasslands, protein-energy supplementation, grazing horizons, grazing down process, beef cattle.

## 4.2. INTRODUCTION

Under grazing conditions, herbage dry matter intake (HDMI) is associated with sward structure, such as the proportion of green leaves (Hodgson *et al.*, 1977; Burns and Sollenberger, 2002) and sward height (SH) (Hodgson *et al.*, 1977; Boval *et al.*, 2007; Da Trindade *et al.*, 2016). Both vertical and horizontal distribution are important to understand the animal-plant interactions (L'Huillier *et al.*, 1986; Marriott & Carrère, 1998; Cangiano *et al.*, 2002), and if supplements are included in the animals´ diet, more complex relationships emerge (da Silva *et al.*, 2009). Among these complexities, different selectivity patterns can be observed. For example, Pötter *et al*. (2010) suggest that supplemented animals (using 0.3-1.5 % body weight, BW, supplementation rates) can be more selective and consume more leaves than unsupplemented animals, while Gomide *et al*. (2009), working with supplementation rates between 0.2 and 1% BW, suggested that it may also affect differentially leaf:stem ratios, light interception, and leaf area index with increasing supplementation rates. Nonetheless, some authors did not find any evidence of differential selectivity between supplemented and unsupplemented animals (Farruggia *et al.*, 2008, working with less than 1% BW supplementation rates) nor effects on sward structural characteristics (Santos *et al.*, 2016, working with 0.5-1.5 %BW supplementation rates) on sown pastures. Therefore, it is unclear to what extent and on which variables does supplementation affect animal selectivity and sward structure, particularly on native grasslands. Particularly, little is known about these processes when animals graze stockpiled herbage of grasslands that combine cool and warm season species and thus constitute horizontally and vertically heterogeneous mixtures of green and dead tissue (Núñez *et al.*, 2022; Cazzuli *et al.*, *2023*).

Some studies of foraging behaviour of ruminants have been carried out on native grasslands of the Pampa Biome (Gonçalves *et al.*, 2009; Da Trindade *et al.*, 2012; Da Trindade *et al.*, 2016), but no studies have been conducted aiming to characterise successive grazing strata, nor its interaction with concentrate supplementation. Experiments carried out on cultivated pastures showed a significant decline of HDMI with high horizontal utilisation levels of the upper leaf stratum by cattle (Benvenutti *et al.*, 2015; Benvenutti *et al.*, 2017; Ison *et al.*, 2019; Ison *et al.*, 2020) where cattle grazed the lower strata of a pasture only when the top stratum became heavily depleted (Benvenutti *et al.*, 2015). Specifically, on native rangelands HDMI decreases with high levels of horizontal utilisation of the upper leaf stratum horizon of preferred plant species (Benvenutti & Poppi, *com. pers*.).

The objective of this study was to characterise the disappearance dynamics of a stockpiled native grassland sward during a grazing-down process and to identify grazing horizons. We hypothesised that, i) during the grazing down process under similar forage allowance, supplemented animals take longer to finish each grazing stratum, than their unsupplemented counterparts, and ii) that in both cases, the upper stratum will be heavily depleted before the subsequent strata are grazed. Additionally, we hypothesised that, iii) some species and/or forage fractions (green tissue and green leaves) are consumed faster than others, regardless of the animals being supplemented at 0.7% BW or not.

## 4.3. MATERIALS AND METHODS

### 4.3.1. General description

All procedures were approved by the (CEUA) Ethics Committee for Experimental Animal Use of INIA (registration number 0009/11).

The experiment was carried out at “Glencoe” Research Station of the National Institute of Agricultural Research (INIA Tacuarembó, Uruguay, S 32° W 57°) on Hapludolls soils with a basaltic origin, with high clay content and medium-high potential rooting depth.

The mean air temperature during the trial was 15.6±6 °C and total accumulated rainfall was 2.4 mm.

Twenty hectares of native grasslands were stockpiled from 28$^{th}$ March to 29$^{th}$ July 2020 (124 days), being grazed at very high stocking rates before the forage accumulation period began, aiming to remove dead material from the summer. The most frequent grass species observed (above 50%) were *Nasella neesiana* (Trin. et Rupr.), *Paspalum dilatatum* (Poir) and *Mnesithea selloana* (Hack.), but there were many others with minor contributions to the forage. Three blocks of native grasslands (1800 $m^2$ each) were chosen out of the initial 20 ha and each block was split into two 900 $m^2$ plots (n=6). Each paddock was grazed at a set stocking rate of 6 heifers of 10.6±0.6 months of age and an initial body weight (BW) of 143±9 kg. Overall, thirty-six female Hereford calves were allotted to each treatment balancing their BW and residual feed intake (RFI) (Berry 2008) expected progeny difference (EPD) to generate six homogeneous groups (6 animals per paddock * 6 paddocks).

There were two treatments: control (C, unsupplemented animals) and supplemented (S) animals (0.7% BW on a dry matter (DM) basis). The concentrate supplement used was corn dried distillers grains with solubles with 35.0% of crude protein (CP), 14.0% of acid detergent fibre (ADF), and 11% of ether extract (EE).

### 4.3.2. Animal feeding

Animals allotted to the S treatment were accustomed to supplement for 10 days before the beginning of the trial, in which the supplementation rate was gradually increased until the target supplementation rate of 0.7% BW was achieved. Likewise, five days before the beginning of the trial both S and C animals were accustomed to graze on nearby paddocks, with a low stocking rate (43% of the target stocking rate) to utilise no more than 10% of the forage DM on offer.

The animals entered their trial paddocks on 30th July 2020 and the trial ended when the average residual pasture height of all of them reached ±2 cm or less, which happened on 10th August (11 days) for both C and S treatments. Supplemented animals were fed daily between 08:15-08:30 a.m., using group troughs, which were placed on the corners of the paddocks, next to the drinking troughs. No supplement leftovers were observed during the experiment.

### 4.3.3. Initial sward characteristics

#### 4.3.3.1. Total forage biomass and its components

To estimate forage biomass, before the trial began (pre-grazing phase) on 21st and 22nd July, forage clippings were made at ground level within a 0.2 x 0.5 m quadrat (22 quadrats per plot). All clippings were performed away from the transects (which will be explained below).

Given the natural heterogeneity of the forage base, two main and distinct "patches" were identified according to their estimation of the visually assessed green forage mass percentage (%G) on the upper stratum, which was estimated with the help of a printed grid of fixed green hues (between 5 and 95%), generated by previous studies (Berretta & Jaurena*, unpublished*) which made it easier to assign %G values. Thus, the following "patches" were identified: dry patches (DP, %G <40%) and green patches (GP, %G >60%). Within each type of patch, 5 (DP) and 2 (GP) subtypes were identified according to their average SH: DP20 (20±2 cm), DP15 (15±2 cm), DP8 (8±2 cm), DP5 (5±2 cm), DP3 (3±2 cm), GP5 (5±2 cm) and GP3 (3±2 cm). Before the clippings were made, five SH and %G estimations were taken randomly within each quadrat. The SH was measured using the same criterion as in the observation points of the transects that will be explained in detail below. In the first clipping round, one of each of the subtypes was clipped on each plot, but in successive rounds the least easily found sub-patch was omitted, until four rounds were performed. Overall, 4 quadrats were clipped for DP20, DP15, DP8, DP5, three for DP3, two for GP5 and one for GP3.

All forage samples were weighed (fresh basis) and afterwards weighed separately into the following fractions: a) dead tissue, b) green tissue, and within the latter, c) grass blade or, d) non-grass blade. Each forage sample was put into an air-forced oven at 60 °C for 72 h, after which they were dry weighed and ground for the nutritive analyses (1 mm sieve).

#### 4.3.3.2. Forage chemical composition

Samples were analysed to estimate the concentration of organic matter (OM), CP, ADF and NDF. Forage CP content (N x 6.25) was estimated using the near-infrared reflectance spectroscopy (NIRS) technique (Model DA 7250, PERTEN Instruments AB, Hagerstem, Sweden), which was previously calibrated by analysing 40% of all samples using an elemental analyser (Model CHN 626, LECO Corporation, Saint Joseph, Michigan, USA) and setting a linear regression between each method ($R^2$ = 0.89). The OM content was estimated by subtracting the ash content (AOAC International, 1990. Nro. 942.05; Furnace 30400, Barnsted/Termolyne, Iowa, USA) from total DM content at 105°C. Both ADF and NDF forage content were analysed using the same NIRS technique already described, previously calibrated by analysing 38% of all samples using the ANKOM Technology Method (A200 Fiber Analyzer, ANKOM Technology, NY, USA) and setting a linear regression between each method ($R^2$ = 0.89 and 0.92 for ADF and NDF, respectively).

### 4.3.4. Sward height and green proportion measurements during the grazing down process

To assess SH and %G every other day, in each paddock, 8 transects of 24 m each and separated by 2.5 m were marked with pickets. The transects were located 5 and 4.5 m away from the surrounding fences and supplement and/or drinking troughs, to avoid areas of excessive trampling. Every 6 m within each transect an iron stake was nailed into the ground, to support a portable iron device on which a tape measure of 6 m was placed successively (4 times/transect), maintaining the same height above the ground (6 cm) and parallel to the floor. This procedure was designed to achieve the greatest precision to find the same point between measuring dates. On each transect there were 48 observation points of 0.06 x 0.06 m and separated 0.5 m between them, thus generating a 2.5 x 0.5 m fixed observation grid. Overall, each paddock presented 384 observation points. On each of these observation points, a 0.06 m wide ruler was placed directly on the ground making sure it stood perpendicularly to the ground. The observations registered on these "points" (0.0036 $m^2$) before and during the grazing activities were SH and %G. The SH was registered as the height in which most of the forage within the area observation point was observed. Both SH and %G were calibrated within observers by training them for several days on adjacent paddocks.

The SH and %G observations were made every other day, starting on 29th July (pre-grazing) and ending on 10th August (last grazing day), generating 7 observation dates. During the grazing down period, we also registered whether each observation point was untouched or was grazed for the first time and if it had been contaminated with faeces or not. This procedure ensured that changes (i.e. reductions) in SH were solely due to HDMI during the grazing down process.

Additionally, at the beginning of the trial (29th July, pre-stocking period) the predominant species of each observation point (384 per paddock) was registered, together with SH and %G. The grass species were classified into their functional types according to Cruz *et al.* (2019) to simplify the analysis. Briefly, these authors first divided native grasslands species into two main groups (C3 and C4 species) after which they were sub classified into four groups (A, B, C and D) of plant functional types (PFT), according to their preferred degree of soil fertility and use intensity, where A and B respond more strongly than C and D to fertilisation or an increase in access to sunlight, i.e. defoliation. The following are some of the most frequent plant species of each group of this study: A-C4, *Axonopus fissifolius* (Raddi) Kuhlm., *Paspalum dilatatum* Poir.; B-C4, *Paspalum notatum Flüggé*, *Botriochloa laguroides* (DC.) Herter; C-C4, *Andropogon ternatus* (Spreng.) Nees, *Mnesithea selloana* (Hack.); D-C4, *Aristida uruguayensis* var. laevis; B-C3, *Poa lanigera* (Nees); C-C3, *Naasella charruana* (Arechav.); D-C3, *Nasella setigera* (Trin. & Rupr.), *Piptichaetium stipoides* (Trin. & Rupr.).

#### 4.3.4.1. Dynamics of herbage disappearance during the grazing-down process

For each observation point and date, the relative SH (RelSH) was calculated as the difference between the SH of two consecutive dates of the same observation points, in relation to the initial value (values between 0 and 1). After that, the numerical characterisation of the grazing down process was calculated as -RelSH, or in other words, the RelSH reduction rate (or residual sward heigh in percentage) which describes the grazing-down process.

Forage mass disappearance (used as a proxy for HDMI) was estimated for all forage fractions and each observation point and date, as the difference between the initial DM (iDM) and the product of iDM by RelSH (HDMI = iDM – [iDM * RelSH]).

Each observation point was classified according to their initial SH and %G values into one of the 7 sub patches defined in the herbage mass calibration. The horizontal utilisation was calculated as a proportion of all possible grazing points (384 in each plot = 100%). The grazing horizons (H) were identified as follows: for the same observation point, the difference between successive SH were calculated; when this difference was > 0, then the grazing horizon was considered to change from $H_i$ to $H_{i+1}$. Horizons were considered when they were first grazed.

### 4.3.5. Animal behaviour

Animal behaviour was registered on 1st, 5th, 7th August throughout all daylight hours (0730-1830 h) with one observer per block using binoculars. Observers rotated at midday, but they were always the same for each block throughout the measurements. The observers registered the activity of all animals of each plot every 15 minutes, classifying them into one of the following: grazing, ruminating, resting, playing/scratching, walking, drinking water or supplement consumption (Montossi, 1995).

### 4.3.6. Statistical analysis

All statistical analyses were performed with the base package of R software (R Core Team, 2017) in combination with Infostat (Di Rienzo *et al.*, 2015).

Several multiple and simple regressions were tested to adjust the best equation to estimate DM biomass (BM), combining the initial original and logarithmic SH and %G data and their interactions, and separating GP and DP or taking all data together (GP+DP). The criteria for choosing the best equations were based on the lowest AIC value in the first place together with the best $R^2$ fitting. After this first draft, three equations were selected for each forage fraction and the mean prediction error (MPE) = (∑ (│(Predicted DM - Observed DM)│) was calculated using the original values. In the end, a simple linear regression was adjusted for each fraction (total, dead, total green and green leaves DM), regardless of the type of patch, using a logarithmic scale for SH and original data for %G. The following equations were used throughout the analyses, for all observation points (384) and observation dates (7):

- log total DM = 2.8305 + 0.8577 * [log SH] - 0.0052 * [%G]
- log dead DM = 2.9120 + 0.9369 * [log SH] - 0.0014 * [%G]
- log green DM = 1.6575 + 0.8619 * [log SH] + 0.0108 * [%G]

- log green blade DM = 1.4062 + 0.9279 * [log SH] + 0.0129 * [%G]

The experimental unit was the plot, and the generation of the weighed means of the variables for both treatment and stratum were generated using the "aggregate" command of the R package.

All DM fractions (total, dead, green and green blade) and their percentage were analysed using a mixed model in which block was used as a random effect, while treatment was the fixed effect.

Since both initial total DM (kg/ha) and the percentage of green blade DM (%) presented differences between treatments ($p<0.05$), they were always tested as covariables throughout the rest of the analyses.

The analysis of the grazing down process, residual sward height, was performed using the following model: treatment x grazing horizon (H) x their interactions with the RelSH as fixed effects, while random effects were block, transect and sub transect, and total DM biomass and %G were covariates. When random effects were not significant, the models were simplified according to Pinheiro & Bates (2000).

Means were considered significant when $p < 0.05$ using Tukey test. When the effect of RelSH was significant ($p<0.05$) different regression models were tested: linear ($y = a + bx$), quadratic ($y = a + bx – cx^2$), squared root ($y = a + \exp(b)x$) and asymptotic models ($Asym + (R0 − Asym) * \exp(−\exp(lrc)x)$, where: *y* is the response variable, *x* is RSH, *a* is the intercept, *b* is the linear coefficient, *c* is the quadratic coefficient, *Asym* is the horizontal asymptote, *R0* is the intercept and *lrc* is the constant rate. The best fitted model was chosen according to the AIC criterion.

Animal behaviour was analysed through a mixed model in which the activity was the fixed effect, and the random effects were block and date.

## 4.4. RESULTS

### 4.4.1. Initial sward characteristics

At the beginning of the trial, swards presented overall similar characteristics between treatments, with a SH of 7 to 8 cm, predominance of dead tissue (72-74%), low CP (6.5 %) and high NDF (58-63%) (Table 1). The frequency of each type of patch was similar between treatments (p<0.05), and no interaction between treatment and strata was found for any sward characteristic (p>0.05). However, total herbage DM and green blade content were 14% and 6% greater in C than S swards, respectively, and total green blade DM also tended to be greater (p=0.056) in C plots. Nonetheless, the initial %G and green DM were similar between treatments, and no differences were found regarding herbage chemical composition (p>0.05, Table 1).

**Table 1**. Initial conditions of the sward of a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

| **Variable** | **Control** | **Supplemented** | **p** | **SEM** |
|---|---|---|---|---|
| Sward height (cm) | 8.12 | 7.21 | 0.104 | 0.34 |
| Green (visual) (%) | 36.9 | 39.8 | 0.286 | 3.07 |
| Total DM (kg/ha) | 1536.8 | 1347.6 | 0.023 | 70.5 |
| Green DM (kg/ha) | 339.8 | 311.9 | 0.186 | 29.2 |
| Green blade DM (kg/ha) | 283.6 | 247.8 | 0.056 | 20.0 |
| Green DM (%) | 27.7 | 25.7 | 0.081 | 0.01 |
| Green blade DM (%) | 84.7 | 79.8 | 0.039 | 0.02 |
| CP content (%) | 6.46 | 6.63 | 0.509 | 0.28 |
| ADF content (%) | 56.0 | 51.3 | 0.101 | 1.81 |
| NDF content (%) | 57.5 | 62.7 | 0.1121 | 1.94 |

DDGS: dried distillers grains with solubles; BW: body weight; DM: dry matter; CP: crude protein; ADF: acid detergent fibre, NDF: neutral detergent fibre; SEM: standard error of mean. Green (visual) (%) was estimated by using a printed grid of fixed green hues (between 5 and 95%), generated by previous studies (Berretta & Jaurena, unpublished).

When analysing the vertical description of the initial condition of the stockpiled sward (Table 2), the upper strata presented less quality in terms of green DM and CP contents, but the opposite was observed for green blade content. Both ADF and NDF contents presented no difference among strata.

**Table 2**. Initial conditions of three strata of the sward of a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

| **Variable** | **lowest** | **medium** | **upper** | **P-value** | **SEM** |
|---|---|---|---|---|---|
| Green DM (%) | 28.3$^{a}$ | 27.2$^{ab}$ | 24.7$^{b}$ | 0.047 | 0.01 |
| Green blade DM (%) | 74.4$^{b}$ | 86.7$^{a}$ | 85.7$^{a}$ | <0.001 | 0.02 |
| CP content (%) | 7.99$^{a}$ | 6.03$^{b}$ | 5.61$^{b}$ | <0.001 | 0.28 |
| ADF content (%) | 53.0 | 52.2 | 55.7 | 0.533 | 1.81 |
| NDF content (%) | 57.0 | 62.7 | 60.6 | 0.338 | 1.94 |

DDGS: dried distillers grains with solubles; BW: body weight; DM: dry matter; CP: crude protein; ADF: acid detergent fibre, NDF: neutral detergent fibre; SEM: standard error of mean.

4.4.2. Vertical and horizontal grazing dynamics

The overall evolution of the relative sward height is presented in Fig. 1, where it can be observed that even though both treatments descended regularly throughout the trial, supplemented animals almost always presented sward heights above their control counterparts. The visually assessed green forage mass content presented little variation throughout the trial, regardless of the treatment (Fig 2).

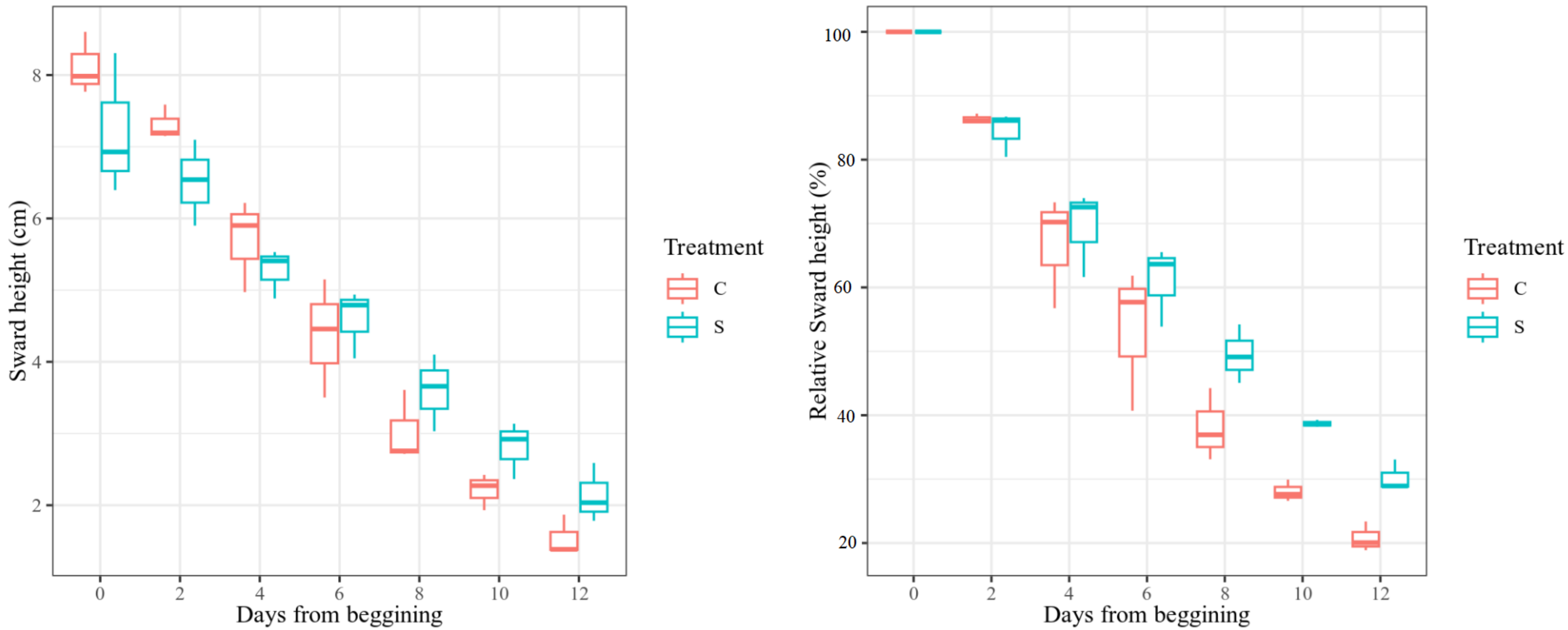


**Figure 1.** Absolute (a) and relative (b) sward height throughout a trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; S, supplemented plots; C, control plots

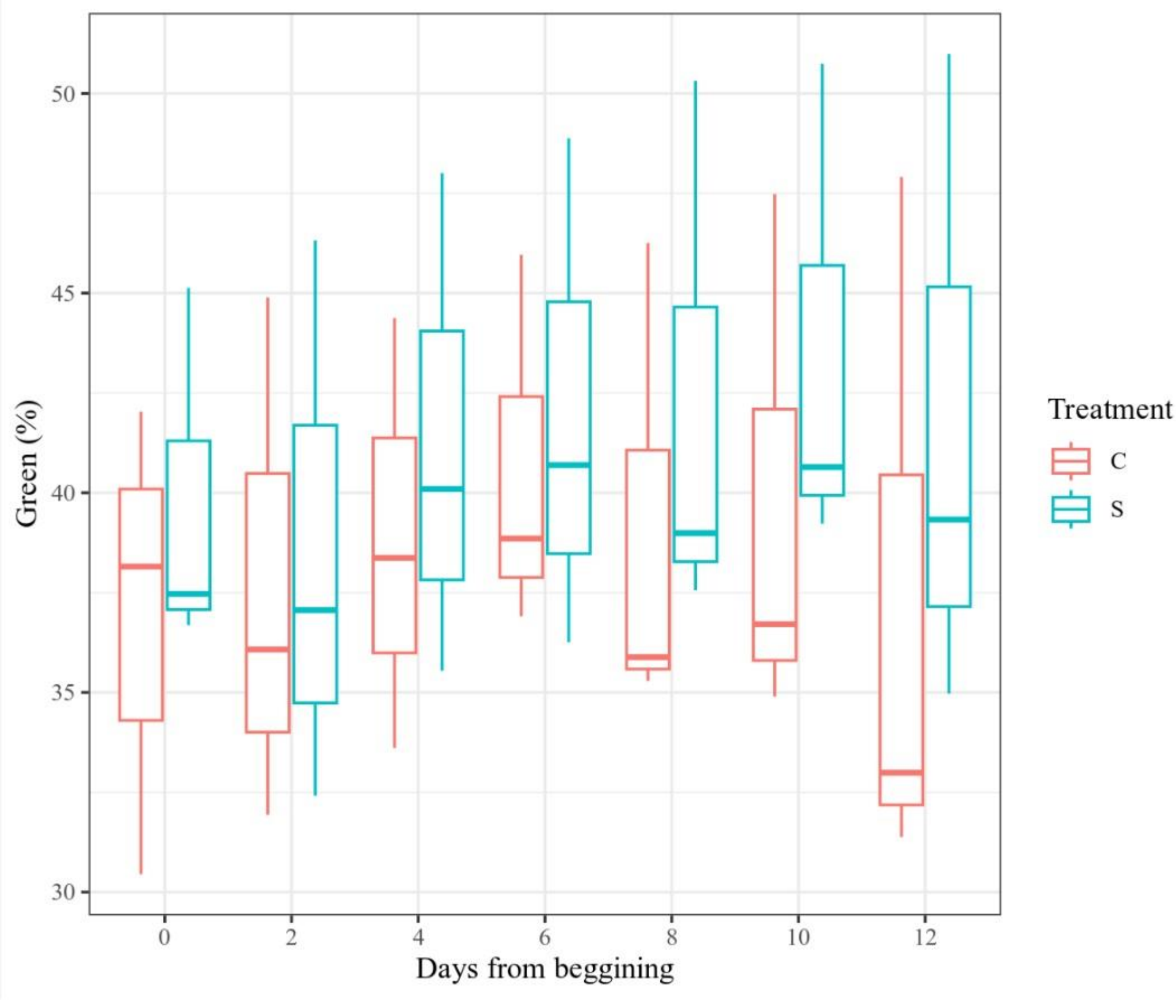


**Figure 2.** Visually assessed green forage mass percentage throughout a trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; S, supplemented plots; C, control plots. The visually assessed green forage mass percentage was done with the support of a printed grid of fixed green hues (between 5 and 95%), generated by previous studies (Berretta & Jaurena, unpublished).

No difference was found between treatments when comparing horizontal dynamics, but an interaction between grazed horizons and residual sward height was detected. When stratum utilisation was plotted against residual sward height (Fig 3), the first horizon presented an asymptotic behaviour, while all the successive horizons behaved logistically.  The second horizon reached an approximately 50% of horizontal utilisation when the first horizon was almost depleted (more than 80% of horizontal utilisation).

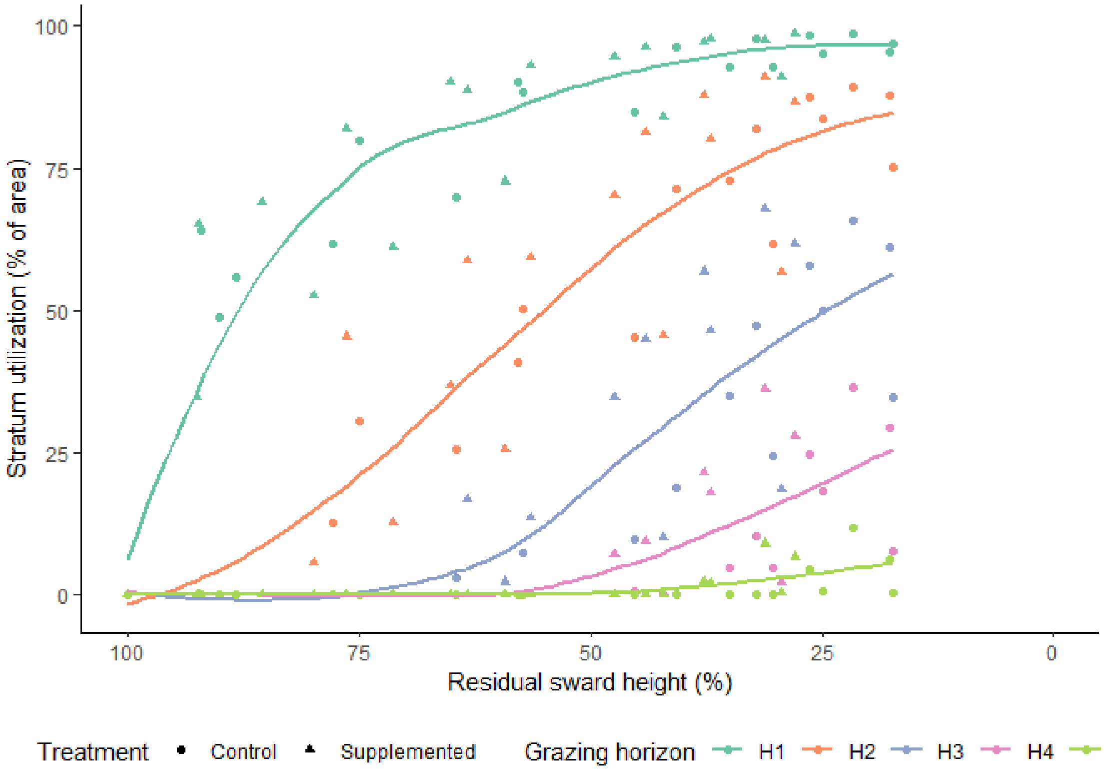


**Figure 3.** Horizontal dynamics (grazing horizons´ utilisation) against vertical (stratum utilisation) grazing dynamics, separated into five grazing horizons of a trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; H, grazing horizon; Circles represent control treatments and triangles represent supplemented treatments.

When analysing DM disappearance (Fig 4), S plots presented lower final DM disappearance when compared to C plots.

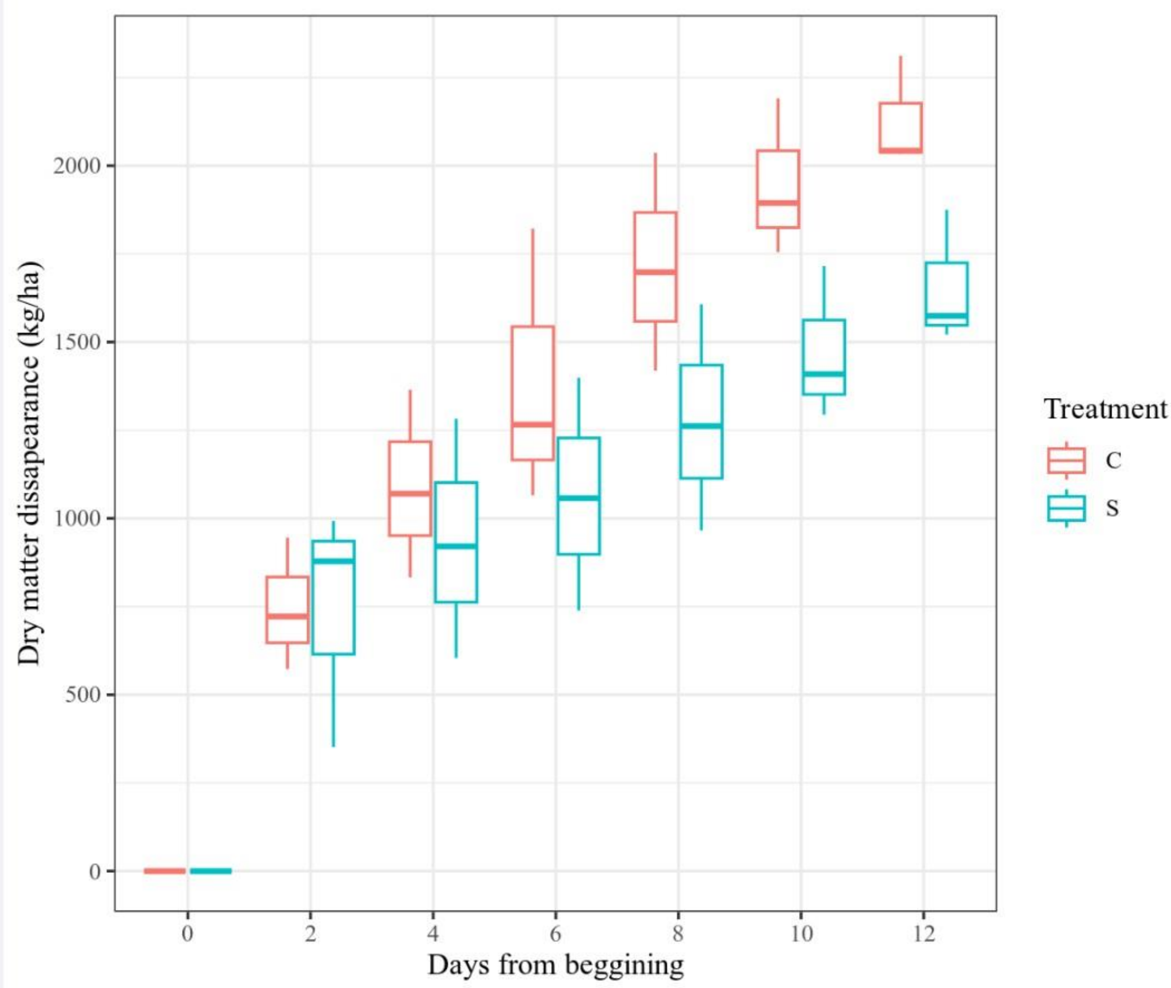


**Figure 4.** Dry matter disappearance (kg DM/ha) throughout a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; S, supplemented plots; C, control plots.

4.4.3. Plant functional type differential analysis

No effect was found for treatment when the relative grazed area (horizontal dynamics) was separated into PFT, yet the grazing horizon and the residual sward height, as well as their interaction, were affected by the evolution in the relative grazed area ($p<0.05$). The accumulated intake of patches of each PTF behaved similarly among them, regardless of the treatment (Fig 5a). The main PFT present in the experiment were all affected by the grazing horizon and the grazing down rate and their interaction ($p<0.05$), and they all fitted a quadratic model. The difference between the forage intake of C3 and C4 species (Fig 5bc) shows that both C and S animals prefer C3 species-dominated patches than C4, since in the first measurement dates this variable is positive and only by the end of the trial does it descend below 0. Additionally, C animals present greater C3>C4 preference, than their supplemented counterparts.

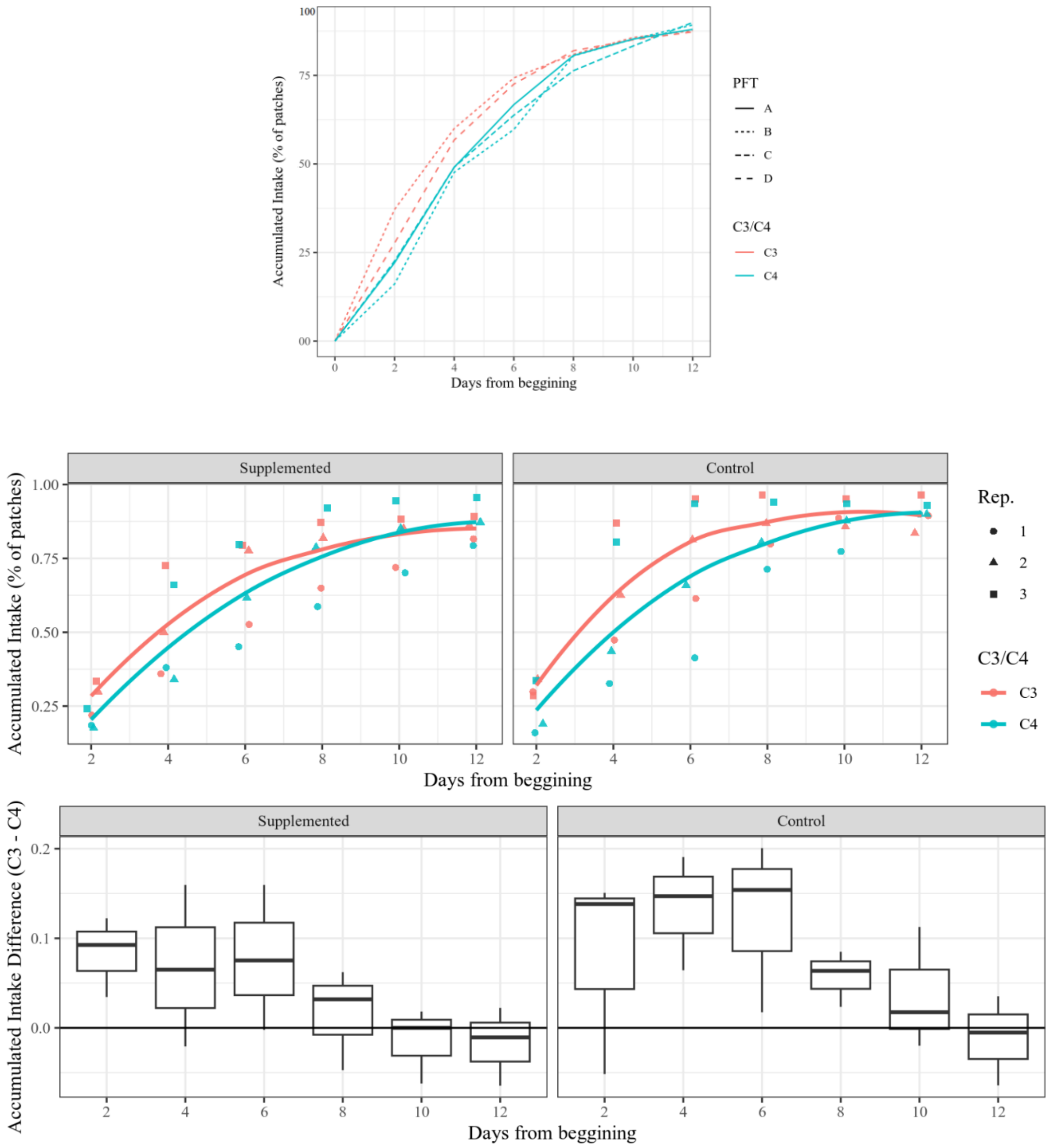


**Figure 5.** Plant functional type intake (a), plant metabolism type intake (b) and accumulated intake difference between C3 and C4 plant types (c) evolutions of a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; C-C3 and D-C4 presented very few registers, and thus were excluded.

### 4.4.4. Animal behaviour

Regardless of the grazing horizon, mean forage disappearance for C animals was generally greater than their S counterparts throughout the trial, especially for the first horizons (Fig 6a), whereas the proportion of each type of "patch" did not present a clear different pattern between treatments (Fig 6b). Finally, on average throughout the trial, C animals spent more time grazing and ruminating ($p>0.05$) than S animals, while the latter in turn rested, played, walked and drank more water ($p>0.05$) than their C counterparts (Table 3).

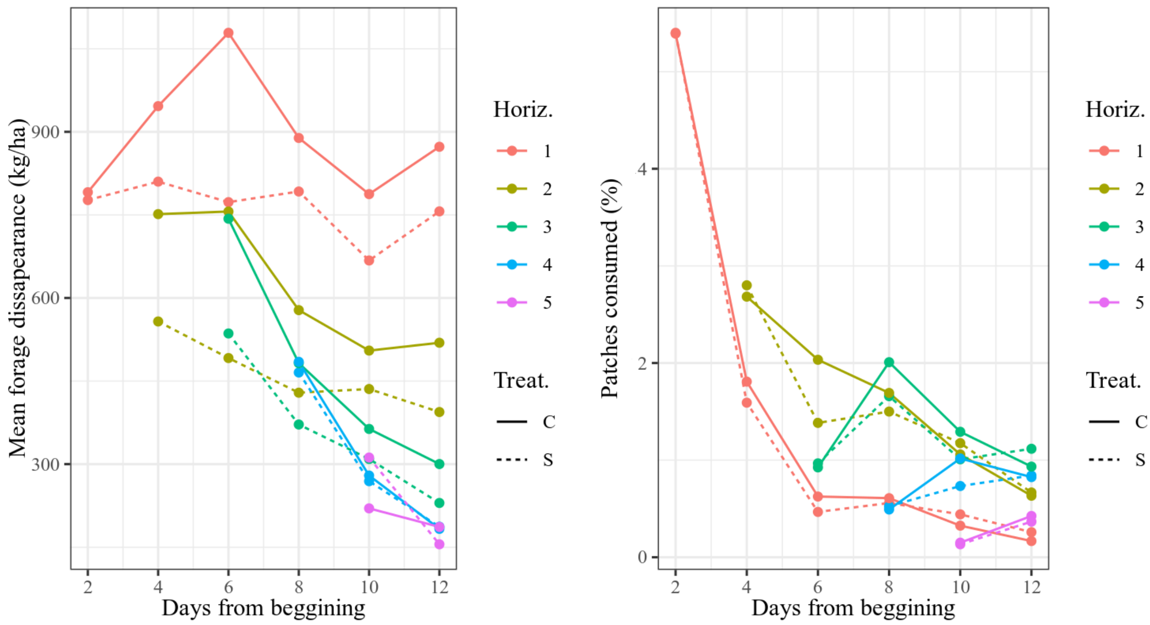


**Figure 6.** Mean forage disappearance (a) and patches consumed (b) evolution by treatment and grazing horizon of a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

DDGS: dried distillers grains with solubles; BW: body weight; S, supplemented plots; C, control plots; Horiz, grazing horizon.

**Table 3**. Animal behaviour activities as a percentage of total time of a grazing down trial with unsupplemented (control) and supplemented (corn DDGS, 0.7% BW) female calves on stockpiled native grasslands during winter.

| Variable | Control | Supplemented | P-value | SEM |
|---|---|---|---|---|
| Grazing time (%) | $60.1^{a}$ | $40.2^{b}$ | <0.0001 | 0.021 |
| Ruminating time (%) | $9.8^{a}$ | $5.5^{b}$ | 0.0001 | 0.008 |
| Resting time (%) | $21.9^{b}$ | $30.6^{a}$ | <0.0001 | 0.018 |
| Recreation time (%) | $3.8^{b}$ | $8.3^{a}$ | <0.0001 | 0.006 |
| Walking time (%) | $2.0^{b}$ | $3.7^{a}$ | 0.0063 | 0.008 |
| Drinking water time (%) | $1.5^{b}$ | $3.2^{a}$ | 0.0018 | 0.005 |
| Supplement consumption time (%) | $0.0^{b}$ | $7.8^{a}$ | <0.0001 | 0.009 |

DDGS: dried distillers grains with solubles; BW: body weight; Measurements were registered during daylight hours.

## 4.5. DISCUSSION

Contrary to our initial hypothesis regarding supplemented animals taking longer to finish each grazing horizon, no differences were found between control and supplemented treatments during the grazing down process. As for the horizontal grazing dynamics, the second grazing horizon was utilised by approximately 50% when the first was heavily depleted for both treatments. When analysing different plant functional type consumption rate, no differences were identified between treatments, yet differences in preference for C3 over C4 species was detected for both treatments and it was more relevant for unsupplemented cattle.

Grazing management based on the horizontal utilisation of the pasture, particularly the top stratum, maximises dry matter intake; a “grazing horizon” is defined as the vertical section of the sward which is being grazed by animals (Benvenutti *et al.*, 2015). This means that the upper grazing horizon will always be associated with deeper and/or heavier bites, compared to the subsequent grazing horizons. One way that animals may compensate for the loss of bite mass is by increasing their time spent grazing. Up to a certain point, that might or might not compensate the lower bite mass (Allden & Whittaker, 1970), particularly at lower grazing horizons. In the present study, control animals did spend significantly more time in grazing activities than supplemented animals (Table 3), but no difference was found between treatments when comparing the utilisation of grazing horizons when residual sward height decreased (Fig 3). Bite mass proved to be more important than bite number, regardless of treatment or grazing horizon (Fig 6), supporting the fact that bite mass is the main variable explaining the changes in dry matter intake through grazing horizons. A possible explanation of this could be that control animals take longer to harvest their bites than supplemented animals do (for example, through a lower bites/minute rate). At any rate, there was a lack of response of supplementation on grazing horizon dynamics.

According to Farruggia *et al.* (2008), who worked with adult beef cows and less than 0.1% BW protein supplementation had no effect on neither the diet selection nor the organic matter intake of beef cows grazing artificially sown pastures. In contrast, even though the animals of both treatments presented greater preference for C3 than C4 species, and our animals were younger and supplementation rate was greater, control animals in this study showed a greater preference than supplemented animals, especially in the first stages of the trial, where selection was exerted with greater freedom. This difference in terms of preference between the trial carried out on sown pastures and ours may be explained, not only by the differences in supplementation rate, but also by the extreme heterogeneity of plants present in the sward, including several PFT within both plant metabolic paths.

The top leafy stratum of grass pastures is expected to be preferred and be associated with high levels of HDMI (Benvenutti *et al.*, 2015). Nevertheless, as animals grazed successive horizons throughout time, visually assessed green forage mass content did not show any distinct pattern, specifically no decrease was observed in either treatment (Fig 2). One possible explanation of this could be associated with the fact that the trial was conducted on a stockpiled forage, from autumn to winter (deferred pasture). For example, Fribourg & Belp (1984) found that both CP and NDF contents of a stockpiled tall fescue did not vary within the stockpiling period -probably related to the fact that this species can retain its quality compared with other cool-season forages - which coincides with our study in terms of NDF but not of CP content. In the deferred conditions of a highly heterogeneous pasture as native grasslands are, it is not as clear as in homogeneous high-quality pastures, that the top stratum is in fact the most green or nutritious (which would explain why the visually assessed green forage mass content did not vary). Nonetheless, the top stratum does present the greater green leaf content, so it plays a slightly different role than the typical upper leafy stratum of other studies.

## 4.6. CONCLUSIONS

No differences were found between the horizontal grazing dynamics between supplemented and control animals as residual sward height decreased: they presented similar grazing horizon switching patterns. In both cases, when the upper grazing horizon was heavily depleted, the subsequent horizon was being depleted by its half. Differences of preference for C3 species over C4 was observed for both treatments, but this effect was more meaningful for control animals. Grazing time never fully compensated for the decline in intake rate during depletion throughout the grazing horizons. Pasture intake declined when the animals transitioned from grazing the top grazing horizons to the lower horizons, irrespective of the level of supplementation.

Among the practical implications of this study, it can be mentioned that since bite depth explained much of the variability observed, and sward height is very important to maximising this variable, managing the sward structure in terms of sward height will be beneficial to maximising individual animal performance, for both unsupplemented and supplemented animals. Additionally, native grasslands paddocks with a greater C3 predominance (“winter vocation” paddocks) will always be preferred over “summer vocation” paddocks, regardless of an eventual supplementation practice.

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# 5. DISCUSIÓN GENERAL

## 5.1. INTRODUCCIÓN

Comprender con más profundidad cuáles son los mecanismos sobre los que se basa una actividad tan relevante para la ganadería sobre campo natural, como lo es la suplementación invernal energético-proteica, es de suma relevancia para que esta práctica siga siendo una tecnología eficaz y eficiente.

En cuanto a lo primero (eficacia), tal como se pudo observar en una revisión de ensayos nacionales previa (Cazzuli, 2017), se concluye que la suplementación invernal sobre campo natural diferido o sin diferir genera, en la amplia mayoría de los casos, respuestas positivas. Es decir, estamos frente a una tecnología cuya predictibilidad de respuesta es muy confiable. Esto, además, quedó también demostrado en el artículo del capítulo 2, en donde el 80 % de los valores de respuesta animal de toda la base de datos se ubicaban por encima de los 0,100 kg/animal/día.

En cuanto a lo segundo (eficiencia), quedó demostrado que son esperables valores de 0,21 ± 0,076 kg/kg, si bien el rango de valores es amplio (0,07-0,40 kg/kg) y es necesario encontrar un balance entre la disponibilidad de forraje y la oferta forrajera para lograr las máximas eficiencias. También se demostró la relevancia que tienen las condiciones climáticas, así como la dependencia más fuerte de la respuesta a la suplementación en comparación con la cantidad de suplemento ofrecido, en determinar los valores de eficiencia. Asimismo, pudieron determinarse las tasas de sustitución de forraje por suplemento y de forraje en relación con un máximo potencial teórico. Estas siempre fueron positivas en estas condiciones (0,3-1,1 kg/kg y 0,1-0,3 kg/kg para forraje por suplemento y forraje en relación con el máximo teórico, respectivamente), lo cual explica también los valores moderadamente altos de eficiencia encontrados en relación con lo que podría observarse sobre otros recursos forrajeros naturales, tales como ambientes semiáridos o pasturas tropicales en un clima con estación seca. Otro aspecto a resaltar fue que, incluso sin suplementar, solamente por trabajar con campos naturales diferidos desde otoño al invierno, en lugar de registrar ganancias promedio negativas, se logró mantener o aumentar el peso vivo de los animales.

Además, pudo establecerse que siempre existe una cierta magnitud de sustitución del forraje por suplemento y, por ende, sustitución de forraje con respecto a un potencial máximo correspondiente a los lotes de animales testigo. También pudo determinarse que existen tres patrones esperables de respuesta a la suplementación —es decir, evolución de la respuesta a la suplementación a lo largo del tiempo (expresado en unidades térmicas)—: uno lineal en el cual la respuesta a la suplementación es constante y dos no lineales en los cuales hay una primera fase de baja respuesta a la suplementación, una segunda fase en la cual respuesta a la suplementación aumenta progresivamente (cuadrático) y una tercera etapa donde vuelve a bajar (Weibull). Cada patrón se asoció a distintas variables. Las respuestas lineales fueron más dependientes del contexto o del escenario del esquema de suplementación, mientras que los modelos cuadráticos se asociaron más a disponibilidad y tasas de sustitución; los de distribución Weibull se asociaron más, sobre todo, con las heladas. Por último, al inicio del período de suplementación, la respuesta en desempeño estuvo más asociada al tamaño de los animales y a la calidad del suplemento, mientras que, hacia el final, estuvo asociada a la ingesta de suplemento y a la ganancia de los animales suplementados, así como a la calidad de la pastura.

Finalmente, se pudo entender un poco más lo que sucede desde el punto de vista de la pastura cuando se suplementa ganado y se sigue el proceso hasta agotar el forraje (*grazing-down experiment*). El poder posicionar el análisis desde el punto de vista de la pastura y no solo del animal ayuda a comprender mejor todo el proceso. En este caso, se halló que la dinámica de pastoreo, en cuanto a los sucesivos horizontes de pastoreo por los que los animales pasaban, no difirió entre tratamientos con y sin suplementación, contrario a las expectativas de acuerdo con la hipótesis planteada. Además, pudo establecerse un valor de utilización del primer horizonte (> 80 %) con respecto al segundo (50 %), con lo cual también se generó un conocimiento local para lo que sucede con el pastoreo de animales con o sin suplementación. También se comprobó una mayor preferencia de especies C3 en comparación a las C4 y se reforzó la relevancia de la estructura de la pastura como determinante del desempeño animal, en ambos casos independiente de si el lote de animales es suplementado o no.

## 5.2. ANÁLISIS CONJUNTO

Al tener las dos visiones —una desde con más foco en el animal, capítulo 2 y 3, y otra más centrada en la pastura, capítulo 4— es posible entender con más profundidad el proceso de suplementación sobre campo natural durante el invierno.

### 5.2.1. Efecto de sustitución

En primer lugar, quedó claro que es inevitable observar cierto grado de sustitución de forraje por suplemento en estos esquemas. Tanto al concentrarnos en la SFE como en la dinámica de defoliación se registró más tiempo de pastoreo de los animales testigo, así como tasas de sustitución siempre mayores a cero. Es de notar que las sustituciones con asociaciones más altas con SFE se dieron cuando se asumían máximas digestibilidades de la pastura (65 %). Lo primero que podría pensarse es que con situaciones otoño-invernales más duras (específicamente con más heladas que maten los tejidos vivos), la digestibilidad sería menor y con esto se lograrían menores SFE. Sin embargo, tal como se observó en el capítulo 2, las mayores SFE se registraron en situaciones con las temperaturas más bajas y mayor cantidad de heladas. Esto plantea la incógnita de en qué magnitud las heladas afectan la digestibilidad y en qué magnitud esta variable está explicada por otros factores, así como el rol último que tiene la digestibilidad como variable explicativa del consumo de forraje y, por ende, la estimación de las tasas de sustitución.

En un estudio publicado por Niyigena et al. (2022) con ovinos consumiendo ensilaje de alfalfa y festuca, se concluyó que el mismo forraje veía reducido tanto su consumo por parte de los corderos como su digestibilidad al cortar el material luego de la ocurrencia de heladas. En concordancia, Narasimhalu et al. (1986), estudiando ensilaje de maíz ofrecido a corderos, cuyas plantas fueran cortadas antes y después de las heladas, también determinaron un descenso en digestibilidad del ensilaje al cortar el material luego de las heladas. Es decir, incluso trabajando con ensilajes, el efecto de las heladas afecta negativamente la calidad del forraje, específicamente su digestibilidad. Sin embargo, las heladas no estarían influyendo solamente de forma directa sobre los tejidos vegetales, sino también en otros parámetros. Ejemplo de esto es lo mencionado por Krysl y Hess (1993), citando a Starker y Holmes (1974), en cuanto a que la formación de las heladas afectó negativamente el tiempo de rumiantes destinado al pastoreo a pesar de observarse una tendencia a un aumento de disponibilidad en el tiempo.

Por otro lado, Bruinenberg (2003) argumenta que, en tapices con gran riqueza de especies —como podría ser el campo natural—, el hecho de que coexista una alta variabilidad de estadios fenológicos al mismo tiempo, combinado con las diferentes digestibilidades individuales de cada especie, hace que las predicciones de digestibilidad de estas bases forrajeras sean poco confiables. En el presente trabajo, las estimaciones de consumo de forraje utilizadas para determinar las tasas de sustitución no fueron medidas, sino que fueron estimadas utilizando modelos predictivos, lo cual relativiza el hecho de tomar la digestibilidad como variable explicativa. Adicionalmente, Jung y Allen (1995) argumentan que para influir positivamente sobre la producción animal, es más relevante la concentración de pared celular y su proporción digerible que la digestibilidad en sí de las plantas.

Si bien la sustitución de forraje por suplemento no fue el foco del análisis del experimento *grazing-down*, desde el momento en que la desaparición del forraje —asociado indirectamente con el consumo— termina siendo mayor en las parcelas testigo, también en esta instancia se concluye que existió una cierta magnitud de sustitución. Esta afirmación es válida tanto para la sustitución de forraje por suplemento como por la depresión del consumo de forraje con respecto al máximo potencial.

5.2.2. Importancia de tamaño del bocado

Existen muchos estudios que examinan el efecto de la estructura de la pastura sobre el comportamiento ingestivo de grandes herbívoros. La altura, la proporción de hojas verdes y la biomasa verde de especies forrajeras estivales están asociadas positivamente con algunos componentes del comportamiento ingestivo de estos animales (Burns y Sollenberger, 2002).

Según Boval et al. (2007), el tamaño de bocado ha demostrado ser la variable más sensible a los cambios en varios aspectos de la estructura de la pastura, como por ejemplo la altura, densidad y disponibilidad de la pastura, expresados como tejido verde, tejido foliar o disponibilidad de biomasa. El bocado en sí es entendido como la unidad básica para comprender el proceso de pastoreo y de hecho se proponen construir estructuras de las pasturas diseñadas para generar «bocados de precisión» (Carvalho et al., 2009).

En el capítulo 4 se concluyó que el tamaño del bocado, determinado particularmente por su profundidad, fue la variable que más explicó la variabilidad observada en el consumo de forraje a través de los distintos horizontes de pastoreo. Tanto es así que, de hecho, es independiente de la presencia o no de suplementación. Barbero et al. (2015), quienes trabajaron con ganado en crecimiento sobre una pastura sembrada con *Brachiaria sp.*, hallaron que diferentes combinaciones de altura del tapiz y tasas de suplementación afectaban el consumo de forraje y que la eficiencia del sistema se vería optimizada si se variaban las tasas de suplementación en función de la altura de las parcelas. En otras palabras, estos autores modificaban la estructura de la pastura a través de la altura para optimizar la eficiencia. Tanto la morfología vegetal como la estructura de la pastura influyen las dimensiones de los bocados individuales (Hodgson, 1985), nuevamente destacándose la importancia del tamaño del bocado y su relación con la estructura de la pastura como variable con una fuerte influencia en la dinámica de pastoreo.

De las variables asociadas con la estructura de la pastura, la altura es muy relevante, como lo es la disponibilidad al momento de un manejo óptimo del campo natural y su consecuente desempeño animal (Da Trindade et al., 2016, Cunha et al., 2023). Si el horizonte superior de pastoreo se asocia con los bocados más profundos y pesados en comparación con los sucesivos horizontes (capítulo 4), se podría pensar que en aquellos patrones de respuesta a la suplementación no lineales (capítulo 3), los puntos de inflexión podrían corresponderse al cambio de pasaje del primer al segundo horizonte, es decir, al consumo del 80 % del área del primero. En el caso de las respuestas lineales y dado que, como se concluyó en el capítulo 3, estas situaciones son más dependientes del contexto específico, o bien no es posible identificar ese pasaje, o bien son otras variables las que están jugando un rol más relevante. Es de destacar que el cambio de horizonte de pastoreo y su relación con el tamaño de bocado solo sería uno de los aspectos a considerar al momento de entender los mecanismos atrás de los patrones de respuesta. Es necesario tener en cuenta que el clima, la asignación de forraje y su calidad, entre otros aspectos, también están influyendo en el comportamiento del complejo animal-pastura.

#### 5.2.3. Suplementación invernal inserta dentro de un sistema productivo

Dado que las situaciones productivas comerciales están insertas en un sistema más amplio de producción y donde tanto antes (acumulación de forraje en otoño) como después (pastoreo sin suplementar en primavera) de la suplementación invernal el sistema continúa, sería deseable conocer cómo impacta dicha actividad en un contexto específico de bioma pampa.

En tal sentido y con foco en un mismo espacio (potrero), para aportar conocimiento durante el momento previo de acumulación otoñal de forraje, existen proyectos de investigación específicamente enfocados en conocer más sobre la dinámica de acumulación de biomasa, su calidad, su composición botánica, etc. (por ejemplo, Núñez et al., 2022).

En el otro extremo, es decir, durante la primavera posterior a la suplementación en el mismo potrero, los estudios sobre campo natural del bioma pampa son menos o no se conoce ningún proyecto de investigación que aborde este tema. Una pregunta de investigación que podría plantearse en tal sentido es: ¿se cumple que, a mayor eficiencia de uso del suplemento, reflejando una mayor utilización durante el invierno y, por lo tanto, menor altura remanente al final del período, menor tasa de crecimiento primaveral? Una menor altura al final del invierno y principio de primavera implicaría menor capacidad de rebrote o, al menos, de su velocidad.

En ciertas condiciones de pasturas sembradas, el efecto del manejo de la asignación de forraje y altura de la pastura de una estación puede ser transferida a la siguiente (Lockhart et al., 1969, King y Stockdale, 1984, Leyshon y Campbell, 1992), mientras que, en otros casos, no parece estar tan claro (Delagarde et al., 2018). Este efecto entre altura pospastoreo y tasa de crecimiento subsecuente podría también reflejarse en cambios en la calidad del forraje (Kibon y Holmes, 1987), ya que distintas tasas de crecimiento estarían asociados a distinta cantidad de hojas verdes, fotosintéticamente activas.

Como próximas investigaciones para determinar cómo evolucionaría el tapiz de campo natural luego de una suplementación invernal (*carry-over effects*), se plantea una situación inicial (fin de invierno) en donde se parta de distintas alturas de forraje y se observa cómo evoluciona la tasa de crecimiento y acumulación de biomasa y hojas verdes durante la primavera.

En cuanto a la investigación acerca del impacto de la suplementación invernal dentro de una empresa ganadera, se plantea el modelado teórico económico de diferentes escenarios, al estilo de lo realizado por De Figueiredo et al. (2007), Soares de Lima et al. (2014), Montossi et al. (2014) y Montossi et al. (2016), esta vez con información y conocimientos nuevos y más específicos. De igual forma, podría modelarse conjuntamente el impacto de la suplementación en términos de indicadores ambientales y sociales, siempre dentro de una visión sistémica de las empresas.

## 5.3. IMPLICANCIAS

La primera implicancia práctica y directa de este trabajo radica en poder utilizar todos los coeficientes generados en sistemas de soporte a la decisión (SSD) para productores, además de su uso puntual para toma de decisiones de productores o de técnicos asesores.

Por otro lado, se detallarán a continuación las implicancias económicas y prácticas.

### 5.3.1. Económicas

Especialmente desde principios del siglo XXI, la ganadería ha tenido que competir con otros rubros (agricultura y forestación) por el uso del suelo, con el costo de la tierra y arrendamientos en constante aumento. Esto hace imperioso volverse más eficiente en todos los procesos involucrados con la producción de carne para mantener el rubro competitivo (Montossi et al., 2016).

Toda aquella tecnología que, al igual que el resto de los factores, mejore la eficiencia, impacta directamente en la economía de los sistemas ganaderos. En particular, para el caso que se suplemente al ganado en crecimiento, situaciones con mejores eficiencias del uso de los alimentos se asocian con los mejores resultados económicos (De Figueiredo et al., 2007). De hecho, Beck et al. (2013) encontraron que si el sistema de producción necesita de la suplementación para mantener la productividad, controlar los costos mediante el ahorro del suplemento tiene un menor resultado económico que suplementar sobre pasturas para mantener la producción.

En el presente trabajo, algunas de las variables asociadas a la base forrajera (asignación forrajera, disponibilidad) afectaron la eficiencia de uso del suplemento, con lo cual estaríamos influyendo sobre el resultado económico, lo que coincide con lo mencionado por Soares de Lima et al. (2014), quienes concluyeron que variaciones en calidad y cantidad de forraje del campo natural frente a un esquema de suplementación energético-proteica definen fuertemente el impacto productivo de la suplementación, lo que afecta el resultado económico.

5.3.2. Ambientales

Cualquier tecnología que apunte a maximizar la eficiencia en el uso de los recursos está alineada con el diseño de sistemas productivos más sostenibles (Paruelo et al., 2023). Específicamente, Kanter et al. (2016) modelaron cómo se vería aumentada la producción de carne equivalente por hectárea en el país si se incluyeran tecnologías utilizadas por aquellos productores que hoy son los económicamente más viables y productivos (una de las cuales es la suplementación) y concluyeron que podría incrementar un 25 %. Esta modelación fue realizada no solo enfocada en aumentar la producción, sino en hacerlo de una forma sustentable ambientalmente. Estudios de Picasso et al. (2014) demuestran que un simple aumento de producción a igualdad del resto de los recursos reduce la huella de carbono tanto por animal como por unidad de superficie. Todo esto significa que un aumento en la eficiencia de uso del suplemento no solo impacta directamente en un mayor ingreso económico del productor, sino que simultáneamente se está aportando al cuidado del ambiente.

Finalente, otro aspecto en donde la sustentabilidad puede ser impactada positivamente a través de la suplementación, es en términos de conservación o mejora de la condición de los campos naturales. La razón de esto es que las parcelas con suplementacíón demostraron una menor presión sobre las especies más palatables (preferencia C3 antes que C4). Además, un diferimiento otoñal (descanso), que es cuando las especies C3 tienen cierta ventaja sobre las C4, podría  lograr una mayor recuperación primaveral de estas especies invernales en relación a las estivales.

# 6. CONCLUSIONES GENERALES

La tecnología de suplementación energético-proteica resultó muy confiable, en el sentido de que las respuestas a la suplementación, como la diferencia en ganancias medias diarias entre animales suplementados y testigo, fueron siempre positivas.

De hecho, un 80 % de los valores de eficiencia de uso del suplemento se ubicaron por encima de 0,15 kg de PV/kg de MS de suplemento, mientras el valor promedio de los 25 ensayos compilados fue de 0,21 ± 0,08 kg PV/kg MS de suplemento. Al observar cuál de las dos variables que componen el cálculo de eficiencia de uso del suplemento influyen más sobre su resultado, se comprobó que es más importante la respuesta a la suplementación o la distancia entre el desempeño de animales con y sin suplemento ofrecido en comparación con la razón a la cual los animales son suplementados, es decir, la cantidad de suplemento ofrecido con relación a su tamaño.

La asignación de forraje afectó negativamente la eficiencia, mientras que su disponibilidad la afectó de manera positiva, lo cual indica que es necesario un balance entre ambas variables si el objetivo es optimizar la eficiencia de la suplementación.

Se constató la influencia que tiene el clima al suplementar animales sobre campo natural, ya que, en inviernos más duros, la respuesta a la suplementación fue mayor (en específico, afectando negativamente a los animales no suplementados) y afectó positivamente a la eficiencia.

No se pudo comprobar que exista una limitante importante en el contenido de proteína del forraje del campo natural durante el invierno en cuanto al desempeño animal. Se estima que su valor nutritivo es superior al de otros campos naturales de otras partes del mundo (como las praderas naturales de Norteamérica o las pasturas tropicales nativas de Australia), lo que sugiere que nuestros campos retienen una proporción elevada, si bien variable, de hojas verdes durante el invierno.

Dado que se estimaron tasas de sustitución moderadas-bajas, se concluye que aún sería posible encontrar caminos para controlar aún más la eficiencia del uso del suplemento. Además, se encontró que la digestibilidad de la pastura de alguna forma afecta la eficiencia, que es una variable afectada multifactorialmente, por el consumo del forraje y suplemento, pero sin olvidar el valor nutritivo del forraje y su estructura.

Pudieron encontrarse tres patrones distintos de respuesta a la suplementación: linear, cuadrática y Weibull. Los modelos lineares no presentaron una asociación definida con un conjunto determinado de variables, lo que los hace más dependientes del contexto. Aquellos patrones de respuesta cuadráticos fueron explicados sobre todo por la disponibilidad y tasas de sustitución, mientras que los patrones Weibull se asociaron más bien con la ocurrencia de heladas. Independientemente del patrón de respuesta, al inicio de los ensayos fue el tamaño de los animales y la relación CP/ME del suplemento lo que más explicó la respuesta a la suplementación, a la cual afectó positivamente. Sin embargo, hacia las fases finales de los ensayos, mayor consumo de suplemento y mayor ganancia de animales suplementados, combinado con pasturas de menor contenido proteico, presentaban respuestas a la suplementación mayores.

Al analizar lo que sucede en las parcelas de campo natural diferido desde un punto de vista horizontal, tanto en situaciones de suplementación como en situaciones testigo por igual, cuando el primer horizonte era consumido casi completamente (> 80 %), el horizonte inmediato inferior había sido pastoreado por la mitad.

Tanto animales suplementados como testigo demostraron mayor preferencia por especies de metabolismo C3 que C4.

El tiempo que los animales dedicaron a la actividad de pastoreo nuca pudo compensar completamente el descenso en la tasa de consumo de forraje a través de los distintos horizontes de pastoreo. El consumo de forraje descendió cuando los animales pasaban de los horizontes superiores a los inferiores, independientemente de la suplementación.

Se comprobó una vez más que la estructura de la pastura, específicamente a través de la altura, es fundamental para maximizar el tamaño de bocado de los animales, que incide directamente en su consumo y, por ende, en su desempeño. Es decir, el manejo de la estructura y arreglo del tapiz permite apuntar a la maximización del desempeño animal individual tanto en presencia como en ausencia de oferta de suplemento energético-proteico.

Analizar con más profundidad lo que sucede en cada fase del proceso de suplementación, particularmente en términos de condiciones de la pastura, podría arrojar más luz sobre las variables que inciden en la eficiencia de uso del suplemento.

Se espera que todos los parámetros estimados en el presente estudio sirvan para alimentar sistemas de soporte a la decisión, que beneficien a los productores, facilitándoles la toma de decisiones en los sistemas ganaderos basados en campo natural.

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