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THE EXPLANATORY POWER OF FORAGE NUTRIENT CONTENT AND SWARD STRUCTURE ON INTAKE, ANIMAL PERFORMANCE AND METHANE EMISSIONS BY SHEEP AND CATTLE IN GRAZING ECOSYSTEMS.

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Dissertação apresentada como requisito para obtenção do Grau de Mestre em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.
Orientadora: Carolina Bremm
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“No dia em que você acreditar em si mesmo, será capaz de qualquer coisa”

(Autor desconhecido)

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[1] Dissertação de Mestrado em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (95 p.) Abril, 2021.

O PODER EXPLICATIVO DOS COMPONENTES NUTRITIVOS DA FORRAGEM E DA ESTRUTURA DO PASTO NA INGESTÃO, DESEMPENHO ANIMAL E EMISSÕES DE METANO POR OVINOS E BOVINOS EM ECOSSISTEMAS PASTORIS.

Autora: Laís Leal da Cunha
Orientadora: Carolina Bremm

RESUMO

A literatura relata que os teores de nutrientes são fatores importantes para predizer o consumo de matéria seca (CMS), desempenho animal (GMD) e emissões de metano (CH_4) de ruminantes (Capítulo I). Este estudo utilizou dados de ovinos e bovinos em pastejo, coletados em cinco experimentos diferentes. Os critérios para os estudos selecionados foram a disponibilização de dados de CMS, GMD, estrutura do pasto, conteúdo de nutrientes da forragem aparentemente ingerida e medição *in vivo* das emissões de CH_4 dos animais. Os dados foram analisados por meio de regressão e modelos mistos para identificar o nível de importância dos componentes nutritivos da forragem sobre o CMS, GMD e emissões de CH_4. Os modelos de regressão foram significativos ($P<0.01$) em relação aos componentes nutritivos da forragem representados pela proteína bruta (R^2: CMS = 0.02; GMD = 0.19; CH_4 = 0.07), fibra em detergente neutro (R^2: CMS = 0.11; GMD = 0.14; CH_4 = 0.01) e fibra em detergente ácido (R^2: CMS = 0.10; GMD = 0.10; CH_4 = 0.01), porém com baixo coeficiente de determinação. Os resultados mostram que em sistemas pastoris existem outros fatores como a estrutura do pasto, representada pela massa de forragem (R^2: CMS = 0.11; GMD = 0.28; CH_4 = 0.36), altura do pasto (R^2: CMS = 0.27; GMD = 0.39; CH_4 = 0.31) e oferta de forragem instantânea (R^2: CMS = 0.19; GMD = 0.19; CH_4 = 0.35), possuem maior poder explicativo do que os componentes nutritivos da forragem. Foram gerados modelos mistos com a inclusão de efeitos fixos (componentes nutritivos da forragem e estrutura do pasto) e aleatórios (e.g., espécie animal, tipo de pastagem, experimento) que apresentaram R^2 de 84.5, 94.9 e 68.0% para CMS, GMD e CH_4, respectivamente. Dentro do total explicado pelo modelo, 12.4, 12.0 e 4.6% foram representados pelos teores de nutrientes da forragem, enquanto que 13.7, 8.8 e 18.4% foram explicados pela estrutura do pasto nos modelos para CMS, GMD e CH_4, respectivamente. Observou-se maior influência dos efeitos aleatórios (i.e. espécie animal, tipo de pastagem e unidade experimental) nas variáveis estudadas (Capítulo II). Os resultados mostram que em sistemas de pastejo, a estrutura do pasto torna-se semelhante ou mais importante do que o conteúdo de nutrientes da forragem na determinação de CMS, GMD e CH_4 em bovinos e ovinos. Além disso, existe uma gama de fatores associados às variáveis aleatórias que não são totalmente compreendidos na literatura, tornando-se um grande potencial a ser estudado, uma vez que o poder explicativo dessas variáveis foi maior do que os efeitos fixos do modelo (componentes nutritivos e estrutura do pasto) (Capítulo III).

Palavras chave: pastagem; modelos mistos; efeitos aleatórios; ruminantes em pastejo; composição química.

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THE EXPLANATORY POWER OF FORAGE NUTRIENT CONTENT AND SWARD STRUCTURE ON INTAKE, ANIMAL PERFORMANCE AND METHANE EMISSIONS BY SHEEP AND CATTLE IN GRAZING ECOSYSTEMS.

Author: Laís Leal da Cunha
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ABSTRACT

The literature reports that nutrients content are important factors to predict dry matter intake (DMI), animal performance (ADG) and methane (CH_4) emissions of ruminants (Chapter I). The study used data from five trials with sheep and cattle grazing different grasslands. The criteria for the selected studies were the availability of data on DMI, ADG, sward structure, forage nutrient content and *in vivo* measurement of animals' CH_4 emissions. The data were analyzed using regression and mixed models to identify the importance level of forage nutrient content in the results of DMI, ADG and CH_4 emissions. The regression models were significant ($P< 0.01$) in relation to forage nutrient content represented by crude protein (R^2: DMI = 0.02; ADG = 0.19; CH_4 = 0.07), neutral detergent fiber (R^2: DMI = 0.11; ADG = 0.14; CH_4 = 0.01) and acid detergent fiber (R^2: DMI = 0.10; ADG = 0.10; CH_4 = 0.01), but with low explanatory power. The results shows that in grazing ecosystems there are other factors, such as sward structure, represented by the herbage mass (R^2: DMI = 0.11; ADG = 0.28; CH_4 = 0.36), sward height (R^2: DMI = 0.27; ADG = 0.39; CH_4 = 0.31) and lastly, herbage allowance (R^2: DMI = 0.19; ADG = 0.19; CH_4 = 0.35), presenting greater explanatory power than nutrient components of the forage. Mixed models were generated with fixed (forage nutrient content and sward structure) and random effects (animal species, type of pasture, experimental unit) that presented R^2 of 84.5, 94.9 and 68.0% for DMI, ADG and CH_4 emissions, respectively. Within the total explained by the model, 12.4, 12.0 and 4.6% were represented by the forage nutrient content and 13.7, 8.8 and 18.4% explained by sward structure in the models for DMI, ADG and CH_4, respectively. There was a greater influence of random (i.e. animal species, type of pasture and experimental unit) on the variables studied (Chapter II). The results show that in grazing systems, the sward structure becomes similar or more important than the nutrient content of the forage in determining CMS, ADG and CH_4 in cattle and sheep. Besides, there is a range of factors associated with random variables that are not fully understood in the literature, making it a great potential to be studied, since the explanatory power of these variables was greater than the fixed effects of the model (nutritional components and sward structure) (Chapter III).

Keywords: pasture; mixed models; random effects; grazing ruminants; chemical composition.

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ABBREVIATIONS LIST

CP: crude protein
HM: herbage mass
DM: dry matter
DMI: dry matter intake
OM: organic matter
CH_4: methane emission
GHG: greenhouse gas
ADG: average daily gain
LW: live weight
MW: metabolic weight
SH: sward height
IHA: instantaneous herbage allowance
ADF: acid detergent fiber
NDF: neutral detergent fiber
ASL: above sea level
S: south
W: west
N: nitrogen
N_2: nitrogen gas
VFA: volatile fatty acids
ME: metabolizable energy
NIRS: near-infrared reflectance spectroscopy
R^2: coefficient of determination
SF_6: sulfur hexafluoride

CHAPTER I

1. INTRODUCTION

Ruminants are create in different systems, based on type of food as grasslands and feedlot systems. The animal nutrition could be conduct in each system separated or the merge of them. Most researches in animal nutrition are conducted on feedlot systems where diets and the nutritive value are mainly composed of concentrates (grains, minerals). In these systems, animals reduce time and energy cost because of short space, then the velocity of ingestion increases during the day and the performance becomes faster when compared to grazing systems. However, in grasslands area animals are reared in pastoral environments where many factors interfere on the ingestion process. The plant-animal interface is the most important study area for understanding how the sward structure can affect animal behavior while grazing.

As far as intake increases, average daily gain and methane emissions also increase. This fact applies to intensive or extensive systems, however; emissions coming from pastoral systems have targeted to mitigate enteric methane through to soil carbon sequestration by plants (O'MARA, 2012). Strategies to mitigate emissions by ruminants in indoor-fed are nutritional-oriented with low methane emission intensity (HRISTOV et al., 2013) although this applicability in grazing becomes less efficient (BEAUCHEMIN et al., 2020). To improve productivity per unit of feed intake, management strategies can optimize animal performance and decrease enteric emissions in grazing ecosystems (STEINFELD et al., 2006).

This dissertation was developed using data from ruminants on grazing trials and describes the explanatory power of forage nutrient content and sward structure to predict dry matter intake, average daily gain and methane emissions by sheep and cattle in pastoral ecosystems. The study was conducted only with grasslands although Chapter I presents a general background of ruminant production on systems showing the difference between them and the reflect on herbage intake, animal performance and methane emissions. A manuscript is present in Chapter II, describing the explanation power that forages nutrient content and the sward structure present on

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intake, animal performance and methane emission. Chapter III presents the final considerations about this dissertation.

2. LITERATURE REVIEW

2.1. Animal production and their scenarios

The world population has been increasing exponentially since the second half of the last century. Nowadays, even under a pandemic scenario due to the coronavirus, the estimate is that the human population will remain increasing until 2050, but at a slower pace (KANEDA et al., 2020). The total demand for livestock products are expect to duplicate by 2050 with the increase in the population density, and this must be accompanied by a significant increase in the productivity of livestock systems, increasing beef production (USDA, 2020).

Brazilian cattle herd increased 0.5% in 2019 compared to 2018, while sheep herd increased 4% in 2019 compared to the last year (IBGE, 2020). Beef production in Brazil is expected to increase as far as domestic demand and export markets increases in 2021 (USDA, 2021; ABIEC, 2020). Brazilian agribusiness increases the gross domestic product (GDP) of the country by 26.6% in 2020 (CEPEA/CNA, 2021) over the decrease of 4.6% in the total GDP in 2020 (IBGE, 2020). This scenario is in agreement with VIDAURRETA et al. (2020) that related a minimal effect on the primary sector of sheep meat production during the crisis caused by coronavirus pandemic in 2020.

Livestock systems differ in feeding strategies as feedlot and grassland systems. Another ways to feed ruminants consists on animals reared on pastures and receiving other ingredients in feed indoors.

2.2. Feedlots

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With the necessity to increase beef production, the land area has been transform into an intensive system producing more grains and animals. Awareness has become advance on animal nutrition, physiology, gene expressions, management, technologies and other factors influencing the efficiency and quality of the systems (NRC, 2016).

The United States and Canada are the principal countries utilizing feedlot systems, wherein cold climates the development of crops are compromise certain time of the year, favoring warm periods for food production. According to DBLITZ (2012), Argentina and Brazil presented a tendency to increase intensive systems numbers over the years. Nowadays, the actual data pointed out a lower proportion of feedlot systems in Brazilian beef farms, where only 14% of slaughters come from herd finished indoors in 2019 (ABIEC, 2020).

Most of the research in Brazil targeting productivity gains of livestock systems are conducted with animals fed indoors (i.e., feedlot systems) where food source are grain-based. These feeding strategies are usually characterized by animals reaching high levels of intake with 80 to 90% based on dry matter (DM) of the diet composed of grain and by-products, forages, additives and minerals. Nutrients such as crude protein (CP) and neutral detergent fiber (NDF) are calculated to meet the animal requirement providing the efficiency and the genetic potential for maximal animal production. The animals receive the food in the trough, chopped on the correct size, without access restrictions, in several meals a day, in a balanced way (NRC, 2016). For this reason, access to food consumption is not a serious problem and the nutritional value becomes important since food within the rumen was assured.

Nowadays, software formulated diets based on fundamental studies such as NRC (2001) and CNCPS (SNIFFEN et al., 1992). These studies calculate with precision the quantity and necessity of ingredients to compose the animal diet for feed intake according to the category and their requirements in a certain period. Given the excess of nutrients consumed by animals are generally excreted wasting energy. Feedlots optimizing the best animal performance, feed efficiency, meat production in a short period and small areas.

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2.3. Grasslands

Grasslands encompass more than 40% of the ice-free land area on earth (O’MARA, 2012; SOLLENBERGER et al., 2020). Livestock occupies 41% of the total land area in Brazil (IBGE, 2017) and comprises the diversity of pasture communities over the areas. Brazil has the second herd size in the world, with approximately 213 million heads mostly reared based on grasslands (O'DONOGHUE et al., 2019; ABIEC, 2020). The grasslands area destined for rearing beef animals in Brazil is approximately 162 million ha, where 86% of animals slaughtered are raised on pastures (ABIEC, 2020). The majority of experiments in Brazil are conducted in intensive systems, which unfortunately do not correspond to the reality of farmers, where most animals are reared based on grasslands managed under overgrazing.

Ruminant production on grasslands is dependent on environmental conditions. The map below (Figure 1) exposes the global distribution of ruminant production systems based on agroecology and land-use (ROBINSON et al., 2018), where the center and southern regions of Brazil are characterized by mixed rainfed humid (MRH) with tree clover, regularly flooded and freshwater. Considering the ecosystems, many factors are related to the environmental influence on animal behavior and their diet. In the pasture, access to food and its availability is variable, depending on the climate, the forage mass, the sward height, the morphological composition of the forage. Spatio-temporal arrangement and distribution of plants on the soil in the same environment define the sward structure (LACA; LEMAIRE, 2000). In vertical dimensions, sward height and the distribution of the components (leaf-stem relation) and forage mass for a horizontal dimension are the most important variables to determine sward structure (CARVALHO et al., 2008). For this reason, aspects relating to the plant-animal interface have greater importance on forage intake than the nutritional value itself, which will be important after the forage is in the rumen.

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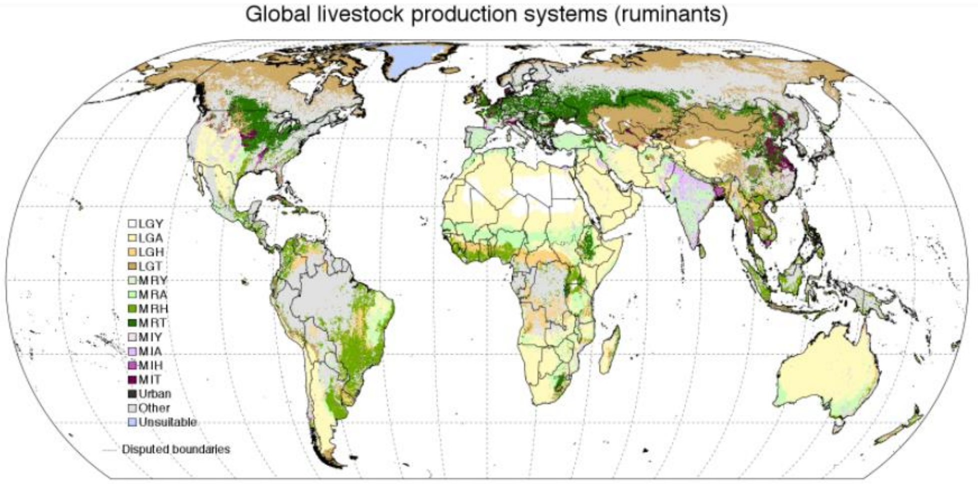

Previewers originally developed by QDR and maintained at https://github.com/GlobalDataverseCommunityConsortium/dataverse-previewers. Feedback and contributions welcome.

Figure 1. Global livestock production systems of ruminants (Robinson et al., 2018).

2.4. *Intake, animal performance and methane emissions*

The literature associating voluntary intake with diet quality is consistent. Van Vuuren (1994) pointed out that forage intake explains around 70% of the variation in animal performance and a range of 10-40% are related to forage nutrients intake by animals. For example, classical models of control of intake in ruminants suggest that high forage quality has a higher passage rate, thus causing less rumen fill (i.e., physical constraints) (POPPI; MINSON; TERNOUTH, 1981). High forage digestibility is strongly associated with the feed digestible NDF fraction (HARPER AND MCNEIL, 2015) and high nitrogen (N) supply to cellulose digesting microbes (VAN SOEST, 1994). On the other hand, when feed is fermented, the by-products of fermentations cause metabolic feedback that depresses voluntary intake (ILLIUS; JESSOP, 1996).

The rumen microbiome allows the use of high-fiber feeds as cellulose and hemicellulose, capable of converting to volatile fatty acids (VFA) used to meet the animal’s energy requirements. Crude protein includes up to 70% of the N in the plant which can be used as availability or in non-protein N forms. Energy and protein are the major components for growth, reproduction and animal production. Under different diets, feedlots or grasslands, voluntary intake is a determinant of nutrient intake by animals and, consequently, animal performance.

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In grazing systems, an adequate sward management strategy is essential to increase animal performance. Studies show that the intensity of methane emissions reduces as long as animal performance increase (SOUZA FILHO et al., 2019; SAVIAN et al., 2014, 2018). BEAUCHEMIN et al. (2020) found that animals characterized with low productivity can present a large environmental impact unlike high-producer animals presenting low relative impact.

In this context, livestock becomes an important scenario for greenhouse gas (GHG) emissions. Enteric fermentation is a biological process that occurs in the rumen and part of them occurs through methanogen microorganisms over the digestive tract pathway that degraded and fermented the food particles intake by ruminants resulting in methane (CH_4) emissions (GIBBS et al., 2000). Methane is the second most abundant anthropogenic GHG and part of the result from livestock and other agriculture practices (Figure 2). Emitted in small quantities than CO_2 (first abundant GHG), CH_4 diffuses to the atmosphere for a shorter period, by the way, it is considering 28-34 times greater for its ability to trap heat in the atmosphereas a global warming potential. In 2020, data shows that 27% of CH_4 emissions come from enteric fermentation being the most important source of this gas (GMI, 2020).

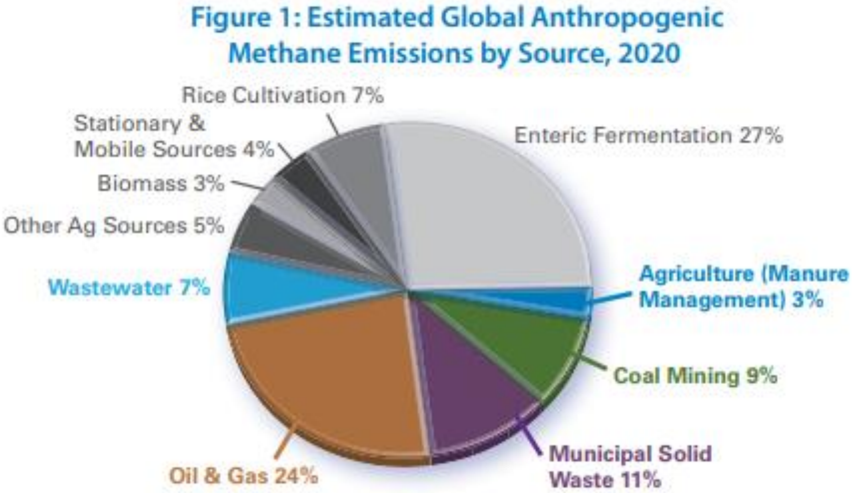

Figure 2. Estimated Global Anthropogenic Methane Emissions by Source (GMI, 2020)

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Most of the strategies proposed to mitigate CH_4 by ruminants are based on intensive management in temperate conditions (KNAPP et al., 2014) focus on nutrition-oriented, thus they miss to impact the ruminants responsible for most of global enteric CH_4. Interestingly, grazing management as a strategy to mitigate such emissions is usually underrated. For example, ZUBIETA et al. (2021) argue that the emission intensity of ruminants can be reduced by 55% when grazing management optimizes both the amount and nutrient content of the herbage ingested by ruminants. The sward structure is important on the intake process by animals grazing, thus over their emissions, offering high nutrient content pastures does not warrant efficient mitigation if animals cannot eat high quantities of nutrients per unit of grazing time. In cases of high nutritive value, the intake increases and the passage rate reflect on CH_4 mitigation (GERE et al., 2021; JANSSEN, 2010). This is because grazers construct their daily intake from space temporal scale (SENFT et al., 1987). In this case, the sward management (SOUZA FILHO et al., 2019; SAVIAN et al., 2014, 2018; ZUBIETA et al., 2021) becomes a strategy to mitigate such emissions. Given this, degraded pastures and overgrazing (BERNDT et al., 2014; TANG et al., 2019) impact negatively on CH_4 emissions and decreases soil C sequestration (O'MARA, 2012), changing the forage quality and the offer of dry matter (TRINDADE et al., 2016).

3. HYPOTHESES

In grazing ecosystems, the sward structure (i.e. sward height and herbage mass) and its nutrient content (i.e. CP, NDF and ADF) explain the variations in forage intake, animal performance and methane emission by sheep and beef cattle.

4. OBJECTIVES

Verify the explanatory power of forage nutrient content and sward structure on daily intake, animal performance and CH_4 emissions by ruminants in grazing ecosystems.

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CHAPTER II

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OS COMPONENTES NUTRITIVOS DA FORRAGEM EXPLICAM O CONSUMO, DESEMPENHO ANIMAL E AS EMISSÕES DE METANO POR OVINOS E BOVINOS EM SISTEMAS PASTORIS?

Destaques

- Os componentes nutritivos da forragem possuem baixo poder de explicação para o consumo, ganho de peso e emissões de CH_4 por ruminantes em pastejo.
- A estrutura do pasto também apresenta baixo poder de explicação para o consumo, ganho de peso e CH_4 emissões por ruminantes em pastejo.
- Fatores aleatórios, como espécie animal, tipo de pastagem e unidade experimental, mostram grande impacto nos modelos de consumo, ganho de peso e CH_4 emissões por ruminantes em pastejo.

Resumo

O teor de nutrientes da forragem é um fator importante na previsão do consumo de matéria seca (CMS), desempenho animal (GMD) e emissões de metano (CH_4) de ruminantes. Este estudo foi conduzido com dados de cinco experimentos de pastejo com ovinos e bovinos em pastagem de azevém anual (*Lolium multiflorum*), mista de azevém e aveia preta (*Lolium multiflorum* + *Avena strigosa*), milheto (*Pennisetum americanum*) e pastagem natural. Analisamos por meio de modelos mistos o efeito do teor de nutrientes da forragem (proteína bruta, fibra em detergente neutro e fibra em detergente ácido) e da estrutura do pasto (altura do pasto, massa de forragem e oferta instantânea de forragem) sobre CMS, GMD e CH_4. Experimento, estação do ano, metodologias de avaliação, espécie animal, tipo de pastagem e unidade experimental, foram considerados fatores aleatórios no modelo. O modelo para CMS apresentou coeficiente de determinação de 84.5%, sendo 12.4% explicado pelo teor de nutrientes da forragem e 13.7% pela estrutura do pasto. O ganho médio diário se ajustou a um modelo com 94.9% de coeficiente de determinação, com 12% de explicação referente ao teor de nutrientes da forragem e 8.9% à estrutura do pasto. O teor de nutrientes da forragem apresentou baixo poder de explicação ($R^2 = 4.6\%$) para as emissões de CH_4 (g dia/ $LW^{0.75}$) em comparação com as variáveis da estrutura do pasto ($R^2= 18.4\%$). O modelo final para emissões de CH_4 apresentou 68% de coeficiente de determinação. Nossos resultados mostram que em ecossistemas pastoris, o teor de nutrientes da forragem ingerido por ovinos e bovinos apresenta relação significativa ($P <0{,}01$) com o consumo de forragem, desempenho animal e emissões de CH_4. No entanto, parte do poder de explicação dos modelos está relacionada à estrutura do pasto (altura do pasto, massa de forragem e oferta instantânea de forragem). Além disso, há uma série de fatores associados a variáveis aleatórias com maior influência nos resultados. Em conclusão, o poder de explicação do teor de nutrientes nos modelos de estimativa de consumo, GMD e CH_4 em ruminantes em sistemas pastoris é igual ou menor do que a estrutura do pasto disponível aos animais. Ressalta-se, ainda, que os efeitos aleatórios apresentam relação forte e significativa com as variáveis dependentes e devem ser considerados em pesquisas futuras.

Palavras-chave: composição química; pastejo; pastagem; efeitos aleatórios; ruminantes; estrutura do pasto.

CAN THE FORAGE NUTRIENT CONTENT EXPLAIN INTAKE, ANIMAL PERFORMANCE AND METHANE EMISSION BY BEEF CATTLE AND SHEEP UNDER GRAZING CONDITIONS?

Highlights

- The forage nutrient content have low explanatory power for intake, average daily gain and CH_4 emissions by grazing ruminants.
- The sward structure also present low explanatory power for intake, average daily gain and CH_4 emissions by grazing ruminants.
- Random effects have an important impact on intake, average daily gain and CH_4 emissions by grazing ruminants.

Abstract

Forage nutrient content is an important factor in predicting the dry matter intake (DMI), animal performance (ADG), and methane emissions (CH_4) of ruminants. Analysis of data was conducted using sheep and cattle measurements from five grazing trials performed in southern Brazil with Italian ryegrass (*Lolium multiflorum*), mixed Italian ryegrass and black oat (*Lolium multiflorum* + *Avena strigosa*) pasture, pearl millet (*Pennisetum americanum*), and natural grassland. We analyzed by mixed models the forage nutrient content (crude protein, neutral detergent fiber and acid detergent fiber) and sward structure factors (sward height, herbage mass and instantaneous herbage allowance) as fixed effects. Trial, season, methodologies, animal species, grassland type, paddock, were considered random effects in the model. Dry matter intake model presented a coefficient of determination (R^2) of 84.5% explained by forage nutrient content (R^2= 12.4%) and sward structure (R^2= 13.7%). The average daily gain model presented 94.9% of coefficient of determination, with 12% explained by forage nutrient content and 8.9% by sward structure. Forage nutrient content had low coefficient of determination (R^2= 4.6%) to predict CH_4 emissions (g day/$LW^{0.75}$) compared to sward structure variables (R^2= 18.4%), totalizing 68% of CH_4 model. Our results show that in grazing ecosystems, the forage nutrient content ingested by sheep and cattle explains a low part of the variation in forage intake, animal performance and CH_4 emissions. Also, the low explanation power was presented to sward factors (i.e., sward height, herbage mass). Besides, there is a range of factors associated with random variables with more influence on outputs. In conclusion, the explanation power of nutrient content to predict DMI, ADG and CH_4 in ruminants based on grazing systems is lower considering the sward structure available for animals.

Abbreviations

CP: crude protein; HM: herbage mass; DM: dry matter; DMI: dry matter intake; OM: organic matter; CH_4: methane emissions; GHG: greenhouse gas; ADG: average daily gain; LW: live weight; MW: metabolic weight; SH: sward height; IHA: instantaneous herbage allowance; ADF: acid detergent fiber; NDF: neutral detergent fiber; ASL: above sea level; S: south; W: west; N: nitrogen; N_2: nitrogen gas; ME: metabolizable energy; NIRS: near-infrared reflectance spectroscopy; R^2: coefficient of determination; SF_6: sulfur hexafluoride.

Keywords: chemical composition; grazing; pasture; random effects; ruminants; sward structure.

1. **Introduction**

Dry matter intake (DMI) is one of the factors that most influences animal productivity (Boval and Dixon, 2012). In feedlot systems, rations contain high levels of concentrates based on total mixed ration or high-grain diets. The formulated diet provides nutrients meeting animal requirements (DiLorenzo et al., 2006) of daily intake, improving the deposits of fat and muscle in reduced time (Silva et al., 2018). The facility to measure residual feed intake can help to understand the nutrients intake by animals analyzing the production efficiency and adjusting the diet as far as animals refuse the food (Bevans et al., 2005). It is well established that feeding grain-based diets are associated with high metabolizable energy (ME) intake, greater digestibility, and lower cell-wall as neutral detergent fiber (NDF) content which leads to less enteric CH_4 (g/kg of DM) as compared with feeding forage-based diets (Johnson and Johnson, 1995). Animals achieve a high DMI and consequently a high average daily gain (ADG).

Feed physical properties (e.g., processing), chemical composition, and digestibility are among the main factors influencing intake by ruminants (Mertens, 1994; Van Soest, 1994), since they determine the rumen environment (Baldwin and Allison, 1983; Kozloski, 2009), digestion, fermentation, ruminal passage rate, and nutrient absorption (Forbes, 2003; Janssen, 2010). From this perspective, NDF and acid detergent fiber (ADF) content of the diet and their intake are critical for achieving the highest animal productivity (Van Soest, 1994) and minimizing greenhouse gas (GHG) emissions (Hristov et al., 2013). However, the forage ingestion process does not work so simply in a pastoral environment (Gregorini et al., 2017). The grazing process encompasses complex interactions between the animal and the environment (Provenza et al., 2015) where the regulation of forage intake is, primarily controlled by short-term mechanisms (Bailey et al., 1996). Animal production under pastures has regularly been related to low herbage intake and low levels of performance explained by chemical and physical constraints (Zubieta et al., 2021).

The sward structure (e.g. herbage mass, sward height, bulk density, leaves and stems proportions) is defined and measured as the distribution and arrangement of above-ground plant parts within a community (Laca and Lemaire, 2000) and it is strongly related to intake rate by grazing animals. There are evidence that the intake rate is reduced in short swards, due to constraints in bite mass by the low bite depth (Flores et al., 1993; Gregorini et al., 2011; Laca et al., 1992). Whereas on tall swards, bite mass and intake rate could decrease as a result of decreasing bulk density (Carvalho, 2013; Fonseca et al., 2013).

In grazing ecosystems, the quantity and quality of forage available for animals infer on intake, consequently on animal performance and CH_4 emissions. Livestock nutritionists agree that the chemical content of feeds are one of the most important variables explaining the variation in daily herbage intake, animal performance, and CH_4 emissions (Bowen et al., 2020; He et al., 2020; Li et al., 2014; Sun et al., 2012; van Lingen et al., 2021), which is true in feedlots, where the animal diet could be balanced based on their nutritional requirements. But is this true in grazing conditions? To answer this question, we used data from five grazing trials measuring intake, performance and CH_4 emissions by beef cattle and sheep grazing on different pastures.

We hypothesized that in grazing ecosystems, the forage nutrient content, (i.e. crude protein, neutral detergent fiber and acid detergent fiber) ingested by sheep and cattle explains a low part of the variation in their intake, performance and CH_4 emissions, and that sward-related factors (i.e. sward height, herbage mass and instantaneous herbage allowance) explain most of the variation of these variables. The aims of this study were i) to verify the explanatory power of forage nutrient content and sward structure on daily intake, performance and CH_4 emissions, and ii) to identify the hierarchical importance of forage nutrient content and sward structure variables in predicting intake, animal performance and CH_4 emission by sheep and cattle in grazing ecosystems.

2. **Material and Methods**

The database composes records from five grazing trials conducted by the Grazing Ecology Research Group at the Federal University of Rio Grande do Sul (UFRGS), southern region of Brazil. All procedures on animals following the recommendations of the Ethics Committee for the Use of Animals (CEUA) of the institution. To uniform the database on this study, the criteria used to select the grazing trials was limited to those studies which used measured *in vivo* CH_4 emissions from ruminants under grazing conditions and when the paddock was considered as the experimental unit with animals as a sample unit. We also considered data from different grasslands and animal species (sheep and beef cattle).

2.1. Site of the grazing trials

Four grazing trials were conducted in the Agronomic Experimental Station (EEA) of UFRGS (30º05’22’’S, 51º39’08’’W; 46 m ASL), and one trial was conducted on a private farm, in São Miguel das Missões district (28º56’14.00’’ S, 54º20’45.61’’ W; 465 m ASL) at Rio Grande do Sul State, southern Brazil. This region is characterized by a warm and humid subtropical climate (Cfa; Köppen–Geiger climate classification system) and an annual average temperature and precipitation of 19°C and 1850 mm, respectively. Throughout the year, summer is warm and winters are cold with frost (Alvares et al., 2013). The soil type of the EEA - UFRGS is classified according to FAO as a sandy clay loam Acrisol and Typic Paleudul (USDA, 1999), and the private farm soil is classified as a clayey Rhodic Hapludox (Soil Survey Staff, 1999).

2.2. Characteristics of the grazing trials

The Table 1 shows information about the five grazing trials as animal characteristics, pasture type, and methodologies used for sward and animal measurements. Basic data of the grazing trials analyzed were the dry matter intake, animal performance, CH_4 emissions, sward structure and forage chemical composition measured in the same period. All trials blocked the effects of soil, land, vegetal, and animal characteristics (type, breed, age, and weight). More details about the field measurements and calculations are described in the following published articles:

Amaral et al. (2016), Savian et al. (2014) and (2018), de Souza Filho et al. (2019), Azambuja et al. (2020), Cezimbra et al. (2021).

Table 1. Data sources with evaluation year, local, animal characteristics, pasture type and methodologies used in the included trials referenced.

Variables								Methodology					Reference
Trial	Local*	Year	Animal				Pasture type	Intake	Bromatological analysis		Forage sample	CH_4	
			Species	Breed	Age (months)	Weight (kg)			CP	NDF and ADF			
1	EEA - UFRGS	2011	Sheep	Texel x Ile de France	11	35 ± 4	Italian ryegrass	N-alkane	Kjeldahl	Van Soest	Hand plucking	SF_6	Savian et al. (2014)
2	EEA - UFRGS	2014/2015	Sheep	Texel x Polwarth	10	24 ± 1	Italian ryegrass	N-fecal	NIRS	NIRS	Hand plucking	SF_6	Savian et al. (2018)
3	Private farm	2013/2014	Beef cattle	Angus x Hereford x Nelore	14	262 ± 5	Mixed Italian ryegrass and black oat	N-alkane	NIRS	NIRS	Hand plucking	SF_6	De Souza Filho et al. (2019)
4	EEA - UFRGS	2011	Sheep	Texel	5	20 ± 2	Pearl millet	N-alkane	Kjeldahl	Van Soest	Hand plucking	SF_6	Amaral et al. (2016)
5	EEA - UFRGS	2012/2013	Beef cattle	Angus x Hereford x Nelore	13	250 ± 20	Natural Grassland	N-alkane	NIRS	NIRS	Hand plucking	SF_6	Cezimbra et al. (2021); Azambuja et al. (2020)

Trial 1: Italian ryegrass (*Lolium multiflorum*); Trial 2: Italian ryegrass (*Lolium multiflorum*); Trial 3: Mixed Italian ryegrass and black oat (*Lolium multiflorum* + *Avena strigosa*) pasture; Trial 4: Pearl millet (*Pennisetum americanum)*; Trial 5: Natural grassland (main plant genera: *Paspalum, Axonopus, Piptochaetium, Coelorachis*, *Aristida, Eryngium, Andropogon, Bacharis* and *Vernonia*); Trial 1 and 4: Kjeldahl analysis based on N value x 6.25 without the use of amylase using AOAC (1975) methods 2036, 1960 and no. 2049; Van Soest and Robertson (1985); Trial 2,3 and 5: NIRS values were obtained by The Walloon Agricultural Research Centre, Belgium (*sensu* Decruyenaere et al., 2009). *EEA-UFRGS: Agronomic Experimental Station of Federal University of Rio Grande do Sul, Brazil

2.3. *Measurements on animals*

2.3.1. *Daily forage intake*

Forage was sampled by hand-plucked method (Johnson, 1978) to estimate the n-alkanes and N-fecal content on herbage. The forage intake in trials one, three, four, and five were estimated by n-alkane methodology (Dove and Mayes, 2006). For that, the animals received twice a day cellulose pellet *per os* dosing containing, on average, 200 mg (for cattle) and 42 mg (for sheep) of dotriacontane (C_{32}) for ten consecutive days, which means five days for animal adaptation with the marker dosification and to achieve a stable content of the marker in the feces, and five days for fecal collection.

For individual animals, fecal samples were collected from the rectum twice a day during the last 5-day collections, and before cellulose pellet administration. Before chemical analysis, fecal samples were dried at 55ºC for 72 h in a forced-air oven, ground with a knife mill through a 1-mm screen. The n-alkanes content were measured in the forage and feces by gas chromatography according to (Dove and Mayes, 2006), and the estimation of dry matter intake by animals was calculated according to (Mayes et al., 1986).

Trial two used the N-fecal method described by Penning (2004), and a specific equation for Italian ryegrass was used for estimating forage intake (Azevedo et al., 2014). Feces collections from three animals per paddock were performed during five consecutive days using bags, which were emptied once per day. The feces were weighed and homogenized and a subsample of 20% of the total was taken, dried at 55ºC for 72 h in a forced-air oven, pooled per animal, ground with a knife mill through a 1-mm screen, and analyzed for dry matter (DM), organic matter (OM), and total nitrogen (N) (AOAC, 1975). The OM intake was transformed to DMI based on forage OM intake to analyze all the data on the same basis.

2.3.2. *Performance*

In the five trials, the live weight (LW) was recorded after fasting animals from solids and liquids for approximately 12 hours. Electronic scales were used to obtain the LW of animals. Average daily gain (ADG; g/animal) was obtained by difference of initial and final LW of the animals, divided by the number of days in the experimental period or the mean of LW in each period according to the trial.

2.3.3. Methane emissions

The five trials used sulfur hexafluoride (SF_6) methodology (Johnson et al., 1994) adapted by Gere and Gratton (2010) to estimate CH_4 emissions. For all trials, in each paddock, three tester animals received a permeation tube filled with SF_6. The SF_6 permeation tube was inserted into the reticulum-rumen of each animal (*per os* dosing*)* for ten days before the beginning of the gas sampling. The average permeation rate varied from 0.793 to 0.9 mg/day for the sheep trials and from 2.0 to 3.62 mg/day for the beef cattle trials.

To collect the gas sampling, stainless steel cylinders (0.5 L) were connected to flow regulators by brass ball-bearing (Gere and Gratton, 2010) to collect during 5 days (Pinares-Patiño et al., 2012). The cylinders were cleaned with high purity nitrogen gas (N_2) and pre-evacuated (<0.5 mb) before sample collection. To quantify the background air, the same mechanism was applied, and three cylinders were placed one meter above ground. After the gas sampling in the field, the pressure in each cylinder was measured, and each sample was diluted with N_2 to facilitate sample removal from the cylinder. The gas chromatography method (Shimadzu, 2010 and 2014) was performed at the Biogeochemistry laboratory in UFRGS and step by step of the analysis and calculations are detailed in the published articles (Amaral et al., 2016; Azambuja Filho et al., 2020; Cezimbra et al., 2021; de Souza Filho et al., 2019; Savian et al., 2018, 2014). All procedures performed here followed the guidelines for use of the SF_6 tracer technique to measure CH_4 emissions from ruminants as proposed by Berndt et al. (2014).

The CH_4 emission (g/day) was calculated using the permeation rate of SF_6 and concentrations of CH_4 and SF6 in breath samples (Johnson et al., 1994). Finally, CH_4 emission was expressed per kg of metabolic weight (g/kg $LW^{0.75}$) and CH_4 per average daily gain (g/kg) referred to CH_4 emission intensity.

2.4. Sward measurements

2.4.1. Sward structure

Measurements of the sward height (SH; cm), herbage mass (HM; kg DM/ha), and instantaneous herbage allowance (IHA; kg DM/kg of LW) were performed to characterize the sward structure in all trials.

The sward height (cm) was measured randomly in the experimental areas using a sward stick (Barthram, 1985).

Herbage mass was measured by cutting herbage samples at ground level in a 0.25m^2 square and dried in a forced-air oven at 55°C for 72 hours to estimate the forage DM. The number of samples varied according to each trial.

Instantaneous herbage allowance was obtained according to the equation of herbage mass divided by stocking rate which is the weight summing of all animals in each paddock divided by the area of the paddock (kg LW/ha). Herbage or forage allowance is defined according to Allen et al. (2011) by the relationship between forage mass and animal live weight per unit area considered the forage-to-animal relationship. It is not consider a sward structure variable specifically but the importance to analyze the forage allowance is due to the relation between forage mass and sward canopy structures characteristics to explain animal performance reducing the wide range across environments (Sollenberger et al. 2005).

More details about these measurements are described in their respective articles.

2.4.2. Forage nutrient content measurements

The crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) composed the forage nutrient content information in all trials.

Forage samples were hand-plucked (Johnson, 1978) simulating the forage ingested by the animals (Bonnet et al., 2011; 2015). Forage samples were collected, dried in a forced-air oven at 55°C for 72h, and then grounded with a centrifugal mill (1 mm screen). To analyze that, trials one and four used traditional methods, which means following AOAC (1975) and Goering and Van Soest (1985) (see methods in Table 1), while trials two, three and five used near-infrared reflectance spectroscopy (NIRS), which were performed by The Walloon Agricultural Research Centre, Belgium (*sensu* Decruyenaere et al., 2009).

2.5. Statistical analysis

All statistical procedures were performed in R software version 4.0.0. The database comprises 569 records of animal measurements from five grazing trials as combined data using the “aggregate” package to corporate the data according to the experimental unit (paddock) and excluding outliers which totalized 84 complete data observations for analyses. Analysis of variance assumptions were tested by observation of QQ plots and residual graphs. To equalize the results for different animal species, LW and ADG were transformed based on metabolic weight. We considered the paddock as the experimental unit to obtain multiple regression models and sward analysis; however, data of individual animals were considered for forage nutrient content analysis.

2.5.1. Regression models considering forage nutrient content and sward structure

Linear models (lm) for intake, animal performance and CH_4 emissions were generated with forage nutrient content (CP, NDF, ADF) and sward structure (sward height, herbage mass, instantaneous herbage allowance) variables. Linear, polynomial and non-linear models were compared and the best fit model was selected by the highest coefficient of determination (R^2) at 5% of the significance level. Linear (yij= a + bx + €ij), quadratic (yij = a + bx + cx2 + €ij) or logarithm (yij = a + log(x) + €ij) models were selected for each variable and the dispersion graphs were created.

2.5.2. Mixed models considering forage nutrient content and sward structure

We tested mixed models to define the most explanatory variables on determining intake, animal performance and CH_4 emission by grazing animals. For this, the “buildmer” package suggested by Voeten (2020) was used to automatically find and compare the largest possible mixed models that converge. Before creating the model, all variables were standardized meaning to put the variables on the same scales. Fully maximal models were defined separately for each dependent variables ($DMI/LW^{0.75}$, $ADG/LW^{0.75}$, $CH_4/LW^{0.75}$). Independent variables were separated into fixed and random effects. Linear and quadratic effects of the nutrient content (CP, NDF, ADF) and sward structure (sward height, herbage mass, instantaneous herbage allowance) were considered fixed effects. Animal species, pasture type, paddock, trial, and season were considered random effects. Firstly, the procedure identifies the maximal model that is capable of converging. Then, automatically, order and backward stepwise elimination procedure was performed based on *Akaike information criterion* (AIC). The final model was created with those effects that still allow the model to converge in the most information-rich effects, minimizing AIC and simplifying for non-significant random effects ($P < 0.05$). The full model and the selected final model can be observed in Table 2.

Table 2. Full and the selected model for dry matter intake per metabolic weight ($DMI/LW^{0.75}$), average daily gain per metabolic weight ($ADG/LW^{0.75}$) and methane emissions per metabolic weight ($CH_4/LW^{0.75}$).

Response	Model	Formula
DMI, ADG and CH_4	Full	*sCP* + *sCP2* + *sADF* + *sADF2* + *sNDF* + *sNDF2* + *sHM* + *sHM2* + *sSH* + *sSH2* + *sIHA* + *sIHA2* + (1|trial) + (1|grassland)+ (1|season) + (1|intake_methodology)* + (1|animal) + (1|paddock)
DMI	Final	*sCP* + *sCP2* + *sADF* + *sHM* + *sIHA* + (1|trial) + (1|grassland)+ (1|animal) + (1|paddock)
ADG	Final	*sCP* + *sNDF* + *sNDF2* + *sHM* + *sSH*+ *sSH2* + *sIHA* + *sIHA2* + (1|grassland)+ (1|animal) + (1|paddock)
CH_4	Final	*sCP* + *sHM* + (1|trial) + (1|paddock)

CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; HM: herbage mass; SH: sward height; IHA: instantaneous herbage allowance; (1|intake_methodology)*: corresponded to N-alkane and N-fecal; *s*: standardize variables; 2: quadratic effect; (1|): random effects

The coefficient of determination of fixed and random effects of the final models were obtained using the “MuMIn” package (Kamil, 2020). Inside fixed effects, the “rsq” (Dabao, 2020) package gives the partial R^2 of each fixed variable in the model. Then, the R^2 for forage nutrient content and sward structure was obtained. Linear models were created with the standardized observed versus predicted (*lme4* package; Bates et al., 2015) data from final mixed models for DMI, ADG and CH_4 emission.

3. **Results**

The Table 3 shows the descriptive statistics of the forage nutrient content, sward structure, daily herbage intake, performance and CH_4 emissions by animals of the five trials analyzed. The forage CP, NDF and ADF content ranged between trials from 9.9 to 26.5%, from 44 to 73%, and from 24 to 34%, respectively. The herbage mass ranged from 716 to 2877 kg of DM per hectare. The instantaneous herbage allowance varied from 1.55 to 4.13 kg of DM per 100 kg of live weight per day. The observed sward height was between 4.2 and 26.5 cm. The outputs are presented in metabolic weight (used in the statistical analysis) and in the most usual variable unit according to literature as forage intake (% LW) ranged from 2.09 to 4.25, ADG from 0.06 to 0.24 kg/animal and CH_4 emissions varied between 13 and 192 g/day and from 194 to 828 g/kg ADG.

Table 3. Mean (± standard deviation) of forage nutrient content, sward structure and outputs in each trial.

Variables	**Trial**				
	1 (*Lolium multiflorum)*	2 (*Lolium multiflorum)*	3 (*Lolium multiflorum+Avena strigosa)*	4 (*Pennisetum americanum*)	**5** (Natural grasslands)
Forage nutrient content (n=300)					
CP	13.3 ± 1.48	19.4 ± 4.33	20.5 ± 3.13	26.5 ± 2.57	9.9 ± 2.23
NDF	60.1 ± 1.50	44.9 ± 2.34	48.2 ± 3.95	56.35 ± 3.44	73.3 ± 2.90
ADF	31.3 ± 1.32	24.1 ± 2.34	25.3 ± 2.49	26.6 ± 2.92	33.9 ± 3.24
Sward structure (n=84)					
HM	2192.01 ± 477.33	1425.70 ± 284.82	2877.41 ± 1188.16	1952.10 ± 367.64	716.28 ± 348.28
IHA	2.25 ± 0.81	1.55 ± 0.42	4.13 ± 2.95	2.38 ± 0.55	1.84 ± 1.32
SH	18.71 ± 2.78	10.46 ± 1.97	26.58 ± 10.15	22.86 ± 4.01	4.24 ± 0.66
Outputs					
Forage intake (n=84)					
% LW	3.43 ± 0.44	2.58 ± 0.38	2.14 ± 0.23	4.25 ± 0.56	2.09 ± 0.63
kg/kg $LW^{0.75}$	0.083 ± 0.011	0.062 ± 0.010	0.091 ± 0.010	0.094 ± 0.014	0.096 ± 0.007

			Average daily gain (n=84)		
kg/animal/day	0.116 ± 0.038	0.088 ± 0.030	1.004 ± 0.142	0.060 ± 0.023	0.242 ± 0.169
kg/kg $LW^{0.75}$	0.008 ± 0.002	0.007 ± 0.003	0.013 ± 0.002	0.006 ± 0.002	0.004 ± 0.002
			Methane emissions (n=84)		
g/day	23.76 ± 4.03	22.74 ± 6.18	192.62 ± 54.93	13.85 ± 2.10	124.99 ± 49.25
g/kg ADG	226.58 ± 66.30	406.54 ± 303.69	194.92 ± 61.326	260.79 ± 99.88	828.97 ± 586.64
g/kg $LW^{\wedge 0.75}$	1.67 ± 0.29	1.69 ± 0.47	2.45 ± 0.67	1.34 ± 0.17	2.29 ± 0.62

Trial 1 and 2: sheep grazing Italian ryegrass pasture; Trial 3: beef cattle grazing mixed Italian ryegrass and black oat pasture; Trial 4: sheep grazing pearl millet pasture; Trial 5: beef cattle grazing natural grassland of Pampa biome; CP: crude protein (%); NDF: neutral detergent fiber (%); ADF: acid detergent fiber (%); HM: herbage mass (kg DM/ha); IHA: instantaneous herbage allowance (kg DM/100 kg LW/day); SH: sward height (cm); % LW: % live weight; kg/kg $LW^{0,75}$: kg of dry matter intake per average daily gain per kg metabolic weight per day; kg/animal/day: kg of LW per animal per day; g/day: g of CH_4 emissions per day; g/kg ADG: g of CH_4 emissions per day per kg of ADG per animal; g/kg $LW^{0.75}$: g CH_4 emissions per day per kg of metabolic weight

3.1. *Forage nutrient content and sward structure*

The Figure 1 shows the relationship between response variables and forage nutrient content obtained in the five studied trials. We observed that forage nutrient content explain part of DMI, ADG and CH_4 emission by beef cattle and sheep grazing different grasslands, presenting a low coefficient of determination. The DMI had a quadratic relation with forage CP content, a logarithm adjust with NDF and linear relation to ADF content. For animal ADG the best fit model was a quadratic relation with CP and NDF and a linear relation with ADF content. For CH_4 emissions, a quadratic relation to CP and a smootling line for NDF and ADF content were presented.

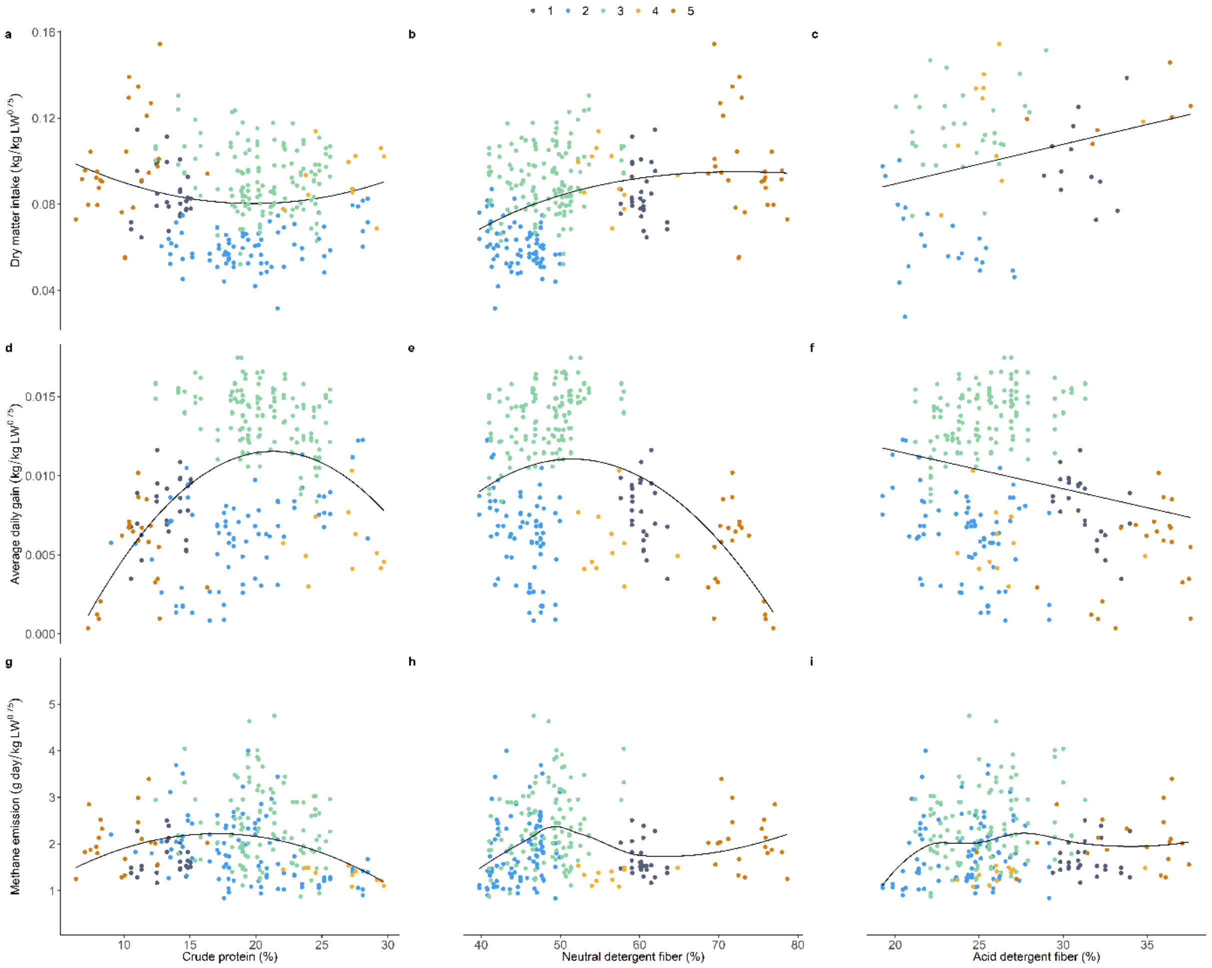

Figure 1. Relations between forage nutrient content variables and outputs. a) Dry matter intake per crude protein ($y= 0.1202 - 0.0047x + 0.0001x^2$, $R^2 = 0.04$, $P = 0.02$); b) Dry matter intake per neutral detergent fiber ($y= -0.05 + \log(0.03)$, $R^2 = 0.09$, $P = 0.002$); c) Dry matter intake per acid detergent fiber ($y= -0.064 + \log(0.0452)$, $R^2 = 0.10$, $P < 0.001$); d) Average daily gain per crude protein ($y= -0.013 + 0.0022x - 0.00005x^2$, $R^2 = 0.21$, $P < 0.001$); e) Average daily gain per neutral detergent fiber ($y= -0.0134 + 0.001x - 0.00001x^2$, $R^2 = 0.20$, $P = 0.019$); f) Average daily gain per acid detergent fiber ($y= 0.0173 - 0.0003x$, $R^2 = 0.08$, $P = 0.004$); g) Methane emission per crude protein ($y= -0.092 + 0.259x - 0.007x^2$, $R^2 = 0.13$, $P = 0.001$); h) Methane emission per neutral detergent fiber ($P > 0.05$); i) Methane emission per acid detergent fiber ($P > 0.05$).

The Figure 2 presents the significant relationship between sward structure and DMI, ADG, and CH_4 emission by beef cattle and sheep grazing different grasslands. The DMI had a positive linear relation to sward height and herbage mass and instantaneous herbage allowance. The ADG presented a logarithm relation to sward height, while the best fit model between DMI and herbage mass and instantaneous herbage allowance was linear. The CH_4 emission had a quadratic relation to sward height and a linear relation to herbage mass and instantaneous herbage allowance.

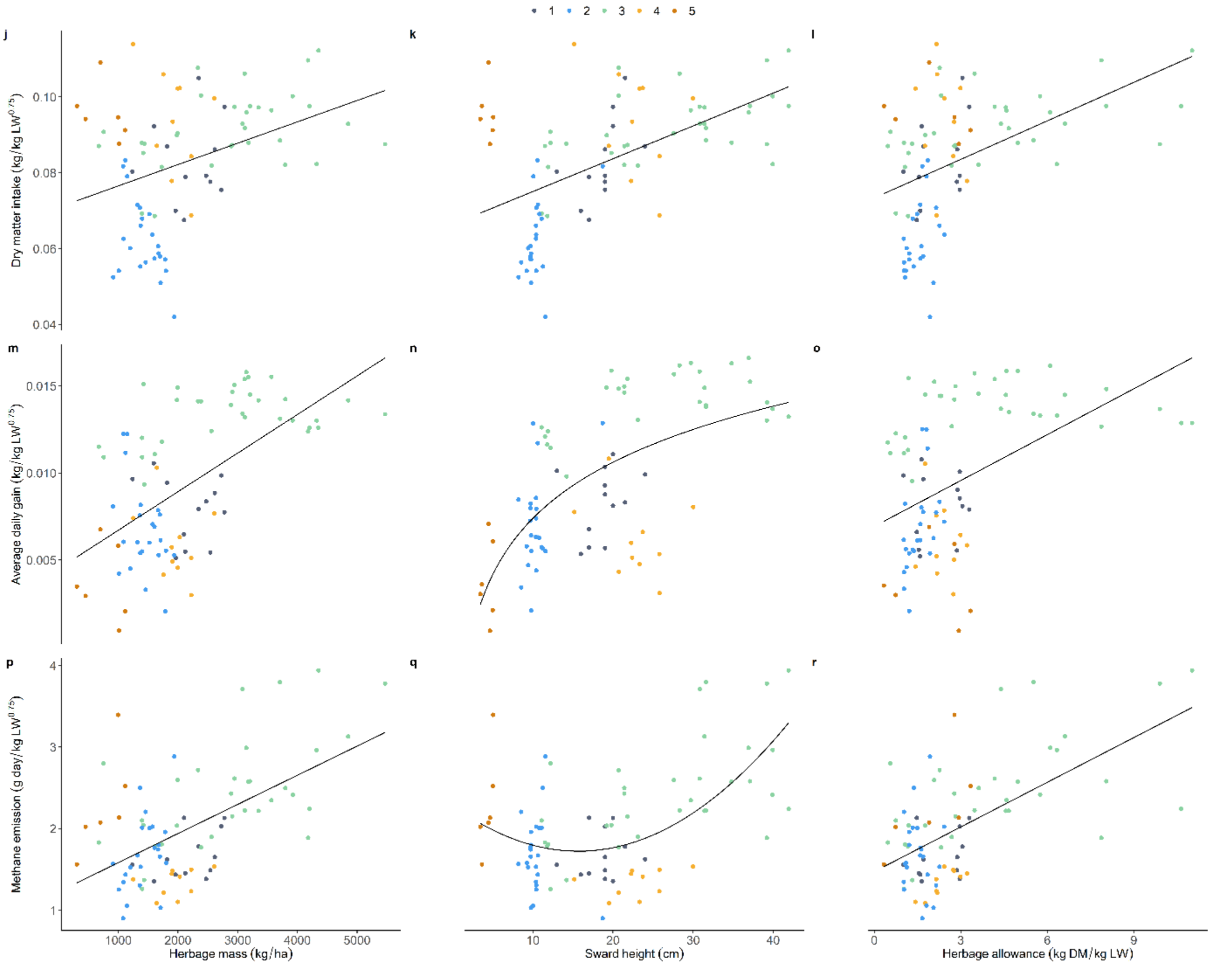

Figure 2. Relationship between sward structure variables and outputs. j) Dry matter intake (DMI) per herbage mass (HM) (y= 0.0709 + 0.000005x, R^2 = 0.11, $P < 0.001$); k) DMI per sward height (SH) (y= 0.0664 + 0.0008x, R^2 = 0.27, $P < 0.001$); l) DMI per instantaneous herbage allowance (IHA) (y= 0.073 + 0.003x, R^2 = 0.19, $P < 0.001$); m) Average daily gain (ADG) per HM (y= 0.004499 +0.000002, R^2 = 0.32; $P < 0.001$); n) ADG per SH (y= -0.003 + log(0.004), R^2 = 0.41, $P < 0.001$); o) ADG per IHA (y= 0.007 + log(0.0008), R^2 = 0.20, $P < 0.001$); p) Methane emission (CH_4 emission) per HM (y= 1.22 + 0.0003x, R^2 = 0.30, $P < 0.001$); q) CH_4 emission per SH (y= 2.285 – 0.071x + 0.002x², R^2 = 0.31, P <0.001); r) CH_4 emission per IHA (y= 1.473 + 0.182x, R^2 = 0.35, $P < 0.001$).

3.2. Multiple models

The linear model for standardized observed versus predicted DMI presented 87% of coefficient of determination (Figure 3a). The Figure 3b shows the coefficients of determination of the mixed model for standardized DMI with 26.1 and 58.3% of the variation explained by fixed and random effects, respectively. Considering the fixed effects, the forage nutrient content and sward structure corresponded to 13.7 and 12.4% of the coefficient of determination, respectively. The final model included the fixed effects of forage CP and ADF content, and sward height. The random effects selected animal species, type of pasture, paddock and trial.

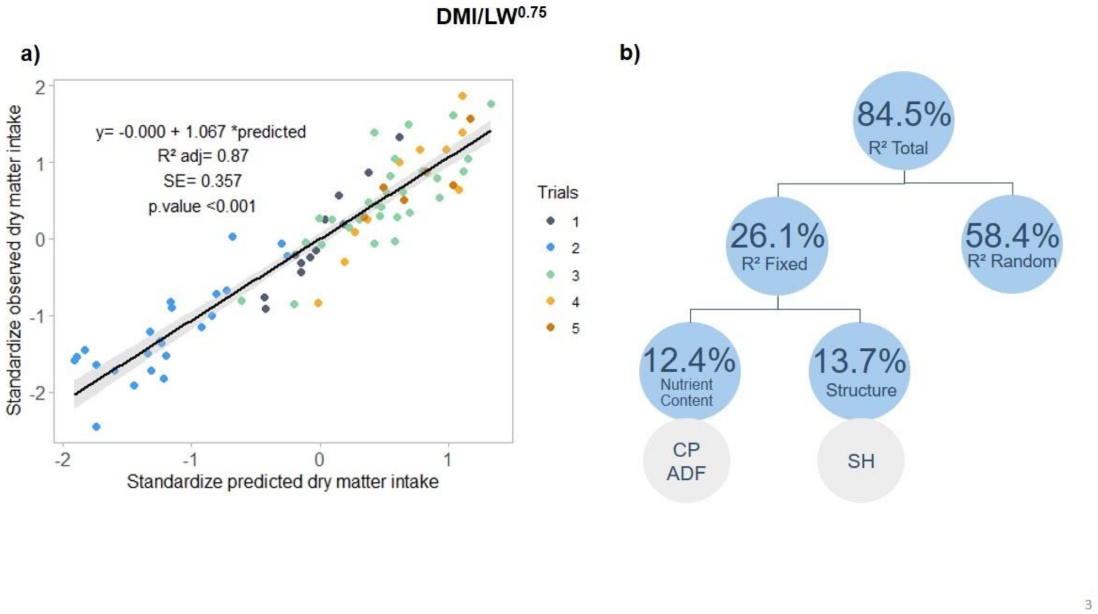

Figure 3. Dry matter intake per metabolic weight. (a) Observed versus predicted value for DMI, and (b) Coefficients of determination of the model (total) and of fixed and random effects. Trial 1: Italian ryegrass; Trial 2: Italian ryegrass; Trial 3: Mixed Italian ryegrass and black oat; Trial 4: Pearl millet; Trial 5: Natural grassland;

Standardized values between predicted and observed ADG are demonstrated in Figure 4a with a close relation between them (R^2= 0.89). The R^2 values for ADG model are demonstrated in Figure 4b with 20.8 and 74.4% corresponding to fixed and random effects, respectively. Forage nutrient content and sward structure explained 12.0 and 8.7% of the fixed effects, respectively. The final model selected CP, NDF (linear and quadratic), herbage mass, sward height (linear and quadratic), and instantaneous herbage allowance (linear and quadratic) as fixed effects ($P < 0.05$). The random effects selected animal species, pasture type and paddock.

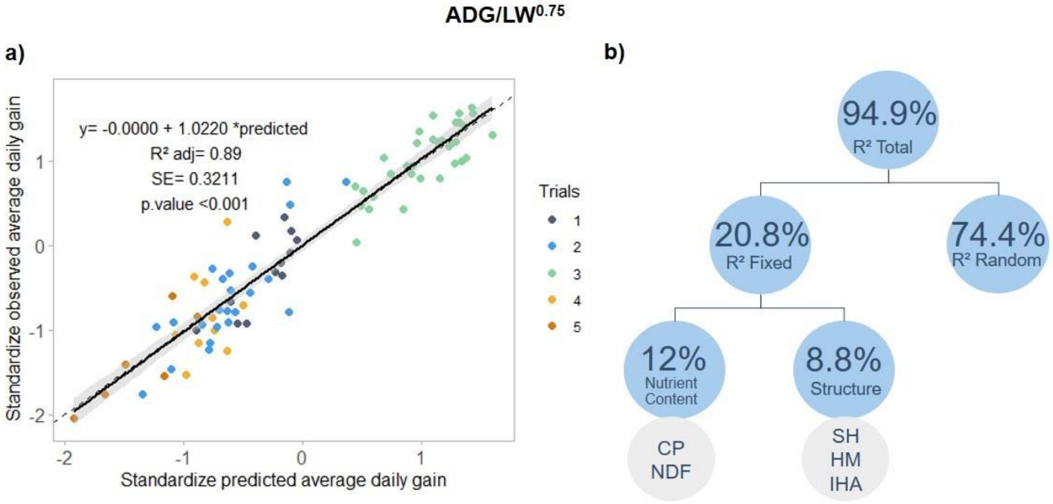

Figure 4. Average daily gain per metabolic weight. (a) Observed versus predicted value for ADG, and (b) Coefficients of determination of the model (total) and of fixed and random effects. Trial 1: Italian ryegrass; Trial 2: Italian ryegrass; Trial 3: Mixed Italian ryegrass and black oat; Trial 4: Pearl millet; Trial 5: Natural grassland;

The predicted versus observed CH_4 emission model (R^2= 0.63) is presented in Figure 5a. The CH_4 model had a coefficient of determination of 23.0 and 44.9% for fixed and random effects, respectively. Only 4.6% of the explanation was equivalent to forage nutrient content as demonstrated in Figure 5b. The sward structure represented 18.4% of the coefficient of determination of the final model for CH_4 emission. The model considered CP (linear and quadratic) and herbage mass as significant effects ($P < 0.05$). Animal species, season and trial were selected as random effects in the model ($P < 0.05$).

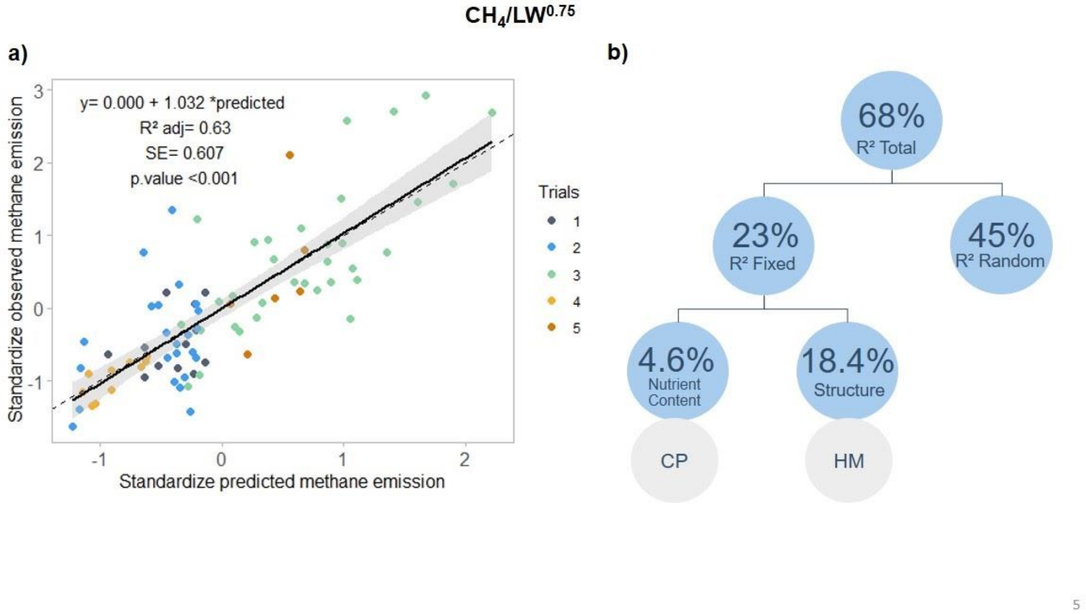

Figure 5. Methane emission per metabolic weight. (a) Observed versus predicted value for CH_4, and (b) Coefficients of determination of the model (total) and of fixed and random effects. Trial 1: Italian ryegrass; Trial 2: Italian ryegrass; Trial 3: Mixed Italian ryegrass and black oat; Trial 4: Pearl millet; Trial 5: Natural grassland

4. **Discussion**

Our initial hypothesis that the nutrient content of the forage ingested by sheep and cattle have a low explanation on DMI, ADG and CH_4 emissions by grazing ruminants was supported by our results. The results of this study demonstrated that forage nutrient content present a low coefficient of determination (R^2 <0.19; Figure 1) on DMI, ADG and CH_4 emissions by sheep and cattle grazing different grasslands. The explanation power of forage nutrient content was similar or lower than sward structure variables. Despite this, during the grazing process, the animal finds the forage nutritive value through the sward structure, limiting the intake according to forage available. These feed actions reflect animal performance and enteric emissions responses.

4.1. Forage intake

Our results reinforce the existence of differences between the indoor-fed and grazing ruminants respecting the forage ingestion capacity and feeding behavior. The classical metabolic and physical constraints of intake (Allen, 1996; Forbes, 2001; Illius and Jessop, 1996) may operate conjunctly, but hierarchically differ under such contrasted feeding conditions (Allden and Whittaker, 1970). For penned animals, the chemical composition of the diet is an important driver of intake. Briefly, the DM content (Fernandez and Rodriguez, 2013), feed digestibility (Hodgson, 1977), NDF content (Arelovich et al., 2008), especially its indigestible fraction (Harper and McNeill, 2015), the type and amount of carbohydrate (Panjaitan et al., 2010), protein (Van Soest, 1994) and the particle size (Aikman et al., 2008; Poppi et al., 1981; Watt et al., 2015; NRC, 2001), affect the rumen fill and passage rate, thus the voluntary intake by animals (Allen, 1996; Forbes and Barrio, 1992).

These diet-related factors are certainly important in a scenario where the feed has been already ingested. Once in the rumen, the chemical composition and digestibility determine the ruminal environment (Baldwin and Allison, 1983; Kozloski, 2009), the digestion kinetics, the fermentation profile, nutrient flow and absorption (Forbes, 2003; Forbes and Barrio, 1992; Illius and Jessop, 1996), all factors triggering the metabolic control of intake (Illius and Jessop, 1996). However, before the chemical constituents of the diet begin altering these physical or metabolic processes, animals first have to search, gather and process (i.e. holding and chewing) the fed material in the mouth and then swallow it. These activities refer to the eating process and are notably different between the feed indoor animals and the grazing ruminants (Allden and Whittaker, 1970).

In grazing systems, our results showed a lower influence of the chemical composition of forage in determining DMI (Figures 1 and 3b). The discrepancy of our results from literature might rely on that the animal-internal mechanism voluntary intake control was developed for feed animals, whose ingestive process differs widely from grazing ruminants. It is expected that animals grazing on better quality pastures (higher CP and lower NDF and ADF) would have greater intake; nonetheless, this is not likely to occur when sward structure and herbage allowances restrict the ingestion process at its smallest scale, i.e. the bite (Allden and Whittaker, 1970; Carvalho, 2015, 2013; LACA et al., 1992). Sward height strongly influences bite and consequently DMI by animals (Boval and Sauvant, 2019; Coleman, 1992). The interaction between plant-animals in grazing systems affects animal selection since they are dependent on the sward structure. Adjusting the sward management through to sward height is possible to optimize the grazing time, allowing animals to select plant parts that contain more leaves, increasing the CP and DMI (Savian et al., 2020).

We observed a significant influence of ADF on DMI (Figures 1 and 3b), which was not expected. Allen et al. (2019) developed an equation to predict DMI based on nutrient content of the diet and observed a high relation between ADF/NDF in forages compared to grain and the filling effects of forage NDF and the digestibility to predict DMI in dairy cows. Our results show a significant correlation between ADF and NDF (r= 0.86, P <0.01). We believe that as far as ADF increases, the DMI increases due to plant maturity indirectly influenced by sward structure. High sward height has more stem increasing the ADF and NDF content on the plant (van Lingen et al., 2021; Villalba et al., 2015).

It is important to highlight that the forage nutrient content values observed in this study (Table 3) correspond to the superior stratum of sward, mainly composed of leaves with a higher quantity of nutrients (CP), selected by animals during the grazing process. Since the grazing process occurs in hierarchical scales which firstly involves the sward structure and secondly, the bite selection (Allden and Whittaker, 1970; Bailey and Provenza, 2008), we could expect an even higher influence of sward structure in DMI responses if the forage samples were collected from available forage and not from hand-plucking methodology. Villalba et al. (2015) described that grazing nutrient content are near of the available in the pasture. However, in heterogeneous grasslands, animals optimize their time selecting and harvesting the forage with quality over the average on the pasture (Laca and Demment, 1991) and they memorize this area to return frequently (Launchbaugh and Howery, 2005). Consequently, the quality of ingested forage can be higher than the quality of the available forage in the pasture.

We suggest more studies that compare the nutrient content in the hierarchical scale of animal behavior. It is necessary to understand how much nutrients are available in the pasture and what faction of it is ingested by grazing ruminants.

4.2. Animal performance

In this study, the nutrient content of the forage apparently ingested by grazing animals explained 12% of the variation in ADG, with the CP and NDF being the components that significantly affected this response (Figure 4b). While it is commonly accepted that diets with higher levels of CP (Xia et al., 2018) and density of digestible nutrients (Fernandez-turren et al., 2020) promote greater ADG, in grazing conditions the daily intake of animals can be restricted by sward structure (i.e., herbage allowance, sward height, herbage mass), even when the animals encounter offer herbage with high nutritive value. Overall, scenarios of high-quality pastures but low herbage intake can explain the low explanatory power of the nutrient content over animal performance in grazing conditions. Carvalho et al. (2015) developed a conceptual model that focuses on the essential factors and processes involved in determining ADG, which energy gain is considered a direct causative factor on ADG and it's regulated by diet quality and daily intake. The sward structure presents an indirect effect on ADG, mainly through its influence on short-term variables such as bite mass and bite rate (Bonnet et al., 2015; Carvalho, 2015).

Our results show a significant influence of sward height, herbage mass and instantaneous herbage allowance on ADG (R^2 = 8.8%). This value represents 42.3% of the coefficient of determination of the fixed effects in the final model for ADG (Figure 4b), highlighting that in grazing conditions, the quantity of food available to grazing animals has improved importance in determining ADG. Sward structure has a direct effect on the bite formation (Bailey and Provenza, 2008) and, consequently, in the higher hierarchical scales of the ingestive behavior of ruminants (i.e. daily intake). Azambuja et al. (2020), studying the animal intake in natural grasslands, observed a high influence of the diversity of vegetal species in the bite mass and nutritive value of bites. Mainly in heterogeneous grasslands, NDF and CP content are related to herbage allowance which affects the ADG (Carvalho et al., 2015; Azambuja et al., 2020). These authors found that in low herbage allowance (4 kg DM/100kg LW), the nutritive value was greater (NDF of 71.5% and CP of 12%) and in high herbage allowance (16 kg DM/100kg LW), the nutritive value was lower (NDF of 77.6% and CP of 9.5%), with high seasonal influence on ADG (Carvalho et al., 2015).

4.3. Methane emission by animals

The CH_4 model demonstrated that the CP content of the herbage apparently ingested explains only 4.6% of the variation in CH_4 production (Figure 5b). This agrees with Hammond et al. (2013), who observed that the CP content of ryegrass and white clover grasses fed to sheep explained a minor share (19%) of the variation in CH_4 yield, or with other studies reporting weak or no association (Jonker et al., 2017; Sun et al., 2011; van Lingen et al., 2021) between the chemical composition of the diet and CH_4 emissions. This lack of association can be attributed to forages with high nutritive value, or to plant-intrinsic factors (i.e., content of secondary compounds, organic acids) that change the fermentation profile in the rumen towards lesser or higher CH_4 production and yield.

In grazing conditions, the hypothesis that improving the quality of the herbage via the manipulation of the sward (e.g., herbage mass, stocking rate; (Dini et al., 2012; Pinares-Patiño et al., 2007, 2003) would increase daily intake, thus reduce CH_4 yield and intensity, was not confirmed. This latter response is likely to occur when animals grazing high quality pastures are not able to efficiently harvest the herbage due to intake-restricting structural attributes of swards (Cezimbra et al., 2021; de Souza-Filho et al., 2019). In other words, animals reaching higher levels of intake from swards facilitating the ingestion process, rather than from pastures of better quality, disrupt the association between quality traits of pastures with CH_4 emissions from grazing animals. As with CH_4 yield, the low explanation power of CH_4 intensity from herbage quality is arguable because not always animals grazing in higher quality pastures achieve greater performances (Cezimbra et al., 2021; Da Trindade et al., 2016; de Souza Filho et al., 2019). Moreover, the performance of grazing animals is highly variable, according to weather conditions. For example, steers ingesting 2% of their body weight from a good quality herbage in summer, can have different performance and resulting CH_4 emissions, compared to the same animal with similar diet quality and level of intake in winter, due to differential energy expenditure (NRC, 2016).

The final model for CH_4 emission presented a total coefficient of determination of 68% (Figure 5b), showing that other factors could be considered in the model to improve the variance of explanation. The level of intake is the variable most explaining CH_4 production (Beauchemin and McGinn, 2005; Hammond et al., 2013; Jonker et al., 2017; Kurihara et al., 1999) and could improve the explanation power of the model. While CH_4 production increases with intake, CH_4 yield (g CH_4/kg DMI) decreases (Hammond et al., 2013; Jonker et al., 2017). Yet, as the average daily gain of ruminants increases with intake, the CH_4 intensity (g CH_4/kg ADG) reduces (Hristov et al., 2013; Pacheco et al., 2014). The CH_4 emissions might have a nonlinear relation to DMI and ADG depending on the grazing environment (see in appendices Figure 6).

Low intake and poor diet nutritive components can cause an impact on higher values of CH_4 emissions especially in grazing systems. The diet quality relates negatively to CH_4 yield (Hegarty, 2009; Shibata and Terada, 2010). Nonetheless, such a relationship is weaker over some feeding conditions (i.e., forages with high nutritive value; Hammond et al., 2013; Jonker et al., 2017; Pinares-Patiño et al., 2003; Sun et al., 2011). As such, since the quality trait of forages has limited scope for CH_4 mitigation (Buddle et al., 2011; Pacheco et al., 2014), promoting higher intake is especially important abating the emission from ruminants.

Most CH_4 mitigation studies were developed for high-yielding animals fed indoors, which has already low CH_4 yield and intensity (Hristov et al., 2013). As such, most of these follow a nutritional-oriented rationale of mitigation (Beauchemin et al., 2008; Beauchemin et al., 2020), which consists of improving the diet quality (e.g., digestibility) as the way to increase voluntary intake and animal performance. However, in pastoral systems conditions this nutritional approach might not be as effective with fed animals, as sward structural characteristics (e.g., sward height, herbage mass) are hypothesized to be more important drivers of voluntary intake of grazing ruminants (Silva and Carvalho, 2005). In other words, the herbage quality as a trigger of the classical metabolic and physical constraints of intake (Allen, 1996; Forbes, 2001; Forbes and Barrio, 1992; Illius and Jessop, 1996) may operate conjunctly, but be hierarchically less important than the sward structure in explaining DMI, ADG and CH_4 emissions of grazing ruminants. This ecological perspective is supported by some studies in which animals grazing on pastures with higher quality (i.e., lower NDF, higher CP or digestibility) had lower herbage intake and the highest CH_4 yield and intensity in relation to pastures offering a sward structure (i.e., sward height or herbage allowance) facilitating the ingestion process of animals (Cezimbra et al., 2021; de Souza-Filho et al., 2020).

4.4. Other factors influencing animal forage intake, ADG and CH_4 emissions

In pastoral ecosystems, some factors influencing the response variables are difficult to control. Many of them are related to environmental conditions. Even though blocked of some factors, random effects such as trial, pasture type, animal species and paddock represented most of the total coefficient of determination to DMI, ADG and CH_4 models.

Even though in our study the live weight was already transformed to metabolic weight, the inclusion of animal species in the model was based on the unbalanced data between sheep and cattle. Animal species were related for many authors as a differential result on production. Cattle have greater DMI than sheep due to body size, greater nutritional requirements and large rumen capacity, even expressed in metabolic weight (Soto-Navarro et al., 2014). Although, the relation between DMI and metabolic weight becomes lower when the forage quality reduces (van Gastelen et al., 2019). Van Lingen et al. (2018) reported a decrease in enteric CH_4 (g/day and g/kg DMI) in response to an increase in the percentage of crude protein in DM in dairy cattle, highlighting that sheep supposedly not be included in this context because the intake is higher to maintain their requirements increasing the passage rate and lower digestibility (Cannas et al., 2019; van Gastelen et al., 2019). We stress that animal species should be considered in the model even though the transformation to metabolic weight was performed.

Another important factor affecting DMI, ADG and CH_4 models is the pasture type. Grasslands are classified according to physiology and morphology differing to C3 and C4 metabolism. Plants with C3 metabolism have greater nutrient content and according to photosynthetic pathways can survive in cool temperatures. Yet, plants with C4 metabolism are adapted to a warm climate with higher content of amino acids and organic acids in their root exudates and lower levels of photosynthetic enzymes (Archimède et al., 2011; Barbehenn et al., 2004; Sivaram et al., 2018). Fibers of C4 grass tend to be more lignified and more resistant to digestion altering the DMI (Wilson, 1994). Archimede et al. (2011) pointed out that C4 grasses presented around 15% greater CH_4 production than C3 grasses due to the increase of cell wall, ADF and lignin causing low digestibility of feed and higher energy loss, decreasing the animal production efficiency. They also mentioned a reduction in CH_4 production in animals fed warm legumes when compared to C4 plants, considering secondary metabolites the principal reduction. The same process has been mentioned in temperate regions despite global warming progress (Shibata and Terada, 2010).

4.5. Contribution of this study to grazing animal science

Under grazing conditions, animals adapt the diet and utilize forage according to the sward structure available to obtain their maintenance requirements. We consider that the southern Brazilian grasslands are not limiting in chemical composition, although this factor is important to ruminant nutrition the main driver in that environment is the quantity of forage, which means offering animals an optimal sward structure to favor high forage intake per unit of time (Carvalho et al., 2013; Schons et al., 2021). For instance, overgrazed native pastures present greater CP content and digestibility than well-managed pastures (da Trindade et al., 2016), but that greater forage nutritive value does not result in greater animal intake and performance (Cezimbra et al., 2021), which was also proven in our work.

Unfortunately, studies considering random variables with influence on DMI, ADG and CH_4 emissions are lacking until today. We encourage grazing animal scientists to go deeper into this topic to better understand which variables we can control to reduce the variance in the results.

5. Conclusions

Pondering upon grazing conditions, our findings highlight that the forage nutrient content, i.e. CP, NDF and ADF, present a low determination value to explain changes in DMI, ADG and CH_4 emission by beef cattle and sheep grazing different grasslands. Also, the explanatory power was similar or lower than sward structure variables, i.e. sward height and herbage mass, to explain changes in DMI, ADG and CH_4 emissions. So, though other variables involving paddock, trial, animal and pasture characteristics have strong explanatory power in our models, the main action to improve animal production and mitigate the environmental impact in pastoral ecosystems is to offer animals an optimal sward structure, and the nutrient content of the forage is just a consequence of that. Finally, considering the wide range of forage nutrient content presented in this dataset (e.g. CP varying from 9.9 to 26.5%) for grazing beef cattle and sheep, these nutritional variables are not capable of explaining changes in DMI, ADG and CH_4 emission, which means that as long as we offer an optimal sward structure, chemical composition of the forage is not a limiting factor.

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CHAPTER III

FINAL CONSIDERATIONS

Our findings demonstrate that before we understand that forage nutrient content is important to explain changes in DMI, ADG and CH_4 emissions, it is necessary to know that forage structure has a direct impact on these results, which occurs because the animal actions result in feed choices. The mind of forage nutrient content for feedlots should not be compared to grassland systems. In this dissertation, we emphasized the difference between those systems and explained the importance of the variables over them.

Some mistakes should be avoided for the next researchers. Database analysis is complex when the data come from different sources, ranging a variety of methodologies, measurement units and all data need to be adjusted and standardize to proceed with the analysis. We encourage researchers to organize sheets with the most information as possible, such as legends about the data to help the next person who will use the information. Also, including most information as possible even if the data will not be necessary at the first moment to process the analysis.

Certain effects were not completely acquaintances influencing the results of random variables. We agree that animal species should be included in the model even though the transformation to metabolic weight was performed. Unfortunately, studies about random variables influencing the response variables are lacking until today. We encourage scientists to go deeper into this topic to better understand which variables we can control to reduce the variation between the results.

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APPENDICES

Appendix 1. Figure 6 from Chapter 2**.**

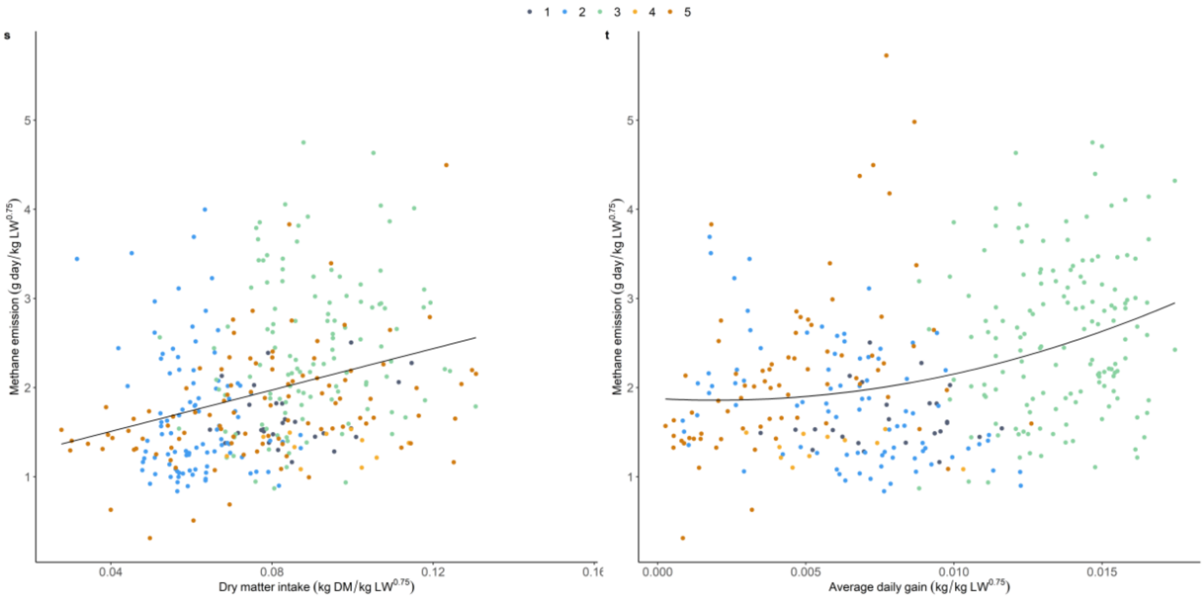

Figure 6. Relationship between CH_4 emission and DMI (a), and ADG (b) of beef cattle and sheep grazing different grasslands. Trial 1 and 2: Italian ryegrass grazed by sheep; Trial 3: mixed Italian ryegrass and black oat grazed by beef cattle; Trial 4: pearl millet grazed by sheep; Trial 5: natural grasslands grazed by beef cattle.

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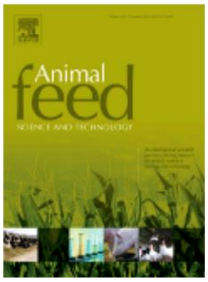

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a. aNDFom-NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

b. NDFom-NDF not assayed with a heat stable amylase and expressed exclusive of residual ash.

c. aNDF-NDF assayed with a heat stable amylase and expressed inclusive of residual

ash.
d. NDF-NDF assayed without a heat stable amylase and expressed inclusive of residual ash.
e. ADFom-ADF expressed exclusive of residual ash.
f. ADF-ADF expressed inclusive of residual ash.
g. Lignin (sa)-Lignin determined by solubilization of cellulose with sulphuric acid.
h. Lignin (pm)-Lignin determined by oxidation of lignin with permanganate.

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VITA

Laís Leal da Cunha, filha de Carlos Alberto de Piero da Cunha e Izair de Vasconcelos Leal, nascida em 12 de julho de 1995, em Canguçu/RS. Cursou o ensino fundamental na E.E.E.M. João de Deus Nunes em Canguçu/RS e o ensino médio e técnico em agropecuária no Instituto Federal do Rio Grande do Sul – Campus Visconde da Graça “CAVG”, na cidade de Pelotas/RS. Em 2013, ingressou no curso de Medicina Veterinária, na Universidade Federal de Pelotas (UFPEL), em Pelotas/RS. Durante os anos do curso desenvolveu atividades de pesquisa e extensão com projetos de desenvolvimento da bovinocultura leiteira (PDBL), controle parasitológico de Equinos e controle de agentes zoonóticos em praças públicas da região Sul do RS junto ao laboratório de doenças parasitárias (LADOPAR) e do grupo de pesquisa em enfermidades parasitárias (GEEP), sob orientação dos professores Diego Moscarelli Pinto, Felipe Geraldo Pappen e Tânia R. Bettin dos Santos. Além disso, atuou na clínica de ruminantes realizando estágio extracurricular no Hospital Clínico Veterinário (HCV-UFPEL). Conclui a graduação realizando o estágio final focado na área de reprodução em bovinos de leite na Universidade de Illinois, Urbana-Champaign, sob orientação do professor Fabio S. Lima. Formou-se em Medicina Veterinária em agosto de 2018 e em abril de 2019 ingressou no Mestrado em Produção Animal pelo Programa de Pós-Graduação em Zootecnia – UFRGS, sob orientação da professora Carolina Bremm. Durante os meses de agosto a dezembro de 2019, trabalhou como pesquisadora assistente na Universidade de Illinois sob orientação do professor Fábio S. Lima, com experimentos baseados em microbioma ruminal de bovinos leiteiros e reprodução de animais domésticos.