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

ANGEL SANCHEZ ZUBIETA

CAN GRAZING MANAGEMENT MITIGATE ENTERIC METHANE FROM PASTORAL ECOSYSTEMS, IMPROVE NUTRITIONAL-WELFARE INDICATORS OF SHEEP AND CREATE A LOW-METHANE *in vitro* RUMEN ENVIRONMENT?

Porto Alegre (RS), Brasil
2020

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

CAN GRAZING MANAGEMENT MITIGATE ENTERIC METHANE FROM PASTORAL ECOSYSTEMS, IMPROVE NUTRITIONAL-WELFARE INDICATORS OF SHEEP AND CREATE A LOW-METHANE *in vitro* RUMEN ENVIRONMENT?

ANGEL SANCHEZ ZUBIETA

Ing. Agrônomo / Instituto Tecnológico Agropecuário # 2
Mestre em Ciência Animal / Instituto Tecnológico de Conkal

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

Orientador: Paulo César de Faccio Carvalho

Porto Alegre (RS), Brasil
Fevereiro, 2020

CIP - Catalogação na Publicação

Sanchez Zubieta, Angel
Can grazing management mitigate enteric methane from pastoral ecosystems, improve nutritional-welfare indicators of sheep and create a low-methane in vitro rumen environment? / Angel Sanchez Zubieta. -- 2020.
237 f.
Orientador: Paulo Cesar de Faccio Carvalho.

Coorientador: Jérôme Bindelle.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, , Porto Alegre, BR-RS, 2020.

1. Grazing management. 2. Methane emission. 3. Sward surface height. 4. In vitro fermentation. 5. Biting behavior. I. de Faccio Carvalho, Paulo Cesar, orient. II. Bindelle, Jérôme, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Angel Sánchez Zubieta
Mestre em Engenharia Agronômica

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOUTOR EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 04.03.2020
Pela Banca Examinadora

Paulo César de Faccio Carvalho
PPG Zootecnia/UFRGS
Orientador

Homologado em: 13/04/2020
Por

DANILO PEDRO STREIT JR.
Coordenador do Programa de
Pós-Graduação em Zootecnia

Juan C. Ku

Juan Ku Vera
Universidad Autónoma de Yucatán - UADY

Anibal de Moraes
Universidade Federal do Paraná- UFPR

Nicolas Di Lorenzo
University of Florida- UFL

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

ACKNOWLEDGEMENTS

To **God**, for putting me in the right place, at the right time, with the right family and in a harmonious environment, allowing and facilitating this accomplishment.

To my mother, the most important person in my life, for looking after me and my siblings; this would have not been possible without her.

To my grandparents, siblings, uncles and entire family, for being there loving me and supporting my goals.

To the Grazing Ecology Research Group (GPEP), through its leader, Professor Paulo Carvalho, for providing opportunities to develop myself in this exciting area of knowledge and experiencing an amazing academic and partnership environment.

To Professor Ênio Prates, for first accepting me as his student.

To my post-graduation colleagues that embraced this research and assisted me at all time; special thanks for Jean, Aleja, Mariana, Anderson, Marcelo, Fernanda, Jussi, Will, Thales, Prof. Carol, Dani, Gugu, Alemao, Jojo; not less important are all the under graduation students from GPEP and outsiders; without them, public research is not possible. I will always be grateful.

To Professor Jerome Bindelle and technicians at Liege University-Agro-Bio Tech, Professor Veerle Fievez and technicians at Ghent University-LANUPRO, for guiding me to conduct part of this research, and Dra. Virginie Decruyenaere at WARC, for NIR analyses. To LacVet, for the blood analysis, especially to Laura Quispe, for her assistance and dedication.

To the staff of the Agronomic Experimental Station (EEA), for always providing me the means to conduct this research.

To CONACYT, for awarding me with the scholarship (171912), especially to the staff of Yucatan for the support provided.

To all the persons that somehow helped and oriented me academically or not, even way time before knowing I was enrolling in this journey; the present is the sum of the past experiences.

Can grazing management mitigate enteric CH_4 from pastoral ecosystems, improve nutritional-welfare indicators of sheep and create a low-CH_4 *in vitro* rumen environment? [1]

Author: Ángel Sánchez Zubieta
Advisor: Paulo César de Faccio Carvalho

Abstract: scientific literature shows that improper grazing management results in low intake of herbage with low nutritive value, low animal outputs and increased CH_4 emissions from pastoral ecosystems. This grazing conditions could cause nutritional imbalance and impair animal welfare (**Chapter I**). This thesis approaches two pillars of sustainable livestock production systems; enteric CH_4 mitigation and promotion of appropriate animal nourishment and welfare, through grazing management. The first trial (**Chapter II**) demonstrated the diversity of bites that animals perform to cope with contrasting sward structures and compound their diet, even in homogeneous *Lolium multiflorum* pastures. It was proved that a grazing management offering animals the opportunity to maximize the short-term intake rate at any time while grazing (Rotatinuous stocking; RN), results in preferential leaf lamina biting behavior, thus in a diet with 14, 12 and 13% higher CP, total soluble sugars and crude fat, respectively, and 24 and 40% lower ADF and ADL, contrary to the diet of animals that are forced to deplete most of the herbage in offer (a traditional rotational stocking; RT). As well, the higher intake (+18%) of a diet with higher nutritive value boosted the daily intake of soluble nutrients (+25%), with this directly affecting animals' blood composition; 17.5, 18 and 6.1% higher glucose, plasma urea nitrogen and albumin, respectively, and 19% lower neutrophil-to-lymphocyte ratio, this latter an indicator of a lower stressful response. In a second trial (**Chapter III**) it was demonstrated that the fermentation of carbohydrates of the RN diet does not result in a low-CH_4 *in vitro* rumen environment, but that the production of N-NH_3, valeric acid and BCFA, from the CP fermentation are increased in 13, 17 and 23% respectively. In **Chapter IV**, through a regression analysis with data from the Grazing Ecology Research Group (GPEP) and published literature, it is suggested that when grazing animals reach around 43 to 57% of the growth usually observed in feedlot, this is, around 0.7 and 0.14 kg LW gain for cattle and sheep, respectively, the CH_4 emission intensity of pastoral ecosystem is as low as from animals of more intense feding systems; around 0.2 kg CH_4/kg LW gain. It is also suggested that for this to happen, sound grazing managements need to be adopted, and that those considering animal behavioral responses (short-term intake rate), as a function of sward structural attributes (e.g. sward height), have proven to be efficient in mitigating emissions, as they promote high levels of intake on individual animal basis. This thesis provide evidences that pasture-based systems can reduce their enteric CH_4 emission intensity to levels of intense-fed animals and boost some nutritional and welfare indicators of animals, but not create a low-CH_4 *in vitro* rumen environment. Further research opportunities are provided (**Chapter V**).

Keywords: CH_4 mitigation; grazing management; sward height; grazing behavior, animal welfare.

---

[1] PhD Thesis in Animal Science, Agronomy Faculty, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. (237 p.) February, 2020.

Pode o manejo do pastoreio mitigar as emissões de CH_4 entérico nos ecossistemas pastoris, melhorar indicadores relacionados à nutrição-estres de ovinos e criar um ambiente ruminal *in vitro* de baixa emissão de metano? [1]

Autor: Ángel Sánchez Zubieta
Supervisor: Paulo César de Faccio Carvalho

RESUMO: a literatura científica mostra que o manejo inadequado do pastoreio resulta em baixo consumo de pasto com baixo valor nutritivo, reduz o desempenho animal e incrementa as emissões de CH_4 dos ecossistemas pastoris. Essas condições de pastejo podem causar imbalancos nutricionais e comprometer o bem-estar animal (**Capítulo I**). Essa tese aborda dois pilares da produção animal sustentável; mitigação do CH_4 entérico e promoção de uma adequada nutrição e bem-estar animal, através do manejo do pastoreio. O primeiro experimento (**Capítulo II**) demonstra a diversidade de bocados que os animais fazem para lidar com estruturas de pasto contrastantes e compor a sua dieta, mesmo em pastagens homogêneas de Azevém anual. Foi provado que um manejo que oferece pros animais a oportunidade de maximizar a taxa de ingestão a qualquer momento durante o pastoreio (pastoreio Rotatínuo; RN), acarreta em consumo preferencial de lâmina foliar, resultando em uma dieta com 14, 12 e 13% maior teor de PC, carboidratos solúveis totais e lipídios totais, respectivamente, e 24 e 40% menor teor de FDA e LAD, contrário à dieta de animais que são forçados a rebaixar a maior parte da forragem ofertada (pastoreio rotativo tradicional; RT). De igual forma, o maior consumo (+18%) de uma dieta com maior valor nutritivo impulsionou o consumo diário de nutrientes solúveis (+25%), sendo isso diretamente relacionado à composição do sangue; 17.5, 18 e 6.1% maior glicose, nitrogênio ureico no plasma e albumina, respectivamente, e 19% menor relação neutrófilo-linfócito, sendo esse último um indicador de uma menor resposta estressante. No segundo experimento (**Capítulo III**) foi demonstrado que a fermentação dos carboidratos da dieta RN não criou um ambiente ruminal de baixo CH_4, mas que a produção de N-NH_3, ácido valérico e ácidos graxos de cadeia ramificada são incrementados em 13, 17 e 23%, respectivamente. No **Capítulo IV**, através de uma análise de regressão com data do GPEP e literatura publicada, é sugerido que quando os animais em pastejo atingem ao redor de 43 a 57% do crescimento observado em fedelhos, entorno de 0.7 e 0.14 kg/GMD para bovino e ovino, respectivamente, a intensidade de emissão de CH_4 é tão baixo quando as observadas nos animais em sistemas de alimentação mais intensivos; entorno de 0.2 kg CH_4/kg GMD. Sugere-se que para isso acontecer, bom manejo do pastoreio precisa ser adotado, e que considerar respostas comportamentais dos animais (e.g. taxa de ingestão), em função da estrutura do pasto (e.g. altura), tem provado eficiência na mitigação de CH_4, por promoverem altos níveis de consumo por animal. Essa tese provê evidencias de que os sistemas pastoris podem reduzir as emissões de CH_4 entérico ao mesmo nível de animais confinados e melhorar alguns indicadores relacionados ao status nutricional e estrese dos animais, mas não criar um ambiente ruminal *in vitro* de baixo CH_4. Futuras oportunidades de pesquisa são provisionadas (**Capítulo V**).

Palavras chave: mitigação de metano, manejo do pastoreio, altura do pasto, comportamento ingestivo, bem-estar animal.

[1] Tese de Doutorado em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (237 p) Fevereiro, 2020.

CONTENT

LIST OF TABLES

CHAPTER II

CHAPTER II

CHAPTER III

CHAPTER IV

LIST OF FIGURES

CHAPTER I

Pag.

CHAPTER II

CHAPTER III

CHAPTER IV

ABBREVIATIONS LIST

Symbol	Description	Symbol	Description
GHG	Greenhouse gases	PUN	Plasma urea nitrogen
CO_2-eq	Carbon dioxide equivalent	CP	Crude protein
CH_4	Methane	NDF	Neutral detergent fiber
TMR	Total mixed ration	ADF	Acid detergent fiber
LW	Live weight	ADL	Acid detergent lignin
EI	Emission intensity	TSS	Total soluble sugars
SSH	Sward surface height	OMD	Organic matter digestibility
RN	Rotatinuous	CF	Crude fat
RT	Rotational	FA	Fatty acid
STIR	Short-term intake rate	SCFA	Short-chain fatty acid
OM	Organic matter	BCFA	Branched-chain fatty acid
ME	Metabolizable energy	FOM	Fermentable organic matter
DM	Dry matter	$N\text{-}NH_3$	Ammonia nitrogen
NEFA	Non-esterified fatty acids	H_2	Hydrogen
BHB	Beta-hydroxybutyrate	C_2	Acetate
N:L	Neutrophil: lymphocyte	C_3	Propionate
OM	Organic matter	C_4	Butyrate

1. CHAPTER I

1.1. GENERAL INTRODUCTION

Livestock is signaled as an important driver of environment and natural resources depletion. Deforestation and biodiversity loss, water use and soil degradation, and nutrients overload and pollution through wastes explain this phenomenon (ROCKSTRÖM et al., 2009). Nonetheless, probably the most controvertible concern was published by FAO in the "**Livestock's long shadow: *environmental issues and options***" report (STEINFELD et al., 2006). On it, this sector is signaled as the main driver of greenhouse gases (GHG) increment in atmosphere, thus to global warming. In an updated report (GERBER et al., 2013), FAO attributed to livestock 18.5 % of anthropogenic GHG, with the methane (CH_4) contributing to most of its emissions budget; 44 %, or according to HERRERO et al., (2013), 65% of non-CO_2 emissions. The CH_4 has a Global Warming Potential (GWP) 28 times higher than CO_2, (IPCC, 2014). Moreover, it has an energy content of 55.22 MJ/kg, thus its emissions by ruminants represent energetic inefficiencies (2 to 12% of gross energy; BLAXTER; CLAPPERTON, 1965; JOHNSON; JOHNSON, 1995). For these reasons, international agreements (The Kyoto Protocol, The Paris Agreement), urge nations to reduce global GHG emission (GAO; GAO; ZHANG, 2017). For doing so, livestock systems have targeted to mitigate enteric CH_4 (MARTIN; MORGAVI; DOREAU, 2010), with special attention put on emissions coming from pastoral ecosystems (BERNDT; TOMKINS, 2013).

Inefficient pasture-based livestock systems have high impact on environment. These have higher emission intensity (EI; kg CH_4 per kilogram of animal output) than intense animal operations (GERBER et al., 2013; HERRERO et al., 2013; OENEMA; DE KLEIN; ALFARO, 2014; RAO et al., 2015). This results from low levels of forage intake and poor forage nutritive value, thus, from depressed animal outputs, all factors associated to excessive grazing intensity (HERRERO et al., 2013; REN et al., 2016). Since grasslands play a vital role in global food supply, this raises concerns, as human population is expected to increase of 9.6 billion in 2050, and with it, the demand for food (ALEXANDRATOS; BRUINSMA, 2012). Furthermore, society demands for high quality products, free of chemicals (BICKELL et al., 2010; DURMIC; BLACHE, 2012), and animal welfare (LI, 2009; LLONCH et al., 2017), must be attended. Evidence showing the narrow range that crops currently have to increase yields (GRASSINI; ESKRIDGE; CASSMAN, 2013; RAY et al., 2012) or of the negative impact that forcing animals from intense-industrial operations to attain superior production levels have on animal welfare and health (LI, 2009; LLONCH et al., 2017), or the debate about livestock use of human edible resources (AIKING, 2014; GARNETT et al., 2017; WAHLQUIST, 2013), raise the question of where might further yield increases will come from. In this regard, provided the scientific literature highlighting the ecosystems services (BELLAVER; BELLAVER, 1999; BOVAL; DIXON, 2012; RUSSELL; BISINGER, 2015) and high animal outputs (SOUZA FILHO et al., 2019; MCCAUGHEY; WITTENBERG; CORRIGAN, 1997) that well-managed grasslands can provide while ensuring food supply, it is reasonable to see pastoral ecosystems as the alternative of attending, conjunctly, all the criteria of future food production.

As enteric CH_4 is the most emitted GHG by pasture-based systems, it’s mitigation on grazing ruminants is necessary. Surprisingly, most of CH_4 mitigation options are nutritional-oriented and were developed for high-yielding animals (indoor-fed), with already low CH_4 EI (HRISTOV et al., 2013). Their applicability in grazing systems, a condition over of which most of high-CH_4 yielding animals are reared, is low, so is their potential to impact global livestock’s emission (BEAUCHEMIN et al., 2008, 2020; CLARK, 2013; PACHECO; WAGHORN; JANSSEN, 2014; RAMÍREZ-RESTREPO et al., 2010). Since most of world’s ruminants graze under sub-optimal feeding conditions, adopting sound grazing managements that optimize their productivity per unit of feed intake, is most important for lowering world’s enteric CH_4 emissions (STEINFELD et al., 2006). This is supported by the increasing evidence showing equivalent or even lower EI from grazing ruminants respect intense-fed animals, when improved pasture management are adopted (AMARAL et al., 2016; ANDRADE et al., 2016; CEZIMBRA et al. Unpublished; CONGIO et al., 2018; DA SILVEIRA PONTES et al., 2018; SOUZA FILHO et al., 2019; DINI et al., 2018; MCCAUGHEY; WITTENBERG; CORRIGAN, 1997; SAVIAN et al., 2014, 2018). From other evidence, it is noticeably that in grazing conditions, high nutritive pastures not always drive the lower CH_4 EI, when DM intake is restricted. Thereby, it is suggested the preponderant role that the pasture structure has for both allowing high DM intake and overall abatement of CH_4 emissions (CEZIMBRA et al. Unpublished; SOUZA FILHO et al., 2019; SAVIAN et al., 2018). These latter reports also show a trade-off between individual DM intake and growth rate, with CH_4 emission per unit of animal output and per hectare.

Under this premise, pastures management targets oriented to maximize individual animal intake are valuable mitigating strategies. CARVALHO (2013) proposed a grazing management concept that offers the best pasture structure (pre- and post-grazing sward surface height; SSH) that allow animals to optimize the nutrients intake per unit of grazing time, thus also to attain maximum daily intake per animal. When compared to a traditional rotational stocking, this innovation in grazing science substantially reduced enteric CH_4 emission from lambs grazing annual ryegrass pastures (SAVIAN et al., 2018). The main driver of such mitigation might be related to the higher daily OM intake. Nonetheless, rumen fermentation pathways might also operate in this direction. Clearly, this management sets in 40% the limit of depletion of the pre-grazing SSH maximizing the intake rate (CARVALHO, 2013). This way, animals are not forced to explore lower parts of the canopy, higher in less preferable steams (AMARAL et al., 2013; BRUNETTI et al., 2016; DIAS et al., 2017; EUCLIDES et al., 2018; ZANINI et al. 2012). The 13 % less CH_4 per kg of digestible OM intake observed in SAVIAN et al. (2018), might have resulted from the occurrence of a low-CH_4 rumen environment (LENG, 2018), provided the preferential leaf lamina harvesting and higher nutritive value of the herbage consumed. This hypothesis, however, was not supported in Savian’s et al. study, since no shifts if rumen fermentation variables were reported. This latter might have resulted from the rumen sampling protocol adopted in that experiment and a limited number of animals sampled given methodological and technical constrains of extracting rumen liquid from non-fistulated animals. Thereby, *in vitro* ruminal studies would assist in determining whether or not such mitigation results from the creation of a low-CH_4 ruminal environment directly from grazing. Understanding how mitigation works for grazing animals is valuable for developing other potential strategies from grass-based systems.

Another dimension of sustainability goes around “ethics” in livestock systems. Grass-origin animal products are recognized as animal-friendly (LOBATO et al., 2014; PROVENZA; KRONBERG; GREGORINI, 2019). Indeed, access to pasture is regarded as a welfare enhancer (e.g. for indoor-fed dairy cows). Nevertheless, grazing managements targeting per hectare productivity and farm profit (FARIÑA; CHILIBROSTE, 2019), limits individual daily intake in 10 % (PEYRAUD; DELAGARDE, 2013), or arguably more, when harvest efficiency reaches 93 % (DOVE, 2010) and no supplementation is used (BARGO et al., 2003). This in turn, might drive shifts in blood constituents associated with nutritional status and stressful responses (DHABHAR et al., 1996; INGVARTSEN; MOYES, 2013; SORDILLO, 2016), even when access to pasture is allowed (COSTA et al., 2015; REN et al., 2016). Overall, certain fuels from carbohydrates and protein metabolism, or hematological profile, can be useful in monitoring the nutritional status and immune responses, as function of feeding systems (SORDILLO, 2016).

This thesis approaches two pillars of sustainable pasture-based livestock systems; mitigation of enteric CH_4 measurement and promotion of appropriate nourishment and welfare through grazing management. The first trial assessed the nutrient intake and nutritional status of animals grazing *Lolium multiflorum* pastures submitted to different grazing management strategies in a rotational stocking. The second scanned the *in vitro* rumen fermentation profile of the herbage apparently consumed by sheep (characterized at the bite scale) in the previous grazing trial. Finally, through a review of literature and a regression analysis, evidence of the mitigation potential that grazing managements have to mitigate CH_4 emission from pastoral ecosystems is provided. This thesis adds to the scientific literature pointing out pastures-based livestock systems as promoters of environment preservation and animal nutrition and welfare.

1.2. LITERATURE REVIEW

1.2.1. Agriculture and global environmental changes: the controversy

Agriculture is a main driver of global environmental changes. From 3.7 billion people in 1961, human population reached 6.9 billion in 2010, and could reach 9.15 in 2050 (ALEXANDRATOS; BRUINSMA, 2012). The exponential growth in food demand accelerated humans' footprint on earth. Over the last 300 years, croplands and pasturelands expanded 5-fold from around three to 15, and from five to 27 million km^2, respectively, with most of land clearing occurring from 1850 to 1950, before the 'Green Revolution' (RAMANKUTTY et al., 2018). Since 1960s, crop yields abruptly increased by the intense use of high-yielding crops (more than 8 thousand varieties for 11 major crops), fertilizers (4-fold increase), agrochemicals, and fossil fuel for soil mechanization and irrigation; globally, *per capita* cereal production increased from 0.29 to 0.39 tons between 1961 and 2014 (RAMANKUTTY et al., 2018, with data from FAO). In 1935, maize yield remained around 1.7 and 2.0 tons/ha in United States and United Kingdom, respectively; since then, yields increased to around 10 and 8 tons/ha, respectively (US Department of Agriculture, 2017). Yet in some regions of Asia, and Latin America and Caribbean the *per capita* food production doubled between 1961 and 2001 (MCARTHUR; MCCORD, 2017).

The Green Revolution also facilitated the expansion of livestock. From 1961 and 2014, the population of cattle and buffaloes, and sheep and goats increased by 62 and 64 %, respectively (RAMANKUTTY et al., 2018) and production increased three-fold (JANZEN, 2011). Productivity gains were significant. For example, the United Stated produce 60 % more milk with 80 % fewer cows than in 1940s (CAPPER; CADY; BAUMAN, 2009). BUTLER (1998) reported that from 1951 to 1995 milk production per cow in the United States increased from around 4,500 to 9,000 liters/year, and INGVARTSEN; MOYES (2013) pointed out that the increase in Denmark went from 6,693 to 8,983 kg of milk per cow/year in 20 years, which represent an annual growth rate of 1.5 % or of 50 % increase in 27 years. Livestock in developing countries also experienced productivity gains. For example, in Brazil, between 1950 and 2006, pasture area passed from 107.6 to158.7

million ha, this is a 1.47 fold increase, while cattle herd had a 3-fold increase, going from 46.8 to 171.6 million heads; productivity gains explained 79% of the 6-fold increase in beef production (MARTHA; ALVES; CONTINI, 2012). Between 2000 and 2010, animal efficiency was also improved; weaning rate went from 57 to 68%, the weaning weight from 160 to 190 kg, the age at first mating from 36 to 30 months, and the slaughter age at 33 months (MCMANUS et al., 2016). Such improvements occurred on farms with intensive forage production, strategic supplementation, health and genetic programs, and operational organization (LOBATO et al., 2014). In Uruguay, from 1985 to 2016, the area allocated to dairy production decreased in 36 %, but milk production increased 3.5 times, explained by higher stocking rate and per cow production (FARIÑA; CHILIBROSTE, 2019).

The food system applied during seven decades succeeded in supplying more than enough food for humans (GODFRAY et al., 2010b). The average available calories per person increased from 2.19 in 1961 to 2.88 Megacal/day in 2013 (RAMANKUTTY et al., 2018). Nevertheless, it failed in warranting food availability, access, utilization and stability (GODFRAY et al., 2010b; PINGALI, 2012); around 2.3 billion people live with under 2,500 kcal, and some 0.5 billion with less than 2,000 kcal, while at the other extreme some 1.9 billion consume more than 3,000 kcal (ALEXANDRATOS; BRUINSMA, 2012). Apart from this, it is especially worryingly that such intense agriculture triggered global environmental changes (PINGALI, 2012), now threatening the future of agriculture and the safe operating space for humanity. The novel study of ROCKSTRÖM et al. (2009), defined the status of some planetary systems (environmental issues) and proposed their operating boundaries, which, if crossed, could generate changes at biosphere level that might compromise human development (Table 1). Authors show that three boundaries have been exceeded, with others near the boundary level. Afterwards, AIKING (2014), stated that food production is an important force underlying such planetary systems, and that livestock production is the linking factor.

Table 1. Ranking of environmental impacts according to the transgression of planetary boundaries.

Rank	Environmental impact	Current Status*
1	**Rate of biodiversity loss**	**>10**
2	**Nitrogen cycle disruption**	**3.45**
3	**Climate change (carbon cycle disruption)**	**1.1-1.5**
4	Phosphate cycle disruption	0.77-0.86
5	Ocean acidification	0.81
6	Land-use change	0.78
7	Freshwater use	0.65
8	Stratospheric ozone depletion	0.50

*Value ≥1 means boundaries already transgressed. Source: Aiking (2014), with data adapted from Rockström et al., 2009).

Diverse phenomena can explain livestock's contribution to environment deterioration and resources depletion. Currently, food production utilizes 26 to 33% of all ice-free land, 70% of fresh water, and 20% of world's produced energy (STEINFELD et al., 2006). Moreover, deforestation (FEARNSIDE, 2005; MARTINELLI et al., 2010), soil degradation (DÍAS-FILHO, 2014), biodiversity loss (BELLAVER; BELLAVER, 1999; MARTINELLI et al., 2010), and nutrient overload and pollution through wastes (GERBER et al., 2013; TAMMINGA, 1996), are also effects of some livestock systems, especially industrialized. Grazing ruminants can also be harmful to environment. This occurs when forage production decouples from animal population density (CARVALHO; BATELLO, 2009). Under this circumstance, herbivory can have serious impacts on productivity, composition and grasslands functioning. Overgrazing reduces soil fertility (physicochemical and microbiological properties; VARGAS et al. (2015), promotes erosion, compaction and desertification (SZOTT et al., 2000). Moreover, since grasslands are the habitat of a great number of soil, terrestrial and aerial species (BOND; PARR, 2010, BILENCA; BIÑARRO, 2004), when degraded, biodiversity loss is a natural consequence (ANDRADE et al., 2015). Methane emission increases in degraded pastures (BERNDT et al., 2014; CEZIMBRA et al., Unpublished; TANG et al., 2019) and soil carbon sequestration reduces (ALLARD et al., 2007; O'MARA, 2012). It also alters the quantity and quality of dry matter consumed (TRINDADE et al., 2012), affecting both primary and secondary production (HODGSON, 1990; ILLIUS; JESSOP, 1996). In the long term, livestock production under these conditions represents the main threat to the sustainability of the whole system (SZOTT et al., 2000).

1.2.2. Food production in the past and trends in future demands

As during the previous century, the food system will have to cope with a crowded and wealthier population. There is a general agreement in literature about the need of doubling food production by 2050 (baseline of 2005). Others, however, consider the production growth experienced from 2005 to 2014, and suggest a need of increase of only 25 to 75 % (RAMANKUTTY et al., 2018). The demand for animal products will be higher than of cereals (+21.5 vs +15.9%; OECD-FAO, 2011). Just in the next decade, *per capita* meat consumption will pass from 32.6 to 35.4 kg; beef will increase by 14% and milk in 22% (OECD-FAO, 2011). This scenario will push an increase in livestock number. For instance, ROSEGRANT et al. (2009) indicate that between 2000 and 2050, the global cattle, and sheep and goat populations will pass from 1.5 to 2.6 and from 1.7 to 2.7 billion, respectively. Interestingly, both higher livestock number and demand for their products will occur at higher rates in countries where livestock represent the main livelihood of rural people (i.e. Asia, South America and Africa), which are also the most vulnerable to climate change (Figure 1; GODBER; WALL, 2014). Projections of future food demands are uncertain though, as the social awareness about health problems associated with meat, or policies promoting less meat in diet, might change future dietary patterns, thus demand of some commodities (RAMANKUTTY et al., 2018).

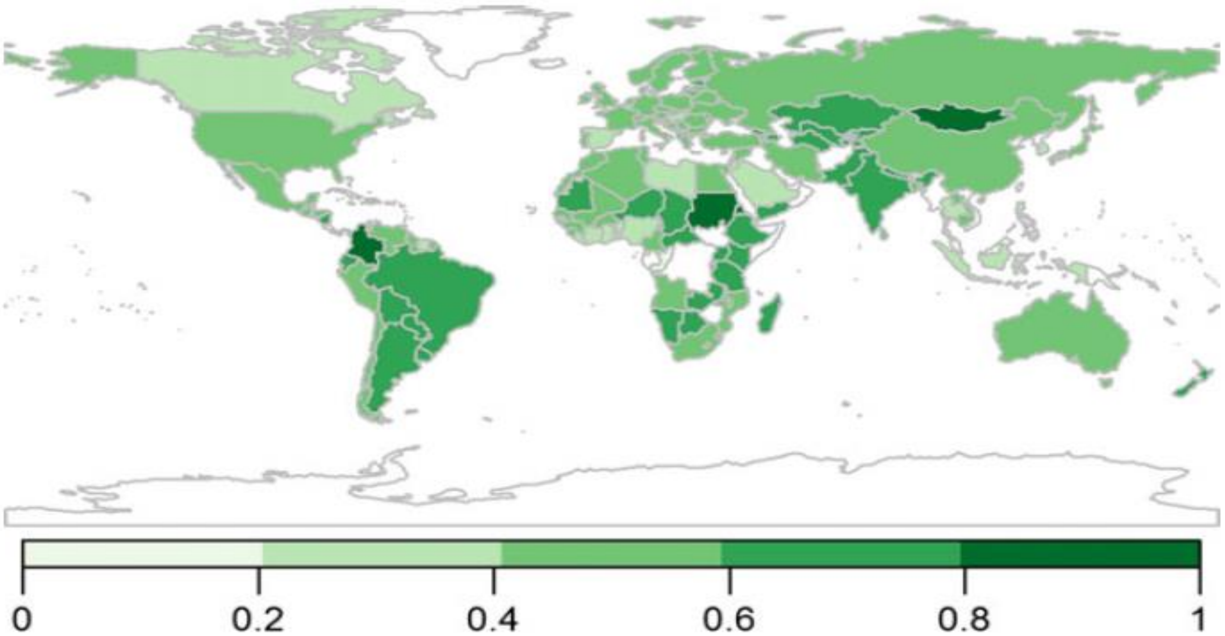


Figure 1. Nutritional reliance on home-produced grazing animals-based food products and level of security; 0 to 1, low to high sensitivity. Nations not included in the analysis are in white. From GODBER; WALL (2014).

1.2.3. Where are the greater opportunities of producing food in a global change scenario and social scrutiny?

Different from the Green Revolution, sustainable intensification and agroecology are key pathways to reduce nation’s uncertainties to global environmental changes and ensure food security (DEFRIES; ROSENZWEIG, 2010; DUMONT; GROOT; TICHIT, 2018; GODFRAY et al., 2010). This implies, not only producing food, but also warranting environmental preservation through less deforestation, soil and water preservation, reestablishment of biodiversity and less GHG emission; a dilemma with which modern agriculture has to cope (GERBER et al., 2013; JANZEN, 2011; MCARTHUR; MCCORD, 2017). To these demands, others, such as providing high quality products (BICKELL et al., 2010; DURMIC; BLACHE, 2012) and animal welfare must be attended (LI, 2009; LLONCH et al., 2017).

Thereby, the scenario is challenging. By 2050, the food system must be able to supply twice the food to feed 9.15 people in the practically the same, or even less area it uses today to produce food to feed 7.6 billion, but under a climate change scenario (GODFRAY et al., 2010a; MCARTHUR; MCCORD, 2017) and society scrutiny (JANZEN, 2011). Some questions arise from this. Where might further yield increase will come from? ¿Are croplands or livestock systems near of their productivity threshold? To address this, it is necessary referring to some evidence. According to DEFRIES; ROSENZWEIG (2010), 80 % of food production increases must come from yield increases. In this regard, (GODFRAY et al., 2010) reviewed literature and observed that for most crops there still a yield gap and, in agreement with RAMANKUTTY et al. (2018), highlighted the importance on plant biologists in developing superior crop varieties for specific growing conditions. Nevertheless, while increasing average crop field yields is possible (13 ton/ha of soybean) in practice it seems difficult to achieve. As an example, just during the past decades yield increases for key crops started a plateau in many regions of the world (GRASSINI; ESKRIDGE; CASSMAN, 2013), in part, due to the experienced degradation of agricultural resources (PINGALI, 2012). Accordingly, RAY et al. (2012) showed that between 1961 and 2008, 24 to 39 % of croplands yields either failed to improve, stagnated after initial gains or collapsed.

In the past, the lack of yield increase was tackled by incorporating land into agriculture. Today, despite possible in some regions, this is not desirable (GODFRAY et al., 2010). For example, CAMPBELL et al. (2008) estimate an area of around 385 to 472 million hectares of abandoned agriculture globally. Such lands are valuable for carbon stock and biodiversity preservation, so it is important to keep them away from agriculture (DUMONT; GROOT; TICHIT, 2018; GODFRAY et al., 2010; RAMANKUTTY et al., 2018). Another concern is about how much cropland yield increments can preserve the environment and natural resources. High-yielding crops were developed to fit most suitable agricultural lands, with high dependency on inputs (i.e. nitrogen, LEMAIRE et al. 2014), thus it is reasonable to think that future crops will mimic this trend. Therefore, even when crops yield increase are possible, the sustainable intensification framework addresses the reduction in harmful effects, but pays little attention on their ability to produce ecosystem services (DUMONT; GROOT; TICHIT, 2018); the same can be inferred for intense livestock systems.

The demand for animal products will be higher than from crops; thus, livestock systems must experience higher yields gains and better adapt towards sustainability (JANZEN, 2011). As with crops, animal breeding represented the main pathway to yield increases in the past, and its participation under the current scenario will be highly valuable (RAO et al., 2015). Breeding programs, mainly targeting high-yielding animals, offer permanent medium- to long-term benefits (WALL; SIMM; MORAN, 2010). In the short-term, feed additives in industrial animal operations promote growth and health, and mitigate enteric methane emissions by ruminants (HRISTOV et al., 2013; KNAPP et al., 2014; KUMAR et al., 2014). However, productive, environmental and human health issues, have converged to promote the concept of “clean, green and ethic” animal production (BICKELL et al., 2010), which urge livestock to move towards systems that involve limited use of drugs, chemicals and hormones, at the same time that stimulate environment conservation and animal welfare (DURMIC; BLACHE, 2012). Nowadays, due to the suspected threat they might represent to human’s health, livestock production is now faced without antibiotics in some countries (EUROPEIAN UNION, 2003; HART et al., 2008), and it is expected that many others will also legislate against non-natural growth promoters and CH_4 mitigating agents (MARTIN; MORGAVI; DOREAU, 2010). These considerations add additional challenges for intense livestock operations (LI, 2009).

Other concern of actual animal production systems is how much they promote animal welfare. For instance, high-yielding animals are more prone to have depressed their health (HUBER, 2018; LI, 2009) and reproductive performance (BUTLER, 1998). Yet, forcing higher production levels on these animals could further compromise their welfare (LLONCH et al., 2017) and other fitness traits (WALL; SIMM; MORAN, 2010). For instance, INGVARTSEN; MOYES (2013) suggest that mastitis has a clear relationship between milk yield and risk of infection and that genetic selection for milk yield will also probably increase the incidence risk of ketosis and lameness. LI (2009) describe how production intensification in China has created a welfare crisis affecting the world’s biggest number of farm animals. Moreover, the author highlighted the sanitary crisis derived from industrial livestock operations that affect human’s health. Thus, expanding industrial livestock systems or pushing high-yielding animals to increase their production level is not desirable in terms of animal welfare.

Moreover, it is also claimed that livestock competes with humans for nutrients by its inefficient conversion of crop into protein (AIKING, 2014; GARNETT et al., 2017;

WAHLQUIST, 2013). Livestock uses ⅓ of global cereal production (HERRERO et al., 2013). For reducing such competition, some advocate shifting dietary habits towards less consumption of ruminant products and increase that of monogastrics, provided their higher feed efficiency. However, it was showed recently that 86% of the dry matter ingested by ruminants, including crops, is made of material actually not eaten by humans (MOTTET et al., 2017), and that producing 1 kg of boneless meat from ruminants uses 2.8 kg of human edible products, compared with 3.2 kg for monogastric systems (MOTTET et al., 2017). Therefore, increasing intake pasture-based ruminants could alleviate the feed vs. food debate.

Summarizing, the productivity gap of specialized-intense crop systems is possible but difficult to attain, the margin of expanding agricultural land is small and environmentally incorrect. Within livestock systems, intensifying the production of already high-yielding animals is prone to impair animal welfare, the harmlessness of products and human health, and increasing the consumption of grain-demanding livestock could worsen competition for human-edible food resources. To these, other environmental restrictions linked to specialized-intense systems arise. When put together, it is evident the narrow role that industrial crops and livestock systems will have in increasing yields to cover the gap of future food demand while attending sustainable development concepts. It is thus obligatory to look at grasslands, especially under integrated crop-livestock operations, as the alternative for providing food and nourishment, while reducing environmental impact, improving animal welfare and reducing competition for human-edible resources.

1.2.4. Grasslands for better matching food production with ecosystem services and social demands

There is an increasing interest by some sector of society, welfare and environmental non-governmental organizations, and research groups in reducing livestock footprint (HERRERO et al., 2011; MCARTHUR; MCCORD, 2017; THORNTON, 2010). Among alternatives proposed are reducing the livestock number or intake of animal protein (AIKING, 2014; GARNETT, 2011; GARNETT et al., 2017; HARTMANN; SIEGRIST, 2017;

MCARTHUR; MCCORD, 2017), substitute animal meat for vegetal protein, laboratory-made meat or alternative food such as insects. However, promoting a shift of dietary patterns towards less consumption of animal products is neither realistic nor desirable, at least for a large sector of society. Here are some considerations. Grasslands of the world extend through 50 million km^2 or 37% of earth surface, especially in least- and sub-developed countries (REID et al., 2004), where most of domestic ruminants locates. Globally, extensive pastoral provide about 7% of beef, 12% of sheep meat and 5% of milk world's production; mixed crop-livestock provide about 20 % of beef and 30% of each of sheep meat and milk; intensive grazing systems provide only about 7, 12 and 5 % of the same commodities (ALEXANDRATOS; BRUINSMA, 2009). Moreover, animal products are important for nourishment, contributing with 17% of calories and 33 % of global protein consumption (HERRERO; THORNTON, 2009).

Economically, almost 75 % of rural poor people depends on livestock for subsistence; it employs at least 1.3 billion people and directly support the livelihoods of 800 million poor smallholder in the developing world (HERRERO; THORNTON, 2009) and 200 more million in more marginal and semi-arid areas (BOVAL; DIXON, 2012). Moreover, grasslands improve the standard of living by providing animal products for medical and others uses (BELLAVER; BELLAVER, 1999; JANZEN, 2011). They also offer services like transport, animal power, companion, sport and opportunities for tourism (BOVAL; DIXON, 2012; DUMONT; GROOT; TICHIT, 2018). Thus, social, economic and cultural development, mainly in rural communities of developing countries, have been historically linked to livestock and grasslands and will continue to be, since access to food, job, health, education and others assets, depends in part on it (BOVAL; DIXON, 2012; JANZEN, 2011; THORNTON, 2010).

When properly managed, grasslands serve as environmental regulators (LEMAIRE et al., 2011; BOVAL; DIXON, 2012; PROVENZA; KRONBERG; GREGORINI, 2019). Among ecosystem services obtained are meliorating soil fertility, water retention, enhancing biodiversity (RUSSELL; BISINGER, 2015). When managed upon integrated crops-livestock systems plus non-tilling, these benefits are enhanced (FISHER; TOZER; ABRECHT, 2012) without affecting crop yields (PETERSON et al., 2019). Yet, integrated systems "duplicate" the agricultural area by producing both cereal and animal protein in different space-temporal arrangements (LEMAIRE et al., 2014). HERRERO et al. (2016) mentioned that carbon sequestration through improved grazing management has the greatest potential to mitigate livestock's GHG emissions (Figure 2). Through modelling, (SILVA et al., 2016) estimated that efficient pasture-based beef systems are associated with lower emission intensities and total emissions when production is decoupled from deforestation and suggested that restoring degraded pastures is the largest opportunity of Brazilian mitigation plans. TORRES et al. (2017) estimated GHG emissions from agroforestry to range between 2.81 to 7.98 t CO_2eq ha^{-1}, and calculated that the number of tress per hectare necessary to offset emissions is of 17 to 44, provided the large amounts of carbon storage in above-ground mass of trees and grass. These data suggest the mitigation potential of grazing management is commonly attributed to carbon sequestration (the off-set emissions pathway), but little attention is put over its potential to reduce CH_4 emissions per animal output (the mitigation pathway).

Healthy pastoral ecosystems are the habitat of a great number of soil, wild terrestrial and aerial species (BILENCA; BIÑARRO, 2004; SUTTIE et al., 2005; BOND; PARR, 2010), of biological controls or pollinators (RUSSELL; BISINGER, 2015). Grasslands bring other benefits. For instance, beef from grazing animals have higher organoleptic and nutritional properties (LOBATO et al., 2014), such as higher content of unsaturated fatty acids (ELGERSMA, 2015), the diversity of herbage species boosts animal welfare (CATANESE et al., 2013), increase their performance and nutritional attributes of their products (PROVENZA; KRONBERG; GREGORINI, 2019). In a unique manner, grasslands offer ecosystem services that any intense crop or livestock system offer. The key driver of such services is biodiversity (BRISKE; WOODWARD, 2016), in turn, controlled by herbivory: location, timing, duration and intensity of grazing (BRISKE et al., 2008; RUSSELL; BISINGER, 2015). Moderate-to-low grazing intensity is mandatory for obtaining such benefits from grasslands (BRISKE et al., 2008; CARVALHO; LEMAIRE et al., 2011; BATELLO, 2009; JANZEN, 2011).

Unfortunately, all these benefits are most likely unknown by general population. Yet, the easy-of-access to non-scientific non-peer reviewed information (e.g. social networks, documentaries, famous people claims, magazines) have made that an increasing sector of consumers claim "louder" the "livestock's long shadow", and confound the differences between industrial operations and pastoral ecosystems. Regarding this, probably, the livestock’s issue most covered by media is its contribution to GHG increment in atmosphere, especially methane (CH_4) emission, thus to global warming (GERBER et al., 2013; SMITH et al., 2008; STEINFELD et al., 2006). Nonetheless, there exist a substantial amount of scientific literature demonstrating the potential of mitigation of GHG, including CH_4, that pastoral ecosystems have when properly managed.

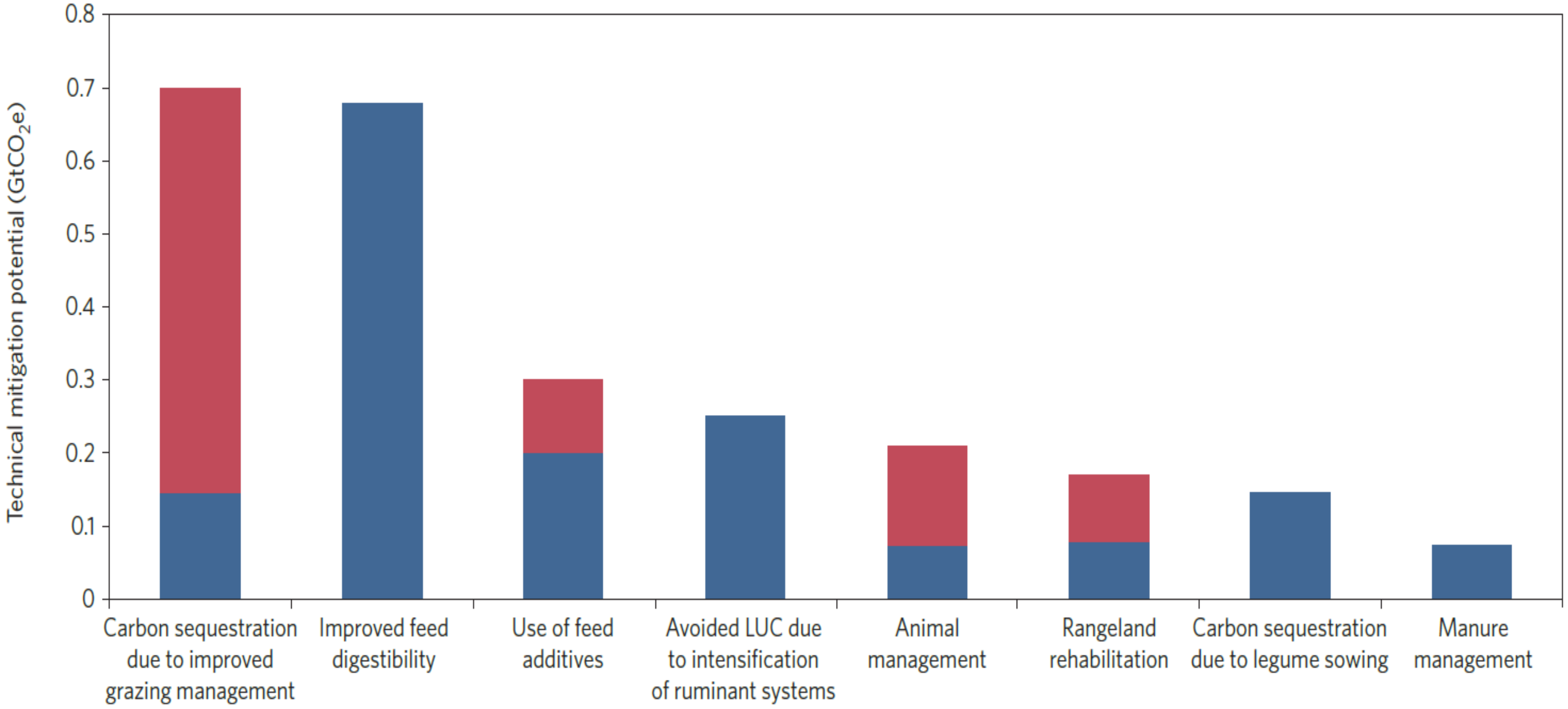


Figure 2. Technical mitigation potential of supply-side options for reducing emissions from livestock sector. Red represents the range for each practice, when available. From HERRERO et al. (2016).

1.2.5. The importance of GHG mitigation: the livestock's long shadow

During the first decade of the 21th century, the annual anthropogenic GHG emissions grew on average at a rate of 1.0 Gt CO_2-eq (2.2%), compared to the 0.4 Gt CO_2-eq (1.3%) per year, from 1970 to 2000; total emissions were the highest in human history and reached 49 Gt CO_2-eq per year in 2010 (IPCC, 2014). The human-induced increase in GHG has caused a 0.8°C to 1.2°C global warming of the atmosphere since pre-industrial times (IPCC, 2018). This scenario incited the international community to sign commitments to "stabilize GHG concentration in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system" (GAO; GAO; ZHANG, 2017). The Kyoto Protocol signed in 1997, aimed to reduce emissions by about 5.2% by 2012, as compared to 1990 levels (UNFCCC, 2008). Thereafter, the Paris Agreement in 2015 proposed to keep the increase in global average temperature to well below 2°C above pre-industrial levels and to pursue efforts to limit the temperature increase to 1.5°C (GAO; GAO; ZHANG, 2017). However, the IPCC (2018) on its latest report states that current abatement commitments are insufficient to achieve a 1.5°C scenario and urged nations to adopt more ambitious compromises by 2050. Now the target is to reduce CO_2 by about 45% from 2010 levels by 2030, reaching 'net zero' around 2050 (IPCC, 2018).

In 2010, 16% of total anthropogenic GHG emissions came from CH_4 (IPCC, 2014). It has shorter lifetime (12 years) but higher radiative forcing than CO_2; hence, its global warming power (GWP) is 28 times that of CO_2 over a 100-years horizon (IPCC, 2014). This makes the CH_4 a suitable target for reducing total anthropogenic radiative forcing. The Food and Agriculture Organization of the United States, through its issue "*Livestock long shadow - issues and options*" first attributed 16.5% of global anthropogenic GHG to livestock (STEINFELD et al., 2006). More recently, (GERBER et al., 2013) reduced this figure to 14.5%; however, these numbers vary among studies (8 to 51 %; HERRERO et al. (2011). Recently, HERRERO et al. (2016) estimated that total emissions from livestock from 1995 to 2005 ranged between 5.6 and 7.5 Gt CO_2eq per year, with enteric CH_4 being the most important source of emissions (1.6 to 2.7 Gt CO_2eq per year). Cattle are responsible for around 77% of enteric CH_4 from ruminants (GERBER et al., 2013). As CH_4 has a GE content of 55.22 MJ kg^{-1}, apart from its environmental issues, its emission by ruminants means energetic losses for the animal (around 2 to 13% of gross energy; (BLAXTER; CLAPPERTON, 1965; JOHNSON; JOHNSON, 1995). For these different reasons, major attention must be paid to its mitigation in livestock systems (BERNDT; TOMKINS, 2013; MARTIN; MORGAVI; DOREAU, 2010).

1.2.5.1. The Brazilian scenario

Since 1990, CH_4 emissions have increased rapidly in Latin America, with this region accounting for 39% of global CH_4 emissions in 2005 (DEFRIES; ROSENZWEIG, 2010). In 2016, Brazilian agriculture (non-CO_2) and land-use change accounted for 21.9 and 51.2 % of total national emissions, respectively. Given its world largest commercial cattle herd of roughly 214 million (IBGE, 2017), 64.8% of agricultural emissions are from enteric CH_4, mainly from beef (86%) and dairy cattle (11%; SEEG, 2019). As a result, and despite that its livestock's' domestic emissions account for 8% of global livestock's' emission (SEEG, 2019), Brazilian grass-based beef industry is under international scrutiny (EUCLIDES FILHO, 2004). In Latin America, it is the largest food producer and makes major contributions to the global food system and to its own economy (FERRAZ; FELÍCIO, 2010); yet it ranks the 7th place of countries with higher emissions (1.9 Gt CO_2-eq, or 3.5% of annual global emissions). Estimates show that by 2025, beef production, consumption and exportation will increase by 23, 18 and 37%, respectively (BRASIL, 2016). Owing to the national commitments, these increases must be accompanied by a 43% reduction by 2030 in overall GHG emissions compared to the 2005 GHG emissions baseline (FEDERATIVE REPUBLIC OF BRAZIL, 2015). For these reasons, major efforts are made in mitigating CH_4 emissions, especially from grazing ruminants (BERNDT; TOMKINS, 2013). Otherwise, countries perceived as high CH_4 emitters might become less competitive on the global market (EUCLIDES FILHO, 2004), since nations, which meat-milk consumption is predicted to increase (i.e. China, main Brazilian beef importer) might adopt a 'green source trade strategy' for their importations (DU et al., 2018).

1.2.6. Methane production and yield

Methane is a byproduct of the microbial fermentation of feed in the rumen (HILL et al., 2016; HOOK; WRIGHT; MCBRIDE, 2010; LENG, 2018). Dietary protein, starch, lipids and fiber are hydrolyzed by the integrated action of enzymes, releasing nitrogenous compounds, simple sugars and free fatty acids into the ruminal environment (LENG, 2014; WEIMER, 1998). Subsequently, these substrates are fermented into microbial biomass, volatile fatty acids, ammonia, intermediaries of unsaturated fatty acids biohydrogenation, H_2 and CO_2 (ELGERSMA, 2015; KUMAR et al., 2014; MATHISON et al., 1998). The synthesis of acetate and butyrate release H_2 in the ruminal environment, while propionate consumes it. When the partial pressure in H_2 rises in the rumen (LENG, 2018), it affects the continuation of fermentation processes (KNAPP et al., 2014) through the reoxidation of reduced cofactors produced during fermentation (MCALLISTER; NEWBOLD, 2008), thereby restricting glycolysis and feed degradation (LENG, 2014). To avoid H_2 saturation, the methanogenic *Archaea* use it as energy to reduce CO_2 to CH_4 (HILL et al., 2016; MATHISON et al., 1998; WEIMER, 1998), which is then released in the rumen and exhaled mainly by eructation.

Numerous reports suggest that the level of intake is the variable most explaining CH_4 production (BEAUCHEMIN; MCGINN, 2006; HAMMOND et al., 2013; JONKER et al., 2017; KURIHARA et al., 1999; MORAES et al., 2014). While CH_4 production increases with intake, CH_4 yield (g CH_4/kg DMI) decreases (HAMMOND et al., 2013; JONKER et al., 2017). Conversely, feed digestibility, as a function of its chemical composition, relates negatively with CH_4 yield (g CH_4 kg of DM or OM intake) over some dietary conditions (HEGARTY, 2009; SHIBATA; TERADA, 2010). The prediction of CH_4 emissions from the chemical composition of forages is weaker than from intake though. For instance, in (HAMMOND et al., 2013), the chemical composition of white clover and perennial ryegrass weakly predicted CH_4 production; the NDF content explained 19% of CH_4 yield variance. Accordingly, (JONKER et al., 2017) obtained minor improvements in the prediction of CH_4 emission from cattle when the model included the pasture quality in addition to intake levels. PINARES-PATIÑO; BAUMONT; MARTIN, (2003) showed no relationship between CH_4 yield and nutrient content of well-managed pastures, and (SUN et al., 2011) observed similar CH_4 yield between chicory and ryegrass, both with a notable difference in chemical composition. Altering the chemical composition of forages has limited scope for CH_4 mitigation (BUDDLE et al., 2011; PACHECO; WAGHORN; JANSSEN, 2014) over some feeding conditions (i.e. forages with high nutritive value), hence promoting higher intake and passage rate are especially important for this purpose. Nevertheless, optimizing herbage nutritive value still relevant, as it may reduce emissions intensity at equivalent or lower level of intake and CH_4 emission (CLARK, 2013; LENG, 2014; PACHECO; WAGHORN; JANSSEN, 2014; RICHMOND et al., 2015; SAVIAN et al., 2018; VAN WYNGAARD; MEESKE; ERASMUS, 2018), and reduce the lifetime emission intensity (CHRISTIE et al., 2016).

1.2.7. The nutritional-oriented strategies for CH_4 mitigation

Mitigation strategies include breeding and herd management, feeding and nutrition, and rumen manipulation (BEAUCHEMIN et al., 2020; COTTLE; NOLAN; WIEDEMANN, 2011; HRISTOV et al., 2018, 2013; PATRA, 2016; SHIBATA; TERADA, 2010; SMITH et al., 2008). Among nutritional-ruminal options are high grain supplementation (JOHNSON; JOHNSON, 1995), feeding corn silage-based diets (HASSANAT et al., 2013; HATEW et al., 2016), adding lipids (BEAUCHEMIN et al., 2008; LYNCH, 2019; MATHISON et al., 1998; MOATE et al., 2016), chemical additives (CAETANO et al., 2016; HULSHOF et al., 2012; NGUYEN; BARNETT; HEGARTY, 2016), natural additives (ARCHIMÈDE et al., 2016; BEAUCHEMIN et al., 2007; PATRA, 2016), feeding forages with higher nutritive value (ARCHIMÈDE et al., 2018; DINI et al., 2018; GERE et al., 2019; MOE; TYRRELL, 1979), as grasses mixed with legumes (HAMMOND et al., 2011, 2013; MOATE et al., 2016; SUN et al., 2015) or with high sugar content (ELLIS et al., 2012). Overall, their aim is to mitigate CH_4 emission by promoting intake and productivity gains (BENCHAAR; POMAR; CHIQUETTE, 2001; MC GEOUGH et al., 2010) and by creating low-CH_4 rumen environment (BEAUCHEMIN et al., 2008; VAN GASTELEN; DIJKSTRA; BANNINK, 2019). The mitigating mechanism seems relevant provided the differences in the digestive and fermentative physiology among ruminant species; strategies promoting fermentation towards a low-CH_4 rumen environment seems to be effective among ruminant species (VAN GASTELEN; DIJKSTRA; BANNINK, 2019).

The mitigation of most of those strategies range from nine to 40 % for indoor-fed animals (BENCHAAR; POMAR; CHIQUETTE, 2001); this is a low to medium potential (GERBER et al., 2013). KNAPP et al. (2014) by reviewing the literature found 2.5 to 15% mitigating potential for some of these strategies applied on dairy cattle, and (CARO; KEBREAB; MITLOEHNER, 2016) predicted a global 16% reduction of CH_4 emission, with the highest abatement rate occurring in Africa, South America and Asia, with 55, 46 and 34%, respectively. (THORNTON; HERRERO, 2010) estimated a 14 and 18% abatement of CH_4 per ton of milk and meat, respectively, when the adoption rate (simulated) of improved feeding practices is of 23%. Despite this, most of these strategies developed upon intensive managements in temperate conditions (KNAPP et al., 2014), and were tested on animals with large intakes of highly nutritive diets, high feed efficiency and low CH_4 EI (HRISTOV et al., 2013; MOTTET et al., 2017). Moreover, their applicability still challenging, especially on grazing systems, given some technical (e.g. have no-effect, limited or short-term effect, reduce diet digestibility, DM intake or animal performance, or increase overall farm emissions), human health legislation issues, economical (e.g. not cost-effective), ethical (e.g. impair welfare or competition for human edible resources) or operational constraints (BEAUCHEMIN et al., 2008; BUDDLE et al., 2011; CLARK, 2013; DOREAU et al., 2014; KUMAR et al., 2014; LENG, 2014; LLONCH et al., 2017; MOATE et al., 2016; NGUYEN; BARNETT; HEGARTY, 2016; PACHECO; WAGHORN; JANSSEN, 2014; RAMÍREZ-RESTREPO et al., 2010).

1.3. CONCEPTUAL MODEL OF THE THESIS

The Rotatinuous stocking (RN) is a pasture management concept that can potentially reduce enteric CH_4 emission intensity from grass-based systems (SAVIAN et al., 2018). The possible mechanism of mitigation is depicted in Figure 1. The leading cause is mostly due to the higher daily intake, this latter, the factor majorly determining CH_4 emissions (BLAXTER; CLAPPERTON, 1965; HEGARTY, 2009; HENRY; ECKARD, 2009; JOHNSON; JOHNSON, 1995). Moreover, chemical attributes of the herbage consumed under the RN management and its associated ruminal digestive and fermentative patterns (low-CH_4 ruminal environment; (LENG, 2018) might explain a share of emissions.

The higher daily intake under RN stocking results from setting the pre- and post-grazing sward heights that allow animals to maximize the short-term intake rate (STIR; g DM minute^{-1}) during grazing (CARVALHO, 2013). Clearly, the daily intake is the product of grazing time and STIR (ALLDEN; MCDWHITTAKER, 1970; HODGSON, 1990), and the STIR is a function of bite mass (g DM per bite) and bite rate (bites min^{-1}). The bite mass is, in turn, the product of bite area, bite depth and bulk density of the grazed sward canopy stratum (CARVALHO et al., 2015). Such bite characteristics are altered by the sward structure (BENVENUTTI et al., 2016; CHACON; STOBBS, 1976; GUZATTI et al., 2017; LACA et al., 1992) and this latter relates with canopy height (HODGSON, 1990; (EUCLIDES et al., 2018; LACA et al., 1992). Thus, through managing pre- and post-grazing sward heights, the RN stocking indirectly set the sward conditions that maximizes the STIR per unit of grazing time (GONCALVES et al., 2009; AMARAL et al., 2013; FONSECA et al., 2012; MEZZALIRA et al., 2017, 2014), hence that can potentially increase the daily nutrient intake and animal performance (Figure 2).

The RN management set in 40% the limit of depletion of the pre-grazing sward height maximizing the STIR (CARVALHO, 2013). As animals are not forced to explore lower parts of the canopy, higher in less preferable steams (AMARAL et al., 2013; BRUNETTI et al., 2016; DIAS et al., 2017; EUCLIDES et al., 2018; ZANINI et al., 2012) of lower digestibility (HODGSON, 1990; VAN SOEST, 1994; ORR et al., 2004). This results by assuming that animals defoliate sward by taking bites in successive layers (roughly 50 % of the tiller height; (GASTAL; LEMAIRE, 2015), from the top to the bottom of the canopy (BAUMONT et al., 2004), and that the chemical composition of the forage follows a vertical gradient (HODGSON, 1990; DELAGARDE et al., 2000).

The hypothetically biting behavior on the upper half strata of the sward, hence almost exclusively leaf lamina harvesting by animals under RN, reasonably may allow higher intake of total digestible nutrients, including soluble sugars, nitrogenous compounds, fatty acids, and consequently lower CH_4 emissions per DM intake. Additionally, the DM intake restriction in the RT could drive shifts in blood constituents associated with nutritional status and stressful responses (DHABHAR et al., 1996; INGVARTSEN; MOYES, 2013; SORDILLO, 2016), even when access to pasture is allowed (COSTA et al., 2015; REN et al., 2016). However, the extent to which grazing management targets aiming to maximize harvest efficiency, but lower individual animal DM intake, triggers stressful responses in grazing animals needs further research.

Questions to be answered

¿How the management of sward height affect the type of bite and the proportion that each one accounts for the accumulated intake by sheep grazing Italian ryegrass pastures in rotational stocking? (Chapter II)

¿Does the biting behavior of sheep grazing Italian ryegrass pastures, with different intensity and frequency of grazing, drive changes in the nutritive value of the herbage apparently ingested? (Chapter II)

¿Does a lenient and frequent grazing management improve blood biochemical indicators of nutrition respect a severe and infrequent management? ¿Does a grazing management promoting high rate of sward heigh depletion (severe grazing) causes stressful responses in lambs grazing high-nutritive Italian ryegrass pastures? (Chapter II)

¿Does the herbage apparently ingested by animals grazing Italian ryegrass pastures with a lenient and frequent grazing promote a low-CH_4 *in vitro* rumen environment? (Chapter III)

¿Can grazing management reduce CH_4 emission intensities of pastoral ecosystems to levels comparable to intense feeding systems? (Chapter IV)

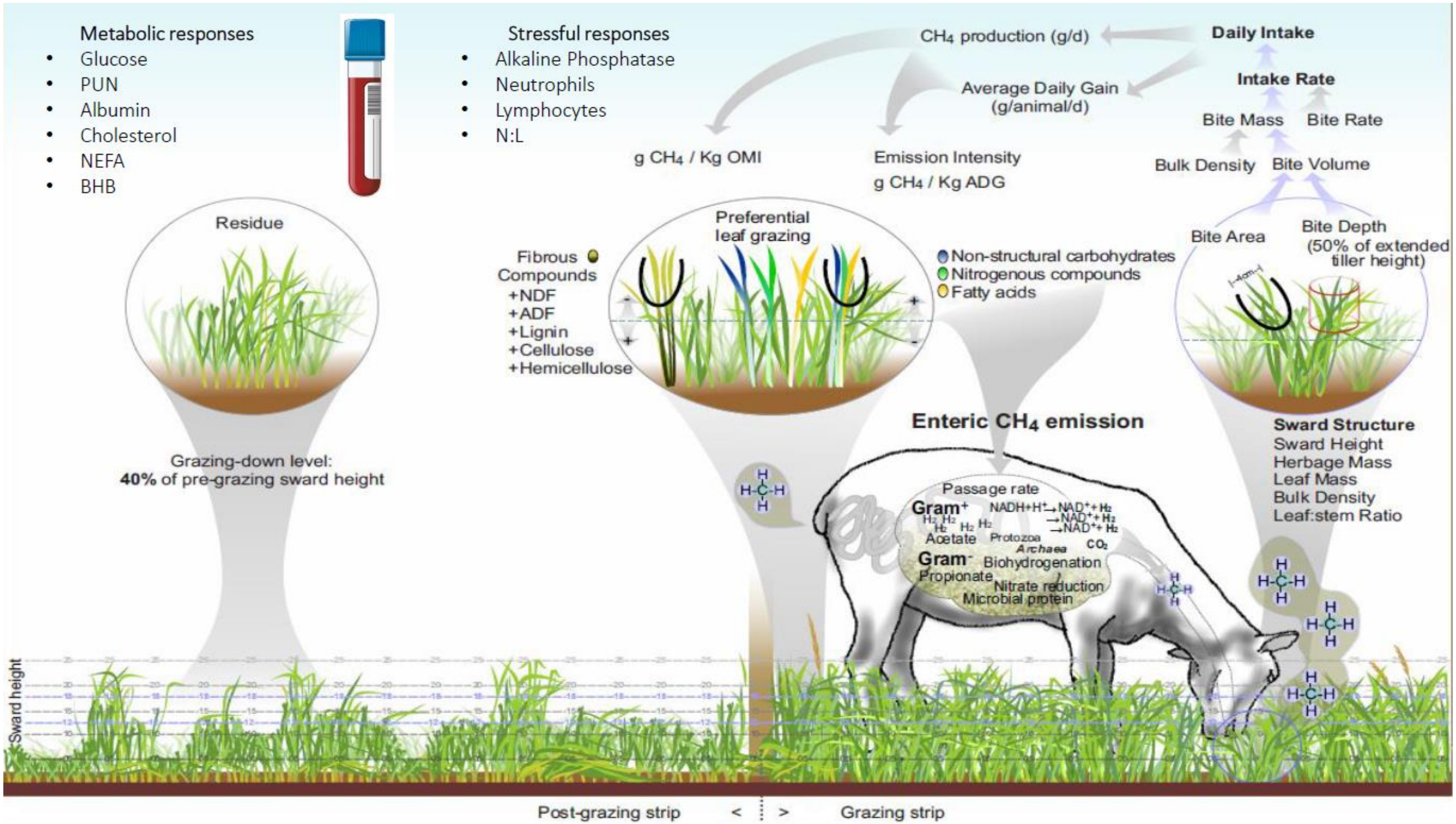


Figure 1. Conceptual model. The influence of pasture structure over the short-term ingestive behavior of animals, the amount and nutritive value of the diet, CH_4 emission and nutritional-welfare indicator of grazing animals. At the right side, the figure mimics the sward surface height target at the beginning of the grazing-down (grazing strip) and at the left side, it show the pasture condition at the end of the grazing-down (post-grazing strip), of *Lolium multiflorum* Lam., pasture; the pre- and post-grazing sward surface height are of 18 and 11 cm, respectively.

1.4. HYPOTHESES

1. The sward height that allow sheep to maximize the intake rate of Italian ryegrasspastures is of 18 cm. In order to sustain this condition, the Rotatinuous stocking sets in 40 % the limit of depletion of this ideal sward height (11 cm at the post-grazing). Differently, for these forage species, the pre-grazing height of 27 cm aims to maximize herbage growth, and the post-grazing height of 7 cm aims to optimize harvest efficiency. These ranges of sward height depletion affects the type of bites and the proportion that each one account for the accumulated intake. It is hypothezised that the preferential leaf lamina biting of the RN animals increases the nutritive value of the herbage (e.g. preferential leaf lamina grazing, thus higher content of soluble sugar, crude protein and lipids, but low content of fiber) and quantity of nutrient ingested, thus improves the nutrition and welfare indicators of grazing sheep, compared to the RT management, in which animals are forced grass non-preferred plant parts, at the canopy bottom. (Chapter II).

2. The diet apparently selected by animals under RN management, characterized by higher proportion of leaf lamina, thus of soluble sugar, crude protein, lipids, but less in fibrous compounds, results in low-CH_4 *in vitro* rumen environment. (Chapter III).

3. Grazing management promoting high levels of DM intake of high nutritive forages and moderate-to-high levels of performance of grazing ruminants display low CH_4 emission intensities, comparable to those of intense-fed animals. (Chapter IV).

1.5. OBJECTIVES

1. Characterize at the bite-scale, the diet apparently consumed by sheep grazing Italian ryegrass pastures under contrasting grazing managements in rotational stocking (Chapter II)

2. Evaluate the effect of grazing management over the nutrients intake and nutrition-stress-related blood parameters of sheep grazing Italian ryegrass pastures (Chapter II)

3. Evaluate the potential that the herbage apparently ingested by sheep under contrasting grazing management has to shift *in vitro* fermentation profile and create a low-CH_4 rumen environment (Chapter III)

4. Provide evidence about the opportunities and potential that some grazing management have to mitigate enteric CH_4 emission by ruminants in pastoral ecosystems (Chapter IV)

2. CHAPTER II[1].

---

[1] Manuscript prepared in accordance with Animal Feed Science and Technology (Appendix 1)

Bite-scale characterization of the herbage apparently consumed, nutrients intake and biochemical-hematological indicators of nutrition and welfare of lambs grazing Italian ryegrass pastures[1]

Abstract

The sward surface height affects the bite characteristics of grazing animals, and infers about the amount and nutritive value of the herbage ingested. We depicted type of bites that lambs perform when grazing Italian ryegrass pastures with contrasting sward grazing managements. The high frequency/low intensity grazing model (RN) was performed at 18 and 11 cm, whilst the low frequency/high intensity (RT) was grazed at 27 and 7 cm, respectively for pre- and post-grazing sward heights. Paddocks were arranged under a complete randomized design and treatments were repeated over two periods. Animals of the RN management performed preferentially leaf lamina biting behavior, on intact plants parts with 20 cm or more, representing 50 % of the accumulated intake. Differently, the RT animals, performed bites preferentially on grazed plants parts with 15 cm or less, or on lying plants, representing 43% of the accumulated intake. The diet of the RN animals had higher CP content, total soluble sugars and crude fat, and reduced content of ADF and ADL, compared to RT animals, forced to grazing-down most of the herbage in offer. A higher intake (+18%) of a diet with higher nutritive value on RN boosted the daily intake of soluble nutrients (+25%) directly affecting animals' blood composition; 17.5, 18 and 6.1% higher glucose, plasma urea nitrogen and albumin, respectively, and 19% lower neutrophil-to-lymphocyte ratio, this latter an indicator of a lower stressful response. The RN management allows animals to ingest most of the daily intake from plants of 20 cm, whereas animals under the RT management perform bites on plants lower than 15 cm. The preferential leaf lamina grazing by animals in the lenient and frequent management drive the increase in the daily intake of soluble nutrients and the improvement of some nutritional and welfare indicators of lambs grazing Italian ryegrass pastures.

Keywords: grazing management target, sward surface height, intake rate, nutrient intake.

2.1. Introduction

The apparent trade-off between individual intake and output, and per hectare yield and farm profit (Dove, 2010; Romera and Doole, 2015), prevents individual maximum DM intake (-10%) directly from grazing, but increases intake per hectare by 15 % (Peyraud and Delagarde, 2013). This is commonly achieved applying higher grazing intensities and forcing animals to collect most of the herbage on offer (Curran et al., 2010; Fariña and Chilibroste, 2019; Penati et al., 2014). It is argued that low herbage residues promotes leafy herbage growth (Fulkerson et al., 1999; Hoogendorn et al., 1992; McEvoy et al., 2009; Peyraud and Delagarde, 2013), which increases its nutritive value, animal output and reduce CH_4 emissions (Boland et al., 2013; DeRamus et al., 2003; Muñoz et al., 2016; van Wyngaard et al., 2018; Wims et al., 2010), among other benefits (DeRamus, 2004).

In rotational stocking, (da Silva et al., 2015) suggest starting the grazing-down when canopy intercepts 95 % of the incident light. At this point of plant physiology mass accumulation (mostly leaf) is almost maximum and senescence still low (Congio et al., 2019, 2018; da Silva et al., 2015; da Silveira et al., 2016). On the other hand, low residual sward heights are proposed as the limit of the grazing-down (Fulkerson et al., 1999; Ganche et al., 2013; McEvoy et al., 2009), which aligns with the target of full exploitation of the grassland area (Peyraud and Delagarde, 2013). When these concepts are applied conjunctly to *Lolium multiflorum* Lam. pastures, for example, the result is a traditional rotational stocking with pre- and post-grazing sward height of 27 and 7 cm, respectively (Savian et al., 2018 Schons et al., unpublished).

Differently from this perspective, Carvalho (2013) proposes a grazing management concept based on animal behavioral responses, that sets the limits of grazing-down based on the sward heights allowing animals to maximize the short-term intake rate (STIR; (Amaral et al., 2013; Fonseca et al., 2013, 2012; Goncalves et al., 2009; Mezzalira et al., 2017, 2014). This management sets in 40% the limit of depletion of the pre-grazing sward height that maximizes the intake rate; pre- and post-grazing sward heights of 18 and 11 cm respectively, for sheep grazing Italian ryegrass (Savian et al., 2018). A consequence is a lenient grazing where animals are not forced to explore lower parts of the canopy, higher in less preferable stems (Zanini et al., 2012; Amaral et al., 2013; Brunetti et al., 2016; DIAS et al., 2017; Euclides et al., 2018) of lower nutritive value (Hodgson, 1990; Van Soest, 1994; Delagarde et al., 2000; Elgersma, 2015; Orr et al., 2004).

When compared with a traditional rotational stocking, this animal-oriented grazing management concept increased the primary and secondary production, demonstrating that it is possible to achieve both targets in the same stocking season and overcome the traditional trade-off found between these variables (Schons et al., Unpublished), and also reduce CH_4 emission intensity of growing lambs (Savian et al., 2018). The higher OM intake might be the leading cause of the improved animal responses. However, whether this contrasting grazing management drives further improvement in the nutritive value of the herbage ingested, when compared to a traditional rotational stocking using high-nutritive pastures, have not been fully investigated. The hypothetically selective grazing on the upper half of the canopy, hence, preferential leaf harvesting could allow animals to select herbage of higher nutritive value, compared with the traditional management, in which animals are forced to grass bottom plant parts.

The amount of nutrients that animals can get directly from pasture is also relevant for animal welfare. Despite grass-origin animal products are recognized as animal-friendly (Lobato et al., 2014; Provenza et al., 2019), grazing managements targeting per hectare productivity and farm profit (Fariña and Chilibroste, 2019) prevent animals to cover their nutritional exigencies (Dove, 2010; Peyraud and Delagarde, 2013) when no supplementation is used (Bargo et al., 2003). This in turn, might drive shifts in blood constituents associated with immune function (Ingvartsen and Moyes, 2013; Sordillo, 2016) and stress (Dhabhar et al., 1996), even when access to pasture is allowed (Costa et al., 2015; Ren et al., 2016).

We conducted two experiments with lambs grazing Italian ryegrass pastures under different managements in a rotational stocking. The first aimed to characterize, at the bite scale, the herbage apparently consumed by animals. In the second, the objective was to analyze the chemical composition of the herbage apparently consumed by animals, and from this, estimate the total nutrient intake by animals. Finally, we assessed the impact both grazing managements over some biochemical and hematological blood variables associated with nutritional and welfare status.

2.2. Material and Methods

2.2.1 Experimental area and pasture establishment

The experiment was conducted at the Experimental Station of the Faculty of Agronomy of the Federal University of Rio Grande do Sul (UFRGS), in Southern Brazil (30°05”22’ S latitude, 51°39”09’W longitude and 46 m above sea level [a.s.l.]). The area presents a typical Paleudult soil type, with 15% of clay and a subtropical humid “Cfa” climate (Köppen classification). An Italian Ryegrass pasture (*Lolium multiflorum* Lam.) was established in April 20th in 2017 and May 23th in 2018, through conventional soil preparation, with 35 kg of seed per hectare and 250 kg of the formula (NPK, 5-30-15) per hectare at seeding, and 200 kg of nitrogen (urea) 30 days after. Areas with low plant population post-emergence were re-seeded manually.

2.2.2 Treatments and experimental design

Two grazing managements were evaluated under a completely randomized design: Rotatinuous (RN) and traditional rotational (RT) stocking, both setting different pre- and post-grazing sward heights as management targets. For the RN, the pre-grazing height of 18 cm is oriented to maximize intake per unit of grazing time (Amaral et al., 2013), while the post-grazing height of 11cm (40% reduction of the initial height) aims to sustain the intake rate at any time until the next strip change (Carvalho, 2013; Fonseca et al., 2012; Mezzalira et al., 2014). For traditional stocking, the pre-grazing height of 28 cm aims to maximize herbage growth and the post-grazing height of 7 cm aims to optimize harvest efficiency (Schons et al., Unpublished).

2.2.3 Animals and pasture management

Twelve crossbred Texel x Corriedale sheep (35 kg ± 4.3 kg of LW) in 2017, and twenty-four Corriedale year-round lambs (41.1 kg ± 3.4 kg of LW) in 2018, were randomly allocated to four and eight paddocks of 0.21 ha each, respectively for each year. Pasture management was similar in both years. Briefly, in order to maintain treatments sward heights, a variable number of regulator animals accompanied the three testers on each paddock (put-and-take technique; Mott and Lucas, 1952). Animals grazed in strips for 24 h, changing to another between 14:00 and 15:00 h. The number and size of strips within paddocks were defined by the herbage growth and target sward heights. Animals first entered the paddock when around ⅔ of the treatment pre-grazing sward height was achieved. Once strips of both treatments achieved the targeted pre-grazing height and animals of both treatments entered their respective paddocks, an adaptation period of 35 day (2017) and 15 days (2018) was considered to start. Afterwards, two evaluation periods occurred between August 21 to 23th (period 1) and September 1 to 2rd (period 2) in 2017, and between September 4 to 8th (period 1) and 15 to 19th (period 2) in 2018, with pasture at full vegetative stage (period 1) or early shoot elongation (period 2), for both years. In both years, the sward height was measured at two-day interval during the adaptation period, and daily during evaluations, with a “sward stick” (150 spots per paddock; Bartham, 1985) at the pre- and post-grazing.

2.2.4 Bite-*scale characterizations of the herbage apparently consumed*

The grazing process, as driver of daily intake and nutritive value of the ingested material, is complex and sensitive to sward structure (Hodgson, 1990; Baumont et al., 2000; Da Silva and Carvalho, 2005). Briefly, the daily intake is the product of grazing time and STIR (Allden and McDWhittaker, 1970; Hodgson, 1990; Laca et al., 1992), with this latter being a function of bite characteristics, in turn, affected by the SSH (Barrett et al., 2001; Benvenutti et al., 2016; Chacon and Stobbs, 1976; Euclides et al., 2018; Guzatti et al., 2017; Hodgson, 1990; Laca et al., 1992). Thus, pre- and post-grazing SSH have a predominant role in the amount and type of bites that animals perform during grazing-down, thus over the daily nutrients intake. Accordingly, we integrated the continuous bite monitoring method and a highly detailed hand-plucking to compose, by bite type, the herbage apparently consumed.

2.2.4.1 Continuous bite monitoring

It is a four-step methodology consisting on animal-observer familiarization, bite-coding grid elaboration, observer training and data recording (for details see Agreil and Meuret, 2004; Bonnet et al., 2015). In 2017, we used this method during two evaluation periods, with three and two days of bite monitoring, respectively. Briefly, four trained observers elaborated the bite-coding grid (Figure 1) during the first week of adaptation to treatment. The observers trained the bite-coding grid until they were able to encode, in real-time, each bite without hesitation (during the 28 days of adaptation); afterwards, evaluations were conducted. On each period, the observation went from the time of strip-grazing change (14:00 to 15:00 hours) until sunset (around 18:15 hours), and continued the next day from sunrise (around 06:15) until the next strip-grazing change. The four observers (one per paddock) evaluated a different animal each day, on each treatment, in alternated fashion. This way, the three tester animals within a paddock were randomly evaluated once by an observer in period one; however, one sheep of each treatment were discarded from analysis as they presented unusual reactiveness to the observer. The second period was conducted similarly, differing only in that a third observation day was not possible due to climatic conditions. Consequently, two out of the three tester animals within a paddock were evaluated once by an observer. Overall, 18 out of 24 possible observations were obtained (n= 9; five in period one and four in period two, for each treatment). The bite encoding was recorded with a digital recorder (Sony recorder Icd-PX240®). The total monitoring time evaluated per animal averaged 10 hours with 45 minutes. The recordings were transcribed using the software (JWatcher®, http://www.jwatcher.ucla.edu/,verified 10 December 2014; The Observer, Noldus Information Technology®, The Netherlands).

2.2.4.2 Bite-scale hand-plucking

The observers simulated at least 20 times each bite type (Figure 1) performed by the animal under the continuous bite monitoring evaluation, according to Bonnet et al. (2011). The bite mass (g DM) was calculated by drying the bite samples at 55°C during 72 h and dividing the dry weight obtained on an electronic scale (0.001 g precision) over the number of simulations. The accumulated intake during the bite monitoring observation time was calculated by multiplying the number of times each bite type occurred by its dry mass. The dry matter ingested per bite type was divided by the accumulated dry matter intake to obtain their relative proportion on the accumulated intake during the bite monitoring time; herein referred as the herbage apparently ingested (Table 1).

2.2.5 Total organic matter intake and digestibility

In 2018, the daily OM intake was estimated on the three tester animals per paddock in two separated periods. We used the fecal crude protein technique (Penning, 2004), as described by Savian et al. (2018). The equation proposed for Italian ryegrass pastures by Azevedo et al. (2014) was used as follows: OM intake = 111.33 + 18.33*fecal crude protein. Each period consisted in total feces collection during five consecutive days. Collecting bags were emptied once per day, the faces were weighed and homogenized and a sub-sample of 20% of the total was taken. Samples were dried at 55 °C for 72 h, pooled per animal, grounded and analyzed for DM, OM and total nitrogen (AOAC, 1980). The organic matter digestibility was calculated using the following equation: OM digestibility = 1 – total amount of feces / OM intake. The digestible OM intake was calculated using the OM intake and OM digestibility. The metabolizable energy (ME) intake was calculated using the model proposed by CSIRO (2007; ME = 0.169* OM digestibility - 1.986). During the first four days of the feces collection, one observer assessed the biting activity of the tester animals during the main morning and afternoon grazing events. Afterwards, the observer simulated, as previously described, at least 20 times each bite of the bite-coding grid of annual Ryegrass (Figure 1). The simulation was performed in alternate fashion, completing one paddock of each treatment per day. At the fourth day of each period, all bites of the bite-coding grid were simulated once on each of the eight paddocks.

2.2.6 Chemical analysis of the herbage apparently ingested and total nutrients intake

We combined the data of 2017 and 2018 to obtain the herbage apparently consumed and total nutrient intake. Clearly, in 2017, we observed the type of bites the animals perform in the RN and RT grazing managements (bite-code grid; Figure 1), and estimated how much each accounted for the accumulated intake during the continuous bite monitoring (herbage apparently ingested; Table 1). Afterwards, in 2018, we estimated the daily OM intake (N-fecal), and each bite of the bite-code grid was *de novo* simulated; this time, the hand-plucked bite samples were put on a cooler with ice immediately after sampling, and within 4 hours stored at -20 °C until freeze-drying. From these simulated bites, we compounded 16 diets of 10 g DM each (2 treatments x 4 paddock x 2 periods) according to Table 1. Diets were crived (1 mm) for NIRS analysis (*sensu* Decruyenaere et al., 2009). Finally, we multiplied the nutrient content (g/kg of DM) of the herbage apparently ingested by the daily DM intake (DM calculated from the OM content of the diets and total OM intake) to calculate the total amount of nutrients that animals would likely ingest by composing their diet as referenced in Table 1.

2.2.7 Biochemical and hematological blood parameters

In 2018, blood samples were taken from 07:00 to 08:30 hours two days after the last fecal collection of each period. Samples were collected both in non- and heparinized-tubes (5 mL) by jugular puncture and immediately put on a plastic fridge with ice until transportation to the laboratory within 6 hours after sampling. Heparinized samples were brought to room temperature, and homogenized. Packed cell volume was assessed by the microhematocrit method, using capillary tubes and a micro centrifuge (Thermo

Scientific®). Red blood cell and leukocyte counting were performed manually, using a hemocytometer. Blood smears of each sample were dried and stained with Diff Quick to perform leukocyte differential count, morphology evaluation and platelet count. All hematological parameters were assessed by the same veterinary clinical pathologist, who was blind to the treatment of each sample.

Non-heparinized samples were brought to room temperature and centrifuged at 3500 rpm x min for 10 minutes. Serum glucose, plasma urea nitrogen (PUN), albumin, alkaline phosphatase, fructosamine and cholesterol were measured by enzymatic colorimetric analysis using commercial kits (Wiener Lab., Rosario, Argentina) in a Wiener lab cm 200 auto-analyzer (Wiener Lab., Rosario, Argentina). Plasma was obtained by centrifugation of heparinized blood, and an aliquot was stored in Eppendorf tubes (1.5ml) and frozen at -20°C until analyzed separately for non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and insulin. NEFA were determined by enzymatic colorimetric analysis (NEFA, Randox, Country Antrim, UK) as well as BHB (D-3 Hydroxybutyrate (Ranbut), Randox, Country Antrim, UK).

2.2.8 Statistical analysis

The data from the pasture was analyzed according to a complete randomized design (ANOVA; 5 % of significance), considering the fixed effect of treatments and random effects of paddock and year, with paddock also considered as the experimental unit. Data of OM intake, digestibility, digestible OM intake, nutrients intake and blood parameters were subjected to analysis of variance at 5% of significance. The model included the fixed effects of treatment, the random effects of animal nested within paddock, and of period (lmer function; R 3.6.0 R Core 202 Team, version 2019). The statistical model was selected considering the best fit model according to the AICs' criteria. A canonical correlation analysis was performed to identify the relation of the grazing managements (RN and RT; independent variable matrix) over the proportion that each bite type observed during the continuous bite-monitoring accounted for the accumulated DM intake (dependent variable matrix), using the library Vegan of R Development Core 202 Team, 2019, version 3.6.0).

2.3. Results

2.3.1 The SSH and bite-scale characterization of the diet apparently ingested

The pre- and post-grazing SSH, and sward height depletion were as pretended in both years (Table 1). This ensured that animals created the sward structure for the observer to hand-pluck the type of bites from swards typical of each treatment. The Figure 2 shows the bite types and their correlation with the grazing management, as estimated during the continuous bite-monitoring performed. The bite "Ve", performed on intact plants of 20 ± 2.5 cm, was the most correlated with the RN management, followed by the "Te", "Ke" and "Va", performed on intact plants between 15 and 25 ± 2.5 cm; together accounted 49.4% of the accumulated DM intake. On the other hand, the bites that correlated the most with the RT management were those performed at the bottom strata of the plant with ≤ 10 ± 2.5 cm, namely "Co", "Ci", "Di", "Du" and "Ra", or to those performed on lying plants, namely "La", "Li", "Le" and "Pança"; together, these bites accounted for 44.8% of the DM intake. Bites “Mix”,” Max”, “Ki”, “Fa” and “Vi”, were associated to both treatments and accounted for roughly 26% of the DM intake.

2.3.2 Herbage apparently ingested, organic matter and total nutrient intake

Table 3 shows the chemical composition of the herbage apparently ingested. The crude protein, total sugar ($P<0.0001$) and crude fat ($P=0.056$) contents increased in the RN, while the fibrous compounds such as ADF and ADL were reduced ($P<0.001$), with no difference for NDF between treatments ($P>0.05$). The OM digestibility was higher in the RN diet ($P<0.0001$). The intake of OM, digestible OM, and of all nutrients, except of ADF ($P>0.05$), were higher in the RN management ($P<0.0001$).

2.3.3 Blood biochemistry and hematology

Table 4 shows blood biochemical and hematological indicators of nutritional status and streesful responses of animals under both managements. The concentration of albumin, glucose and plasma urea nitrogen increased in the RN animals ($P<0.02$), the NEFA tended to increase ($P=0.09$), and the alkaline phosphatase, fructosamine, cholesterol and BHB were unaffected ($P>0.05$). The lymphocyte counting and the neutrophil-to-lymphocyte ratio increased in the RN animals ($P<0.05$). The other hematological variables were unaffected ($P>0.05$).

2.4. Discussion

We illustrate the diversity of bites (Figure 1) that animals perform to cope with contrasting sward structures and compound their diet, even in homogeneous Italian ryegrass pastures. The pre- and post-grazing SSH and depletion rate were similar between 2017 (year of bite-scale characterization of the herbage apparently ingested) and 2018 (year of herbage sampling for chemical analysis and total nutrients intake estimation). Despite this, non-accounted sward characteristics, animal or climatic factors, might have led animals in 2018 to deviate their biting behavior from that observed in 2017, therefore to possible over- or under-estimation of the nutritive value of the herbage ingested. Nonetheless, we considered the hand-plucking and continuous bite monitoring methodologies employed to be adequate for describing the biting mechanism underlying the differences in the nutritive value of the herbage ingested between grazing management, as they have proven accuracy in estimating intake, indirectly through short-term animal behavioral responses, when key criteria are attended (Agreil and Meuret, 2004; Bonnet et al., 2011, 2015; Soares et al., in preparation).

2.4.1 The biting behavior and nutritive value of the herbage apparently ingested

The RN management allowed animals to exert preferential leaf lamina grazing on annual ryegrass plants of 20 ± 2.5cm, and to increase the DM intake from these bites and to reduce it from bites allocated at the bottom of the sward, which were more correlated to the RT management (Figure 2). This behavior was expected, since around this sward height sheep maximizes the intake rate (Fonseca et al., 2013), and also by assuming that animals defoliate the sward taking bites in successive layers (Gastal and Lemaire, 2015), from the top to the bottom of the canopy (Baumont et al., 2004). Moreover, animals select leafs when are not forced to explore lower parts of the canopy, higher in sugars, CP and lipids (Elgersma, 2015), but lower in less preferable steams (Amaral et al., 2013; Barrett et al., 2001; Brunetti et al., 2016; Chacon and Stobbs, 1976; Dias et al., 2017; Euclides et al., 2018; Zanini et al., 2012), with higher fiber content and lower digestibility (Hodgson, 1990; Orr et al., 2004). In agreement, in this study and in Savian et al. (2018), it was observed 14 % higher CP content in the herbage apparently ingested by RN animals. The CP content of the herbage consumed was within the range reported for ryegrass pastures grazed by sheep (Cosgrove et al., 2015; Vasta et al., 2012) or cattle (McEvoy et al., 2009; Wims et al., 2010) and considered to not limit voluntary intake (Van Soest, 1994) on either treatment. The increase (+12%) of total soluble sugar of the RN, but similar NDF, probably resulted from the intrinsically low fiber content of the ryegrass at the stages of growth evaluated. Despite this, the less lignified fiber of the RN diet is consistent with the 2.2 % higher OM digestibility ($P<0.05$; Table 3); this small difference is capable to drive LW gains (Cosgrove et al. 2015) or milk yield (Peyraud and Delagarde, 2013). As with soluble sugar, the 13% increase in crude fat was expected, with values within the range of other grazing trials with ryegrass (Bonanno et al., 2016; Lourenço et al., 2007). Several works showing the vertical gradient of forages' chemical composition (Cano et al., 2004; Delagarde et al., 2000; DIAS et al., 2017; Elgersma, 2015; Hodgson, 1990; Hoogendoorn et al., 1992; Moreira et al., 2004; Orr et al., 2004) support our results indicating that the increased nutritive value of the herbage apparently ingested by RN animals results from the preferential leaf lamina biting behavior, provided the lower sward height depletion.

2.4.2 Organic matter and total nutrients intake

The individual OM intake increased in the RN treatment (Table 3) in the same pattern reported by Savian et al. (2018). According to Dove (2010), as profit derives from outputs per hectare, balancing nutrient supply with requirements should not be attempted on an individual-animal basis, instead a daily penalization of around 10 % of individual intake (DM basis) should be targeted (Peyraud and Delagarde, 2013). In some dairy systems though, the average herbage utilization reaches 93 % (Curran et al., 2010), thus, the intake restriction could arguably surpass 18 %. Conversely, Schons et al. (Unpublished) recently demonstrated that both the herbage production and harvest efficiency are maximized conjunctly with the lenient and frequent RN management. Thereby, we suggest that maximizing individual nutrients intake directly from grazing would occur without the trade-off between individual intake and per hectare harvest efficiency and farm profit. While it is true that offering the pasture conditions permitting animals maximize the STIR rate does not necessarily warrants maximum daily intake, the conditions are ideal for this to happen (Amaral et al., 2013; Carvalho, 2013; Fonseca et al., 2012; Gonçalves et al., 2009; Mezzalira et al., 2014). This is especially timely for high yielding animals (i.e. cows whose intake capacity increases in 0.18 kg/kg of peak milk on good quality pastures; Peyraud et al., 1996), under time-limiting scenarios. Clearly, the daily competence of grazing with other time-consuming behaviors (i.e. ruminating, idling, socializing, displacement), human interventions (i.e. nocturnal housing, milking) or weather conditions (i.e. rain and risk of fouling, heat stress, low pasture growth), could reduce the available eating time and accentuate intake restrictions, specially under scenarios of low STIR (Allden and McDWhittaker, 1970; Barrett et al., 2001; Poppi, 2011).

The 18% higher OM intake of a diet with higher content of soluble nutrients boosted their daily intake (Table 3). In the RN management, the intake of total soluble sugars increased by 28%. Moreover, higher intake of a forage with less fibrousness (less ADF and ADL), resulted in higher intake of digestible OM (+20%). According to CSIRO (2007), the digestible OM intake determines the energy use efficiency for productive purposes. For the RN management, higher OM intake supported higher LW gain and feed conversion efficiency (Schons et al., Unpublished), and better carcass composition (Savian et al., Unpublished), over a 150-days grazing experiment. Higher growth rates were also observed by Cosgrove et al. (2015) with sheep grazing high-sugar ryegrasses.

Increasing CP intake is usually attempted as a mean of improving animal performance; RN animals ingested 29% more of protein. Yet, given the OM digestibility and the CP content, higher than 75 and 21%, respectively for both managements, losses of the ingested-to-metabolizable protein are actually expected (Poppi and McLennan, 1995), especially in the RN management. The rapid/potentially degradable CP of ryegrasses causes that up to 50 % of it be deaminated and lost as ammonia in the rumen (Huntington and Archibeque, 2000), and then excreted in manure (Peyraud and Delagarde, 2013). Nitrogen excretion in feces was 20% higher in RN animals ($P<0.05$; data not shown). This contributes to higher GHG emissions from pastures, as proved in Savian et al. (2019). In that work, the RN management resulted in a 27 and 47 % increase in CO_2-eq emitted from fecal CH_4 and N_2O, per animal and per hectare, respectively; nonetheless, these emissions represented less than 1% of the CO_2-eq emitted as enteric CH_4 (g/ha/day), which was 61 % lower in the RN management (Savian et al., 2018). Despite this, most of the N excreted by animals ingesting forages with high amount of readily soluble N is excreted via urine (Wang et al., 2019; Zhao et al., 2016); hence, further studies are needed to assess how much the urinary N further increase in overall GHG emissions in the RN, including the trade-off between such emissions and the carbon stocked in soil due to increased pasture growth (Shons et al., Submitted). Differently from animals grazing temperate pastures, animals grazing tropical species would be beneficiated with a management promoting higher intake of readily digestible N compounds (McSweeney et al., 1999; Vendramini et al., 2008).

The intake of crude fat by RN animals increased in 25%, respectively. In (Lourenço et al. (2007), the fatty acids (DM basis) of perennial ryegrass grazed by sheep accounted for 68 % of the crude fat content, and DIAS et al. (2017) concluded that leafy grazing is an effective way to increase their intake by lactating cows. Elgersma et al. (2004) observed very quick changes in milk fatty acid composition, in part associated to the greater depth of the grazed horizon; the human-health enhancer C18:2 *cis9-trans-11* reduced in 36 % within a week. Similarly, Coppa et al. (2015) showed a more constant milk fatty acid composition when cows were moved to another paddock before complete herbage utilization, with the opposite management leading to variable and decreasing milk fatty acids content, which is undesirable for dairy farmers receiving incentives for milk quality. From this perspective, it is interesting the benefits that the preferential leaf lamina biting behavior at any time while grazing could bring for milk quality traits.

2.4.3 Nutrition-stress-related blood parameters

Glucose is a short-term indicator of energetic metabolism (Ginane et al., 2015; Ingvartsen and Moyes, 2013). Its concentration on both RN and RT animals (Table 4) are within reference values of adult sheep (Kaneko et al., 2008), and respectively, equivalent to those of animals fed 2.0 and 1.0 times the daily energy exigencies (P>0.05; Caldeira et al., 1999). As well, the RN animals had values similar than the reported for sheep grazing annual ryegrass at a low grazing intensity Macari et al. (2011), and its higher concentration respect RT animals coincides with the findings of Ren et al. (2016) and Costa et al. (2015), comparing high and low grazing intensities, or with animals with body condition scores of 3.0 to 4.0 or 1.2 to 2.0 (P<0.05; Caldeira et al., 2007). Glucose concentration responds positively to the digestible OM intake and associates with growth rate (Raja et al., 1981), which agrees with the higher OM intake (Table 3) and LW gain observed previously on the RN management (Schons et al., Unpublished).

The fructosamine is a glycated protein formed from glucose and mainly albumin, and owing albumin’s half-life of around 14-16 days (Tóthová et al., 2018), the fructosamine is proposed as a marker of the previous 1 to 3 week glucose and albumin concentration (Caré et al., 2018). The 2 % (P>0.05) fructosamine increase in the RN management, despite higher glucose and albumin, could indicate either an eventual meal bout previous blood sampling, encouraged by the non-depleted sward structure, or its eventual insensibility to capture glucose acute oscillations (Jensen et al., 1993), as observed with lactating sheep in the transition period and postpartum (Filipović et al., 2011), or with cows after the first month in lactation (Caré et al., 2018), once the more challenging energy deficit has passed. The latter scenario is more plausible, since other metabolites, also responding to feeding time, were not affected by grazing treatment. Another reason might be that the single glucose sampling did not allow an accurate referencing of glycemia of the previous weeks, in response to the between-day variation in DM intake, likely to occur in grazing conditions.

The serum concentration of cholesterol, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) were within values previously reported for sheep (Caldeira et al., 2007; Costa et al., 2015; Macari et al., 2011), but lower than the reported by Kaneko et al. (2008) for BHB. Energy intake restriction reduces cholesterol levels (Fernandes et al., 2012), triggers the mobilization of fatty acids from adipose tissue and increases serum concentration of NEFA (Ingvartsen and Moyes, 2013) and shortens propionate production (Kronfeld, 1971). This leads to the formation of ketone bodies, mainly BHB (Braun et al., 2010; McGuffey, 2017). In this study, however, the grazing management did not affect their concentration. In partial accordance, Costa et al. (2015) observed no changes in cholesterol, as in this study, but also observed a 34% increase ($P<0.05$) of NEFA between animals with DM intake differences of 10.2%, imposed by contrasting grazing intensities; in this study NEFA tended to increase in RT animals ($P=0.09$). Our study converge with that of Caldeira et al. (2007), who found no effect of feeding sheep to target body condition scores of 1.25 and 3.0 over NEFA concentration, or between animals fed 1.0 or 2.0 times daily energy requirements (Caldeira et al., 1999), although in the latter study, BHB differed between groups. With 18% shortage in OM intake in the RT management, the increase in BHB was not evident, as in Caldeira et al. (2007) with animals differing 2.75 units in their body condition score, or as in Costa et al. (2015) with animals with the above-mentioned intake differences. We suggest that substantial changes in basal cholesterol, NEFA and BHB concentration in blood of low productive non-metabolically challenged adult sheep most likely occur when imposing energy restrictions below maintenance, which is not the case of our study, as animals of both treatments gained weight (data not shown).

The albumin accounts for around 50 % of plasma protein and is indicative of mid-term protein status (9 days; Tóthová et al., 2018), as PUN is of readily dietary N availability (Kenny et al., 2001; Marini et al., 2004). Both variables were above the superior limit of 30 g/L and 20.7 mg/dL, respectively, reported by Kaneko et al. (2008) for adult sheep. Both metabolites response directly to dietary protein, thus they show excessive N intake in both treatments, with the RN animals having increased the albumin in 9.4% and PUN in 14.5% ($P<0.05$). PUN level on RN and RT animals, 26.9 and 31.2 mg/dL, respectively, are comparable to the value reported by Speijers et al., 2004) on sheep grazing temperate pastures with a CP content similar of this study (25 % CP and 33.1 mg/dL of PUN). Lower albumin might also be explained by higher infestation of *Haemonchus contortus* (Braun et al., 2010), as previously observed in RT animals (Schons et al., Unpublished). PUN level is positively related with milk urea nitrogen ($r = 0.82$, $P < 0.001$; Butler, 1998). Thereby, high MUN or PUN levels, as confirmed by farmers where the RN management is adopted (Carvalho, personal communication), could associate with reproductive inefficiencies (Butler, 1998; Elrod and Butler, 1993). For instance, the PUN levels that have shown to impair sheep reproductive traits are of 14.6 mg/dL (BISHONGA et al., 1996; Fahey et al., 2001; McEvoy et al., 1997), which is well below of values here reported. Cattle seem to be more tolerable. Elrod and Butler (1993) and Sinclair et al. (2000) associated impaired reproductive traits to dairy cows with 15 to >19 md/dL of PUN, or with ≥ 25 mg/dL (Ferguson et al., 1993), which agrees with the meta-analysis threshold value of 19.3 mg/dL suggested by Raboisson et al. (2017). Despite this evidence, others studies did not observe these negative effect, though at PUN concentrations below the reported in this study with sheep (Chapa et al., 2001; Garcia-Bojalil et al., 1994; Kenny et al., 2001). PUN-related reproductive inefficiency is perceived as a problem of intense dairy farming (Butler, 1998). However, Fariña and Chilibroste (2019) noticed the poor reproductive performance of Holstein cows on some temperate pasture-based herds in Uruguay, and Wittwer et al. (1999) confirmed this by associating high PUN levels with low conception rates in grazing dairy herds during spring, in Chile. Preventing measures can be easily adopted though. For instance, Albaaj et al. (2017) confirmed the association between reproduction inefficiencies and high PUN levels before the time of artificial insemination, as also suggested by the meta-analysis of Raboisson et al. (2017). Thus, a strategy would be avoiding feeding regimes increasing PUN level at that time, with especial attention put on animals with negative energy balance, over of which the effect urea could be exacerbated (Chapa et al., 2001; Garcia-Bojalil et al., 1994).

Stressful responses (immunosuppression) result from the activation of the pituitary-adrenal axis (Dhabhar et al., 1996; Tornquist and Rigas, 2010). Within immune cells, neutrophils participates in phagocytosis, produce reactive oxygen species (highly toxic for engulfed bacteria) and antibacterial enzymes as part of the innate immune system (Sordillo, 2016). In situations of long-term stress, the neutrophil to lymphocyte ratio increases (Dhabhar et al., 1996), as a signal of increased levels of glucocorticoids in plasma (Bayes and Kramer, 2010; Tornquist and Rigas, 2010). Small nutritional imbalances, such as feeding animals monotonous diets (Catanese et al., 2013) or delaying stall-feeding (González et al., 2009), can affect neutrophils formation and function (Ingvartsen and Moyes, 2013), as also suggested for animals under poor nutrition

(Collier et al., 2017). In this study, with 18% less OM intake (P>0.001) of the RT animals, we found a 19.5% increase in the N:L ratio (P<0.0486), which supports our hypothesis that grazing systems aiming to maximize herbage harvest efficiency, hence assuming a penalization of individual daily intake, could impair animal welfare, even when access to pasture and no supplementation is allowed. This response was also observed by Costa et al. (2015) with sheep managed under severe grazing intensity, even with LW gains going from 59 to 73 g/animal, and by Ramírez-Restrepo et al. (2010), with grazing hoggets with 36 % lower LW gains (65 vs 102 g/d) than their counterparts with improved nutrition from pasture. In Ren et al. (2016) an overgrazing condition triggered differential expression of hepatic proteins involved in immune response and inflammatory cytokines of sheep. The absolute value and proportion of other leukocytes (i.e. eosinophil, basophils and monocytes), remained similar between treatments, and within the reference values reported by Bayes and Kramer (2010).

Low concentration of alkaline phosphatase can be associated with feed intake restriction, as occurred with high stocked grazing sheep (Thamsborg and Hauge, 2001). Nonetheless, its decrease in RT animals was only of 7.6 % and not significant. Similarly, Caldeira et al. (1999) found no difference on its concentration between animals fed 1.0 and 2.0 times energy requirements for maintenance, but a 44 % decrease respect those fed 0.3 times requirements. As with serum energy metabolites, it is conceivable the necessity of a deeper feed restriction to actually decrease its concentration.

2.5. Conclusion

The high frequency/low intensity grazing model (RN) permitted animals to compound their diet preferentially from bites performed on plant with 20cm, thus to preferentially ingest leaf lamina, with higher nutritive value, when compared to a low frequency/high intensity grazing model (RT), whose daily DM intake is majorly composed from bites performed from plant of 15 cm or lower. The excessive sward height depletion in the RT grazing results in lower individual intake of organic matter and soluble nutrients, and negatively effects of some nutritional and welfare-related blood variables of animals with low nutrients exigencies and grazing highly nutritive pastures.

2.6 Consulted literature

Agreil, C., Meuret, M., 2004. An improved method for quantifying intake rate and ingestive behaviour of ruminants in diverse and variable habitats using direct observation. Small Rumin. Res. 54, 99–113. https://doi.org/10.1016/j.smallrumres.2003.10.013

Albaaj, A., Foucras, G., Raboisson, D., 2017. Changes in milk urea around insemination are negatively associated with conception success in dairy cows. J. Dairy Sci. 100, 3257–3265. https://doi.org/10.3168/jds.2016-12080

Allden, W., McDWhittaker, I., 1970. The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. Aust. J. Agric. Res. 21, 755. https://doi.org/10.1071/AR9700755

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

AOAC. 1980. Official Methods of Analysis (13th Ed.). Association of Official Analytical Chemestry. Arlintong, VA. USA. 1018 p.

Azevedo, E.B., Poli, C., David, D.B., Amaral, G.A., Fonseca, L., Carvalho, P.C.F., Fischer, V., Morris, S.T., 2014. Use of faecal components as markers to estimate intake and digestibility of grazing sheep. Livest. Sci. 165, 42–50.

https://doi.org/10.1016/j.livsci.2014.04.018

Bargo, F., Muller, L.D., Kolver, E.S., Delahoy, J.E., 2003. Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. J. Dairy Sci. 86, 1–42. https://doi.org/10.3168/jds.S0022-0302(03)73581-4

Barrett, P.D., Laidlaw, A.S., Mayne, C.S., Christie, H., 2001. Pattern of herbage intake rate and bite dimensions of rotationally grazed dairy cows as sward height declines. Grass Forage Sci. 56, 362–373. https://doi.org/10.1046/j.1365-2494.2001.00286.x

Barthram, G. T. 1985. Experimental techniques: the HFRO sward stick. In: the Hill Farming Research Organization Biennial Report, 1984/1985. HFRO, Penicuik, pp.29-30.

Baumont, R., Cohen-Salmon, D., Prache, S., Sauvant, D., 2004. A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. Anim. Feed Sci. Technol. 112, 5–28. https://doi.org/10.1016/j.anifeedsci.2003.10.005

Baumont, R., Prache, S., Meuret, M., Morand-Fehr, P., 2000. How forage characteristics influence behaviour and intake in small ruminants: a review. Livest. Prod. Sci. 64, 15–28. https://doi.org/10.1016/S0301-6226(00)00172-X

Byers, S.R. and Kramer, J.W., 2010. Normal hematology of sheep and goats. *Schalm's Veterinary Hematology*, *6*, pp.836-842.

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

BISHONGA, C., ROBINSON, J.J., MCEVOY, T.G., FINDLAY, P., AITKEN, R.P., ROBERTSON, I., 1996. Excess dietary urea intake in ewes and its effect on ovulation rate and embryo development. Jpn. J. Vet. Res. 44, 139–151.

Boland, T.M., Quinlan, C., Pierce, K.M., Lynch, M.B., Kenny, D.A., Kelly, A.K., Purcell, P.J., 2013. The effect of pasture pregrazing herbage mass on methane emissions, ruminal fermentation, and average daily gain of grazing beef heifers1. J. Anim. Sci. 91, 3867–3874. https://doi.org/10.2527/jas.2013-5900

Bonanno, A., Di Grigoli, A., Mazza, F., De Pasquale, C., Giosuè, C., Vitale, F., Alabiso, M., 2016. Effects of ewes grazing sulla or ryegrass pasture for different daily durations on forage intake, milk production and fatty acid composition of cheese. animal 10, 2074–2082. https://doi.org/10.1017/S1751731116001130

Bonnet, O., Hagenah, N., Hebbelmann, L., Meuret, M., Shrader, A.M., 2011. Is Hand Plucking an Accurate Method of Estimating Bite Mass and Instantaneous Intake of

Grazing Herbivores? Rangel. Ecol. Manag. 64, 366–374. https://doi.org/10.2111/REM-D-10-00186.1

Bonnet, O.J.F., Meuret, M., Tischler, M.R., Cezimbra, I.M., Azambuja, J.C.R., Carvalho, P.C.F., 2015. Continuous bite monitoring: a method to assess the foraging dynamics of herbivores in natural grazing conditions. Anim. Prod. Sci. 55, 339. https://doi.org/10.1071/AN14540

Braun, J.P., Trumel, C., Bézille, P., 2010. Clinical biochemistry in sheep: A selected review. Small Rumin. Res. 92, 10–18. https://doi.org/10.1016/j.smallrumres.2010.04.002

Brunetti, H.B., Carvalho, L. da R., Chiavegato, M.B., Silva, S.C. da, 2016. Sward structure, light interception and herbage accumulation in forage peanut cv. Belmonte subjected to strategies of intermittent grazing management. Acta Sci. Anim. Sci. 38, 395. https://doi.org/10.4025/actascianimsci.v38i4.32207

Butler, W.R., 1998. Review: Effect of Protein Nutrition on Ovarian and Uterine Physiology in Dairy Cattle. J. Dairy Sci. 81, 2533–2539. https://doi.org/10.3168/jds.S0022-0302(98)70146-8

Caldeira, R.M., Almeida, M.A., Santos, C.C., Vasques, M.I., Portugal, A.V., 1999. Daily variation in blood enzymes and metabolites in ewes under three levels of feed intake. Can. J. Anim. Sci. 79, 157–164. https://doi.org/10.4141/A97-083

Caldeira, R.M., Belo, A.T., Santos, C.C., Vazques, M.I., Portugal, A.V., 2007. The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Rumin. Res. 68, 233–241. https://doi.org/10.1016/j.smallrumres.2005.08.027

Caré, S., Trevisi, E., Minuti, A., Ferrari, A., Loor, J.J., Calamari, L., 2018. Plasma fructosamine during the transition period and its relationship with energy metabolism and inflammation biomarkers in dairy cows. Livest. Sci. 216, 138–147.

Carvalho, P.C.D.F., 2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? Trop. Grasslands - Forrajes Trop. 1, 137. https://doi.org/10.17138/TGFT(1)137-155

Catanese, F., Obelar, M., Villalba, J.J., Distel, R.A., 2013. The importance of diet choice on stress-related responses by lambs. Appl. Anim. Behav. Sci. 148, 37–45. https://doi.org/10.1016/j.applanim.2013.07.005

Chacon, E., Stobbs, Th., 1976. Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. Aust. J. Agric. Res. 27, 709. https://doi.org/10.1071/AR9760709

Chapa, A.M., McCormick, M.E., Fernandez, J.M., French, D.D., Ward, J.D., Beatty, J.F., 2001. Supplemental Dietary Protein for Grazing Dairy Cows: Reproduction, Condition

Loss, Plasma Metabolites, and Insulin. J. Dairy Sci. 84, 908–916. https://doi.org/10.3168/jds.S0022-0302(01)74548-1

Collier, R.J., Renquist, B.J., Xiao, Y., 2017. A 100-Year Review: Stress physiology including heat stress. J. Dairy Sci. 100, 10367–10380. https://doi.org/10.3168/jds.2017-13676

Congio, G.F.S., Batalha, C.D.A., Chiavegato, M.B., Berndt, A., Oliveira, P.P.A., Frighetto, R.T.S., Maxwell, T.M.R., Gregorini, P., Da Silva, S.C., 2018. Strategic grazing management towards sustainable intensification at tropical pasture-based dairy systems. Sci. Total Environ. 636, 872–880. https://doi.org/10.1016/j.scitotenv.2018.04.301

Congio, G.F.S., Chiavegato, M.B., Batalha, C.D.A., Oliveira, P.P.A., Maxwell, T.M.R., Gregorini, P., Da Silva, S.C., 2019. Strategic grazing management and nitrous oxide fluxes from pasture soils in tropical dairy systems. Sci. Total Environ. 676, 493–500. https://doi.org/10.1016/j.scitotenv.2019.04.186

Coppa, M., Farruggia, A., Ravaglia, P., Pomiès, D., Borreani, G., Le Morvan, A., Ferlay, A., 2015. Frequent moving of grazing dairy cows to new paddocks increases the variability of milk fatty acid composition. Animal 9, 604–613. https://doi.org/10.1017/S1751731114003000

Cosgrove, G.P., Taylor, P.S., Jonker, A., 2015. Sheep performance on perennial ryegrass cultivars differing in concentration of water-soluble carbohydrate. J. New Zeal. Grasslands Assoc. 77, 123–129.

Costa, J.P.R., Silva, W.L. da, Paula, G.C. de, Tsuzukibashi, D., Malheiros, E.B., Ruggieri, A.C., Negrão, J.A., 2015. The effect of grazing intensity and supplementation on performance, stress indicators and metabolic profiles of finishing lambs. Semin. Ciências Agrárias 36, 3863. https://doi.org/10.5433/1679-0359.2015v36n6p3863

CSIRO, 2007. Nutrient requirements of domesticated ruminants. CSIRO publishing.

Curran, J., Delaby, L., Kennedy, E., Murphy, J.P., Boland, T.M., O’Donovan, M., 2010. Sward characteristics, grass dry matter intake and milk production performance are affected by pre-grazing herbage mass and pasture allowance. Livest. Sci. 127, 144–154. https://doi.org/10.1016/j.livsci.2009.09.004

da Silva, S., Sbrissia, A., Pereira, L., 2015. Ecophysiology of C4 Forage Grasses—Understanding Plant Growth for Optimising Their Use and Management. Agriculture 5, 598–625. https://doi.org/10.3390/agriculture5030598

Da Silva, S.C., Carvalho, P.C. de F., 2005. Foraging behaviour and herbage intake in the favourable tropics/sub-tropics. Grassl. a Glob. Resour. 1, 81–95.

da Silveira, M.C.T., Júnior, D. do N., Rodrigues, C.S., Pena, K. da S., Júnior, S.J. de S., Barbero, L.M., Limão, V.A., Euclides, V.P.B., da Silva, S.C., 2016. Forage sward structure of Mulato grass (Brachiaria hybrid ssp.) subjected to rotational stocking strategies. Aust. J. Crop Sci. 10, 864–873. https://doi.org/10.21475/ajcs.2016.10.06.p7568

Decruyenaere, V., Lecomte, P., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim. Feed Sci. Technol. 148, 138–156. https://doi.org/10.1016/j.anifeedsci.2008.03.007

Delagarde, R., Peyraud, J.., Delaby, L., Faverdin, P., 2000. Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Anim. Feed Sci. Technol. 84, 49–68. https://doi.org/10.1016/S0377-8401(00)00114-0

DeRamus, H.A., 2004. Grazing Management of Ruminant Animals in Sustainable Agriculture. Outlook Agric. 33, 93–100. https://doi.org/10.5367/000000004773973073

DeRamus, H.A., Clement, T.C., Giampola, D.D., Dickison, P.C., 2003. Methane Emissions of Beef Cattle on Forages. J. Environ. Qual. 32, 269–277. https://doi.org/10.2134/jeq2003.2690

Dhabhar, F.S., Miller, A.H., McEwen, B.S., Spencer, R.L., 1996. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. J. Immunol. 157, 1638–1644.

Dias, K.M., Schmitt, D., Rodolfo, G.R., Deschamps, F.C., Camargo, G.N., Pereira, R.S., SBRISSIA, A.F., 2017. Fatty acid profile in vertical strata of elephant grass subjected to intermittent stocking. An. Acad. Bras. Cienc. 89, 1707–1718. https://doi.org/10.1590/0001-3765201720150272

Dove, H., 2010. Balancing nutrient supply and nutrient requirements in grazing sheep. Small Rumin. Res. 92, 36–40. https://doi.org/10.1016/j.smallrumres.2010.04.004

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

Elgersma, A., Ellen, G., Tamminga, S., 2004. Rapid decline of contents of beneficial omega-7 fatty acids in milk from grazing cows with decreasing herbage allowance. Grassl. Sci. Eur 9, 1136–1138.

Elrod, C.C., Butler, W.R., 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein1. J. Anim. Sci. 71, 694–701.

https://doi.org/10.2527/1993.713694x

Euclides, V.P.B., Carpejani, G.C., Montagner, D.B., Nascimento Junior, D., Barbosa, R.A., Difante, G.S., 2018. Maintaining post-grazing sward height of Panicum maximum (cv. Mombaça) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. Grass Forage Sci. 73, 174–182. https://doi.org/10.1111/gfs.12292

Fahey, J., Boland, M.P., O'Callaghan1, D., 2001. The effects of dietary urea on embryo development in superovulated donor ewes and on early embryo survival and development in recipient ewes. Anim. Sci. 72, 395–400. https://doi.org/10.1017/S1357729800055892

Fariña, S.R., Chilibroste, P., 2019. Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. Agric. Syst. 176, 102631. https://doi.org/10.1016/j.agsy.2019.05.001

Ferguson, J.D., Galligan, D.T., Blanchard, T., Reeves, M., 1993. Serum Urea Nitrogen and Conception Rate: The Usefulness of Test Information. J. Dairy Sci. 76, 3742–3746. https://doi.org/10.3168/jds.S0022-0302(93)77716-4

Fernandes, S.R., Monteiro, A.L.G., Dittrich, R.L., Salgado, J.A., Silva, C.J.A. da, Silva, M.G.B. da, Beltrame, O.C., Pinto, P.H.N., 2012. Early weaning and concentrate supplementation on the performance and metabolic profile of grazing lambs. Rev. Bras. Zootec. 41, 1292–1300. https://doi.org/10.1590/S1516-35982012000500029

Filipović, N., Stojević, Z., Mašek, T., Mikulec, Ž., Prvanović, N., 2011. Relationship between fructosamine with serum protein, albumin and glucose concentrations in dairy ewes. Small Rumin. Res. 96, 46–48. https://doi.org/10.1016/j.smallrumres.2010.11.003

Fonseca, L., Carvalho, P.C. de F., Mezzalira, J.C., Bremm, C., Galli, J.R., Gregorini, P., 2013. Effect of sward surface height and level of herbage depletion on bite features of cattle grazing Sorghum bicolor swards. J. Anim. Sci. 91, 4357–4365. https://doi.org/10.2527/jas2013-5602

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

Fulkerson, W.J., Slack, K., Havilah, E., 1999. The effect of defoliation interval and height on growth and herbage quality of kikuyu grass (Pennisetum clandestinum). Trop. Grasslands 33, 138–145.

Ganche, E., Delaby, L., O'Donovan, M., Boland, T.M., Galvin, N., Kennedy, E., 2013. Post-grazing sward height imposed during the first 10 weeks of lactation: Influence on early and total lactation dairy cow production, and spring and annual sward characteristics. Livest. Sci. 157, 299–311. https://doi.org/10.1016/j.livsci.2013.08.001

Garcia-Bojalil, C.M., Staples, C.R., Thatcher, W.W., Drost, M., 1994. Protein Intake and Development of Ovarian Follicles and Embryos of Superovulated Nonlactating Dairy Cows. J. Dairy Sci. 77, 2537–2548. https://doi.org/10.3168/jds.S0022-0302(94)77195-2

Gastal, F., Lemaire, G., 2015. Defoliation, Shoot Plasticity, Sward Structure and Herbage Utilization in Pasture: Review of the Underlying Ecophysiological Processes. Agriculture 5, 1146–1171. https://doi.org/10.3390/agriculture5041146

Ginane, C., Bonnet, M., Baumont, R., Revell, D.K., 2015. Feeding behaviour in ruminants: a consequence of interactions between a reward system and the regulation of metabolic homeostasis. Anim. Prod. Sci. 55, 247. https://doi.org/10.1071/AN14481

Goncalves, E.N., Carvalho, P.D.F., Kunrath, T.R., Carassai, I.J., Bremm, C. and Fischer, V., 2009. Plant-animal relationships in pastoral heterogeneous environment: process of herbage intake. *Revista Brasileira de Zootecnia*, *38*(9), pp.1655-1662.

González, L.A., Correa, L.B., Ferret, A., Manteca, X., Ruíz-de-la-Torre, J.L., Calsamiglia, S., 2009. Intake, water consumption, ruminal fermentation, and stress response of beef heifers fed after different lengths of delays in the daily feed delivery time. J. Anim. Sci. 87, 2709–2718. https://doi.org/10.2527/jas.2008-1709

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

Hodgson, J. (1990). *Grazing management. Science into practice*. Longman Group UK Ltd.

Hoogendoorn, C.J., Holmes, C.W., Chu, A.C.P., 1992. Some effects of herbage composition, as influenced by previous grazing management, on milk production by cows grazing on ryegrass/white clover pastures. 2. Milk production in late spring/summer: effects of grazing intensity during the preceding spring per. Grass Forage Sci. 47, 316–325. https://doi.org/10.1111/j.1365-2494.1992.tb02277.x

Huntington, G.B., Archibeque, S.L., 2000. Practical aspects of urea and ammonia metabolism in ruminants. J. Anim. Sci. 77, 1. https://doi.org/10.2527/jas2000.77E-Suppl1y

Ingvartsen, K.L., Moyes, K., 2013. Nutrition, immune function and health of dairy cattle. animal 7, 112–122. https://doi.org/10.1017/S175173111200170X

Jensen, A.L., Petersen, M.B., Houe, H., 1993. Determination of the Fructosamine Concentration in Bovine Serum Samples. J. Vet. Med. Ser. A 40, 111–117.

https://doi.org/10.1111/j.1439-0442.1993.tb00607.x

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. eds., 2008. *Clinical biochemistry of domestic animals*. Academic press.

Kenny, D.A., Boland, M.P., Diskin, M.G., Sreenan, J.M., 2001. Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and on embryo survival in heifers. Anim. Sci. 73, 501–511. https://doi.org/10.1017/S1357729800058471

Kronfeld, D.S., 1971. Hypoglycemia in Ketotic Cows. J. Dairy Sci. 54, 949–961. https://doi.org/10.3168/jds.S0022-0302(71)85951-9

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

Lobato, J.F.P., Freitas, Ak., Devincenzi, T., Cardoso, L.L., Tarouco, J.U., Vieira, R.M., Dillenburg, D.R., Castro, I., 2014. Brazilian beef produced on pastures: Sustainable and healthy. Meat Sci. 98, 336–345. https://doi.org/10.1016/j.meatsci.2014.06.022

Lourenço, M., Van Ranst, G., De Smet, S., Raes, K., Fievez, V., 2007. Effect of grazing pastures with different botanical composition by lambs on rumen fatty acid metabolism and fatty acid pattern of longissimus muscle and subcutaneous fat. animal 1, 537–545. https://doi.org/10.1017/S1751731107703531

Macari, S., Carvalho, P.C. de F., Oliveira, L. de, Devincenzi, T., Albuquerque, C., Moraes, A. de, 2011. Recria de borregas sob diferentes métodos de pastoreio em azevém anual em sucessão a lavoura. Pesqui. Agropecuária Bras. 46, 1401–1408. https://doi.org/10.1590/S0100-204X2011001000038

McEvoy, M., O'Donovan, M., Kennedy, E., Murphy, J.P., Delaby, L., Boland, T.M., 2009. Effect of pregrazing herbage mass and pasture allowance on the lactation performance of Holstein-Friesian dairy cows. J. Dairy Sci. 92, 414–422. https://doi.org/10.3168/jds.2008-1313

McEvoy, T.G., Robinson, J.J., Aitken, R.P., Findlay, P.A., Robertson, I.S., 1997. Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. Anim. Reprod. Sci. 47, 71–90. https://doi.org/10.1016/S0378-4320(96)01627-2

McGuffey, R.K., 2017. A 100-Year Review: Metabolic modifiers in dairy cattle nutrition. J. Dairy Sci. 100, 10113–10142. https://doi.org/10.3168/jds.2017-12987

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

86, 1159–1168. https://doi.org/10.1111/1365 -2656.12698

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

Moreira, F.., Prado, I.., Cecato, U., Wada, F.., Mizubuti, I.., 2004. Forage evaluation, chemical composition, and in vitro digestibility of continuously grazed star grass. Anim. Feed Sci. Technol. 113, 239–249. https://doi.org/10.1016/j.anifeedsci.2003.08.009

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

Orr, R.J., Rutter, S.M., Yarrow, N.H., Champion, R.A., Rook, A.J., 2004. Changes in ingestive behaviour of yearling dairy heifers due to changes in sward state during grazing down of rotationally stocked ryegrass or white clover pastures. Appl. Anim. Behav. Sci. 87, 205–222. https://doi.org/10.1016/j.applanim.2004.01.009

Penati, M.A., Corsi, M., Congio, G.F. de S., Almeida, P.C. de, Goulart, R.C.D., Shiota, M.M., 2014. Effects of post-grazing forage mass on a beef cattle grazing system on Tanzânia grass pastures. Rev. Bras. Zootec. 43, 296–300. https://doi.org/10.1590/S1516-35982014000600003

Peyraud, J.L., Comeron, E.A., Wade, M.H., Lemaire, G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows.

Penning, P.D., 2004. Animal-based techniques for estimating herbage intake. In: Penning, P.D. (Ed.), Herbage Intake Handbook seconded., British GrasslandSociety,Reading,pp.53–93.

Peyraud, J.L., Delagarde, R., 2013. Managing variations in dairy cow nutrient supply under grazing. animal 7, 57–67. https://doi.org/10.1017/S1751731111002394

Poppi, D.P., McLennan, S.R., 1995. Protein and energy utilization by ruminants at pasture. J. Anim. Sci. 73, 278. https://doi.org/10.2527/1995.731278x

Provenza, F.D., Kronberg, S.L., Gregorini, P., 2019. Is Grassfed Meat and Dairy Better for Human and Environmental Health? Front. Nutr. 6. https://doi.org/10.3389/fnut.2019.00026

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raboisson, D., Albaaj, A., Nonne, G., Foucras, G., 2017. High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold. J. Dairy Sci. 100, 7581–7587. https://doi.org/10.3168/jds.2016-12009

Raja, R.H., Moss, R., Murray, R.M., Leng, R.A., 1981. Growth and glucose metabolism in young calves grazing tropical pasture-the effects of supplementation with maize or cottonseed cake. Trop. Anim. Prod. 105, 115.

Ramírez-Restrepo, C.A., Barry, T.N., Marriner, A., López-Villalobos, N., McWilliam, E.L., Lassey, K.R., Clark, H., 2010. Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. Anim. Feed Sci. Technol. 155, 33–43. https://doi.org/10.1016/j.anifeedsci.2009.10.003

Ren, W., Hou, X., Wang, Y., Badgery, W., Li, X., Ding, Y., Guo, H., Wu, Z., Hu, N., Kong, L., Chang, C., Jiang, C., Zhang, J., 2016. Overgrazing induces alterations in the hepatic proteome of sheep (Ovis aries): an iTRAQ-based quantitative proteomic analysis. Proteome Sci. 15, 2. https://doi.org/10.1186/s12953-016-0111-z

Romera, A.J., Doole, G.J., 2015. Optimising the interrelationship between intake per cow and intake per hectare. Anim. Prod. Sci. 55, 384. https://doi.org/10.1071/AN14424

Savian, J.V., Priano, M.E., Nadin, L.B., Tieri, M.P., Schons, R.M.T., Basso, C., Pontes Prates, A., Bayer, C., Carvalho, P.C. de F., 2019. Effect of sward management on the emissions of CH4 and N2O from faeces of sheep grazing Italian ryegrass pastures. Small Rumin. Res. 178, 123–128. https://doi.org/10.1016/j.smallrumres.2019.08.011

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

Sinclair, K.D., Kuran, M., Gebbie, F.E., Webb, R., McEvoy, T.G., 2000. Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. J. Anim. Sci. 78, 2670. https://doi.org/10.2527/2000.78102670x

Sordillo, L.M., 2016. Nutritional strategies to optimize dairy cattle immunity. J. Dairy Sci. 99, 4967–4982. https://doi.org/10.3168/jds.2015-10354

SPEIJERS, M.H.M., FRASER, M.D., THEOBALD, V.J., HARESIGN, W., 2004. The effects of grazing forage legumes on the performance of finishing lambs. J. Agric. Sci. 142, 483–493. https://doi.org/10.1017/S0021859604004496

Thamsborg, S.M., Hauge, E.M., 2001. Osteopenia and Reduced Serum Alkaline Phosphatase Activity in Grazing Lambs Naturally Infected with Gastrointestinal Nematodes. J. Comp. Pathol. 125, 192–203. https://doi.org/10.1053/jcpa.2001.0498

Tornquist, S.J. and Rigas, J., 2010. Interpretation of ruminant leukocyte responses. *Schalm's veterinary hematology. Ames: Wiley-Blackwell*, pp.307-13.

Tóthová, C., Mihajlovičová, X., Nagy, O., 2018. The Use of Serum Proteins in the Laboratory Diagnosis of Health Disorders in Ruminants, in: Ruminants - The Husbandry, Economic and Health Aspects. InTech. https://doi.org/10.5772/intechopen.72154

Van Soest, P.J., 2014. *Nutritional ecology of the ruminant*. Cornell university press.

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2018. Effect of concentrate feeding level on methane emissions, production performance and rumen fermentation of Jersey cows grazing ryegrass pasture during spring. Anim. Feed Sci. Technol. 241, 121–132. https://doi.org/10.1016/j.anifeedsci.2018.04.025

Vasta, V., Pagano, R.I., Luciano, G., Scerra, M., Caparra, P., Foti, F., Cilione, C., Biondi, L., Priolo, A., Avondo, M., 2012. Effect of morning vs. afternoon grazing on intramuscular fatty acid composition in lamb. Meat Sci. 90, 93–98. https://doi.org/10.1016/j.meatsci.2011.06.009

Vendramini, J.M.B., Sollenberger, L.E., Adesogan, A.T., Dubeux, J.C.B., Interrante, S.M., Stewart, R.L., Arthington, J.D., 2008. Protein Fractions of Tifton 85 and Rye-Ryegrass Due to Sward Management Practices. Agron. J. 100, 463–469. https://doi.org/10.2134/agronj2007.0143

Wang, C., Zhao, Y., Aubry, A., Arnott, G., Hou, F., Yan, T., 2019. Effects of concentrate input on nutrient utilization and methane emissions of two breeds of ewe lambs fed fresh ryegrass. Transl. Anim. Sci. 3, 485–492. https://doi.org/10.1093/tas/txy106

Wims, C.M., Deighton, M.H., Lewis, E., O'Loughlin, B., Delaby, L., Boland, T.M., O'Donovan, M., 2010. Effect of pregrazing herbage mass on methane production, dry matter intake, and milk production of grazing dairy cows during the mid-season period. J. Dairy Sci. 93, 4976–4985. https://doi.org/10.3168/jds.2010-3245

Wittwer, F.G., Gallardo, P., Reyes, J., Opitz, H., 1999. Bulk milk urea concentrations and their relationship with cow fertility in grazing dairy herds in Southern Chile. Prev. Vet. Med. 38, 159–166. https://doi.org/10.1016/S0167-5877(98)00121-4

Zhao, Y.G., Gordon, A.W., O'Connell, N.E., Yan, T., 2016. Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep offered fresh perennial ryegrass (Lolium perenne)1. J. Anim. Sci. 94, 5321–5331. https://doi.org/10.2527/jas.2016-0541

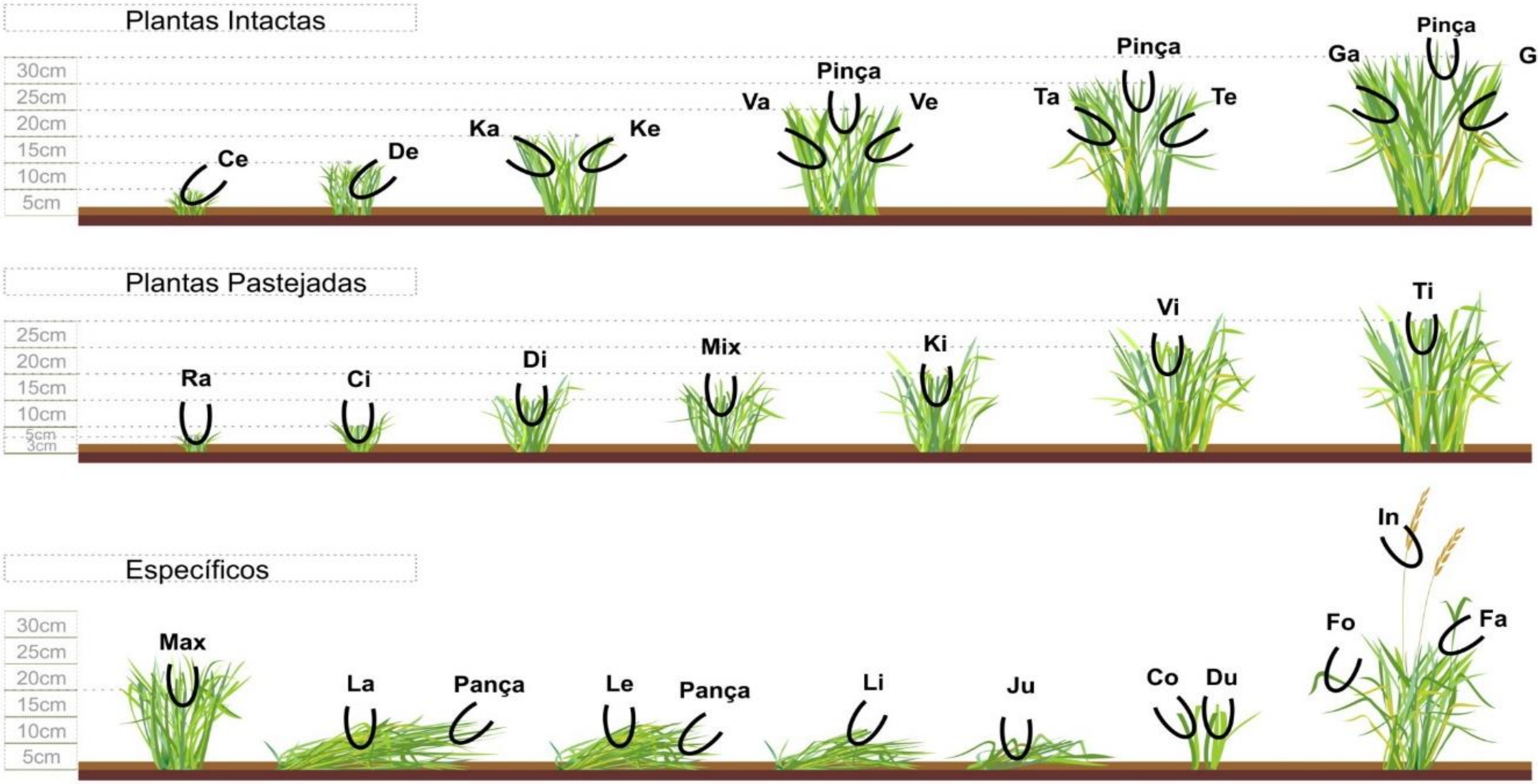


Figure 1. Bite-code grid of *Lolium multiflorum* Lam., performed by sheep grazing under contrasting grazing managements in rotational stocking. The pictogram illustrates the "ideal bite" for each bite category. The consonants "C, D, K, V, T and G" indicates, respectively, plants of 5, 10, 15, 20, 25 and ≥ 25 cm (± 2 cm, except for "Ga" and "Ge" with a deviation of +5 cm). Otherwise indicated, the vowels "a" and "e" after the consonants indicate bites performed on "intact" leafs, with "a" also indicating dense bites, while "e" being indicative of less dense bites. The vowel "i" indicates "grazed" leafs with the presence or not of stems. The bite "Ra" is a bite performed on plants ≤3cm with minimum or no presence of leafs. The "Max" and "Mix" are bites allocated on grazed/non-grazed leafs, with the presence or not of stem, at sward height ≥ or ≤ 15 cm, respectively. The "Pinca" is a bite allocated at the tip (superficial) of a single or no more than two leafs, intact or grazed, and at any sward height; the "Panca" is the same bite but on a lying plant. The "La" and "Le" are bites on lying leafs ≥ or ≤ 20 cm, respectively, with average density of 2 to 3 leafs. The "Li" is a bite on 2 to 3 grazed lying leafs of any horizontal length. The "Co" and "Du" are bites performed on a single or on two defoliated stems, respectively, at sward height between 5 and 15 cm. The "Fo" is a bite performed on a single leaf within the canopy, whereas the "Fa" is a bite performed on the "flag" leaf below the inflorescence. The "In" is a bite on inflorescence. The "Ju" is a bite given on a trampled plant, in which animals manipulate and gather between 2 and 4 leafs.

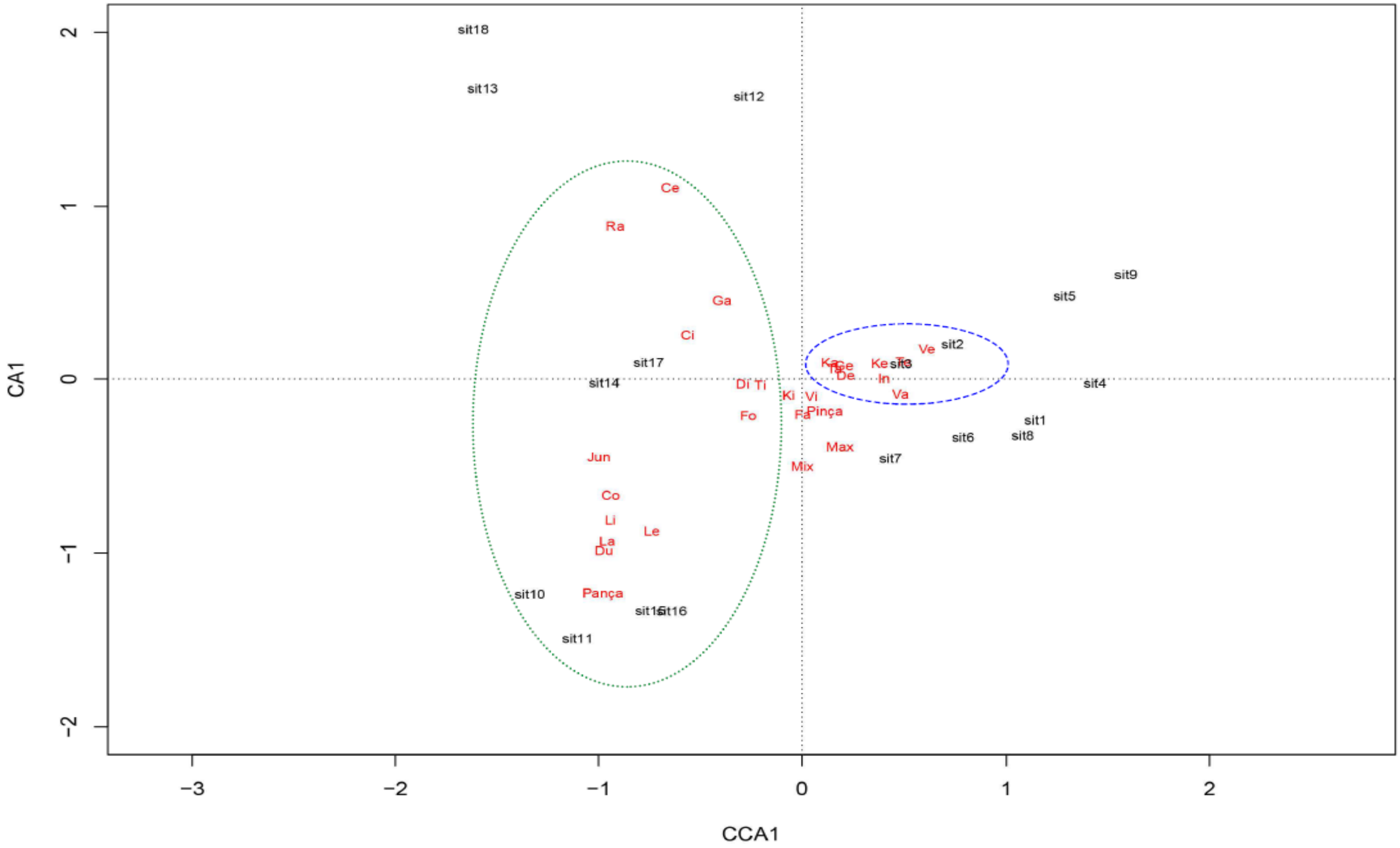


Figure 2. Canonical Correlation Analysis (CCA). Ordination diagram of the bite types with the grazing management: RN (blue dotted line), RT (green dotted line).

Table 1. Sward surface height (cm) and sward height depletion (%) of Italian ryegrass pastures grazed by lambs under contrasting grazing management strategies in a rotational stocking.

Variables	RN	RT	SD	P-value
Pre-grazing	19.4	27.3	4.36	<0.0001
Post-grazing	12.2	6.9	3.03	<0.0001
Sward height depletion	37.3	74.7	0.20	<0.0001

RN = Rotatinuous stocking; RT = Traditional rotational stocking; SD= Standard deviation.

Table 2. Bite-scale characterization (relative proportion that each bite accounted for the accumulated DM intake during the bite-monitoring time in 2017) of the diet apparently ingested by sheep grazing Italian ryegrass pastures under contrasting management in rotational stocking.

Bite Type	Full vegetative[1]		Early shoot elongation[2]	
	RN, %	RT, %	RN, %	RT, %
Ce	0.91	2.21	0.47	4.75
Ci	4.48	13.11	2.41	12.22
Co	0.01	0.23	0.01	0.38
De	3.86	2.10	2.75	2.27
Di	8.12	13.25	6.34	13.44
Du	--	0.05	0.00	0.12
Fa	0.23	0.17	0.19	0.26
Fo	0.08	0.14	0.08	0.13
Ga	0.26	0.96	1.06	1.98
Ge	2.05	1.96	5.04	2.55
In	0.02	0.00	0.00	0.01
Jun	--	1.41	0.00	1.72
Ka	0.24	0.52	1.07	0.39
Ke	13.79	4.19	8.60	6.27
Ki	6.03	7.24	5.84	6.17
La	--	1.68	0.08	1.68
Le	0.17	3.19	0.97	3.87
Li	0.01	3.80	0.25	3.92
Max	4.08	3.50	8.53	4.93
Mix	11.29	10.62	10.25	10.92
Panza	--	0.04	0.00	0.28
Pinza	0.40	0.37	0.30	0.18
Ra	0.40	12.07	0.41	6.41
Ta	1.15	1.37	1.87	0.95
Te	10.65	4.31	11.95	3.03
Ti	0.51	1.41	1.47	1.44
Va	2.67	1.01	2.96	0.92
Ve	25.59	5.91	22.56	5.61
Vi	2.70	3.17	4.53	3.22

RN = Rotatinuous stocking; RT = Traditional rotational stocking. [1]Diet composed with bites hand-plucked in 2018 during the vegetative stage of growth (period 1). [2]Diet composed with bites hand-plucked in 2018 during the early shoot elongation stage of growth (period 2).

Table 3. Chemical composition of the herbage apparently ingested and total nutrient intake by sheep grazing Italian ryegrass pastures under contrasting grazing managements in rotational stocking

Variable	RN	RT	SD	P-value
Chemical composition, g/kg				
OM	896	883	13.7	0.036
CP	254	220	21.8	<0.0001
NDF	358	367	38.9	0.4086
ADF	259	321	55.9	0.009
ADL	23	33	10.8	0.027
TSS	144	126	12.3	0.0086
CF	41	37	5.8	0.056
OMD, g/kg OM	771	755	155.7	<0.0001
Intake, g/animal/day				
OM,	835.95	680.90	153.7	<0.0001
CP	237.1	169.5	50.5	<0.0001
NDF	333.8	281.6	62.6	<0.0001
ADF	241.7	248.4	51.7	0.5864
ADL	21.5	25.3	7.5	<0.0001
TSS	135.2	96.9	27.6	<0.0001
CF	38.1	28.6	8.7	<0.0001
Digestible OM	643.5	513.6	117.0	<0.0001

RN = Rotatinuous stocking; RT = Traditional rotational stocking. OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, TSS= total soluble sugars, CF= crude fat. SD= standard deviation.

Table 4. Biochemical and hematological parameters of sheep grazing Italian ryegrass pastures under contrasting grazing management in a rotational stocking.

Absolute Neutrophils	RN	RT	SD	Reference values	P-value
Biochemistry					
Albumin (g/dL)	3.76	3.53	0.585	2.4 – 3.0[1]	0.011
Alkaline phosphatase (U/L)	260.1	231.4	89.03	68 – 387[1]	0.335
Fructosamine (umol/L)	254.3	233.3	50.54	--	0.175
Glucose (mg/dL)	61.0	50.4	11.602	50 – 80[1]	0.0007
PUN (mg/dL)	30.8	25.3	6.916	8 – 20[1]	0.0003
Cholesterol (mg/dL)	75.6	84.5	23.37	52 – 76[1]	0.478
BHB (mmol/L)	0.327	0.309	0.1275	0.55[1]	0.170
NEFA (mmol/L)	0.12	0.24	0.164	--	0.092
Hematology					
Total Leucocytes	6853.0	6231.1	1125	4000 - 8000[2]	0.11
Neutrophils	1183.6	1272.8	517	700 – 6000[2]	0.76
Lymphocytes	3755.8	3358.7	875	2000 – 9000[2]	0.05
Neutrophils, %	17.46	20.44	7.59	10 – 50[2]	0.133
Lymphocytes, %	55.3	53.9	9.97	40 – 55[2]	0.109
N:L	0.33	0.37	0.193	-	0.018

RN = Rotatinuous stocking; RT = Traditional rotational stocking. [1]Reference value from Kaneko et al., 2008. [2]Bayes and Kramer (2010). N:L= Neutrophil to Lymphocyte ratio. SD= Standard deviation.

3. CHAPTER III[1].

---

[1] Manuscript prepared in accordance with Animal Feed Science and Technology (Appendix 1)

Implications grazing management on *in vitro* CH_4 production and fermentation profile of the herbage apparently consumed by sheep grazing Italian ryegrass pastures[1]

Abstract

Setting pre- and post-grazing sward surface heights (SSH) that allow animals to maximize the short-term intake rate set conditions for preferential leaf lamina grazing. Conversely, grazing managements with higher pre-grazing and lower post-grazing SSH, aiming to balance optimal herbage accumulation with harvest efficiency, force animals to collect most of the forage in offer, including less digestible plant parts. We incubated herbage samples had-plucked from paddocks, in a way to represent at the bite scale, the herbage ingested under two rotational stocking managements with different pre- and post-grazing SSH; 18 and 11 cm (RN) and 27 and 7 cm (RT), respectively. We tested the extent to which differences in chemical composition drive shifts on *in vitro* rumen fermentation profile including CH_4. The RN diet increased the net production of SCFA at 6 and 10 h of incubation, but changes in CH_4 production were not evident. The net production of C2 increased marginally in the RN diet, while its proportion was not affected ($P>0.05$). The production and proportion of C4, valeric acid and BCFA increased in RN diet, and in a time-dependent fashion ($P<0.0001$). The C3 remained unaffected by treatment. The fermentable organic matter (FOM) slightly increased at 10 h of incubation on the RN diet. The $N\text{-}NH_3$ increased in the RN diet, and in both treatments at 24 h of incubation. The grazing management shifted the *in vitro* fermentation profile of the herbage apparently ingested by lambs grazing Italian ryegrass pastures, but changes were small and biologically unable to create a low-CH_4 rumen environment, but notably changed the production and proportion of by-products of protein degradation and fermentation.

Keywords: sward surface height, enteric methane, *Lolium multiflorum*, grazing management.

3.1. Introduction

Animal commodities produced in pastoral ecosystems grazed by ruminants under sub-optimal feeding conditions display high carbon footprints (Gerber et al., 2013; Herrero et al., 2013; Oenema et al., 2014; Rao et al., 2015; Steinfeld et al., 2006), with most of the CO_2-eq emitted coming from enteric methane (CH_4). Some nutritional-oriented CH_4 mitigation strategies are proposed to reduce methane produced by ruminal fermentation (Beauchemin et al., 2008; Berndt and Tomkins, 2013; Clark, 2013; Hristov et al., 2018, 2013; Kumar et al., 2014; Smith et al., 2008), but they have only a limited applicability under grazing conditions. These, mitigate emissions indirectly by increasing DM intake and animal outputs (Clark, 2013; Hristov et al., 2013; Pacheco et al., 2014) or directly by shifting rumen fermentation towards less CH_4 yield (Beauchemin et al., 2008; Grainger and Beauchemin, 2011; Leng, 2018; van Gastelen et al., 2019).

Some natural processes in the rumen act as H_2 sinks and directly affect CH_4 production (Janssen, 2010; Leng, 2018; Mathison et al., 1998). After methanogenesis, propionate production (Janssen, 2010), biohydrogenation of unsaturated fatty acids (Beauchemin and McGinn, 2006) and microbial cell growth (Czerkawski et al., 1966; Leng, 2014) are the most important and likely to occur naturally on pasture-fed or grazing animals. Ultimately, a low-CH_4 rumen environment is most likely to occur with increased intakes of diets high in readily fermentable carbohydrates and low in poorly digestible fiber, rich in unsaturated fatty acids, that facilitate digestibility and passage rate, and promote fermentation pathways deviating H_2 from methanogenesis (Janssen, 2010; Leng, 2018). Nonetheless, while in theory this is sound, in practice, over many feeding conditions (Hammond et al., 2013; Pinares-Patiño et al., 2003; C.S. Pinares-Patiño et al., 2007; Sun et al., 2011), better quality pastures do not result in lower CH_4 per kilogram of dietary or animal input, with the latter being especially interesting in grazing conditions (Cezimbra et al., Unpublished; de Souza Filho et al., 2019; Pinares-Patiño et al., 2003; Richmond et al., 2015).

Previously in this thesis (Chapter IV), we characterized, at the bite scale, the diet apparently consumed by sheep grazing *Lolium multiflorum* Lam. pastures. We observed that a grazing management allowing animals to maximize the short-term intake rate at any time while grazing, promotes preferential leaf lamina harvesting, when compared to a grazing management aiming to maximize herbage accumulation and harvest efficiency. The former, also led to higher intake of water-soluble carbohydrates, nitrogenous compounds and fatty acids, but less fibrous compounds (Chapter IV). This condition could naturally create alternative H_2 sinks, hence a low-CH_4 rumen environment, as suggested by the 13 % reduction in CH_4, as measured with the SF_6 technique, emitted per kg of digestible OM intake observed by Savian et al. (2018) with sheep. However, proving this directly on grazing animals by extracting rumen liquid via esophagus or fistulated animals has enormous technical, economical and ethical constrains; thereby, *in vitro* procedures are possible alternatives.

In this study, we take as reference those diets characterized in Chapter IV, and simulated directly from pasture, the type of bites the animals perform under both contrasting grazing managements. From these bite samples, we composed and determined the chemical composition of the diet apparently consumed from the sward and conducted *in vitro* incubations of the herbage to determine its CH_4 production, fermentation and biohydrogenation profile. We hypothesized that the diet apparently selected by lambs under RN management, characterized by a higher proportion of soluble leaf lamina (soluble compounds), results in low-CH_4 *in vitro* rumen environment.

3.2. Material and methods

We managed a flock of sheep grazing an Italian Ryegrass (*Lolium multiflorum* Lam.) pasture at the Experimental Station of the Faculty of Agronomy of the Federal University of Rio Grande do Sul (UFRGS), in Southern Brazil. The pasture was conducted to set the conditions for collecting directly from pasture herbage samples characterizing the biting behavior of animals. Afterwards, with the collected herbage, an *in vitro* fermentation procedure of the forage samples was carried out at the Department of Animal Science and Aquatic Ecology of the University of Ghent. Animal procedures were approved by the Animal Ethics Committee of corresponding institutes.

3.2.1. Pasture management

Amaral et al. (2013) defined the pre-grazing sward height of 18 cm, to be optimal for sheep to maximize the intake rate on *Lolium multiflorum* Lam., pastures, and Carvalho (2013), delimited in 40 % the grazing-down limit of this optimal sward height, for allowing animals to sustain the intake rate. The above defined a management with swards heights of 18 and 11 cm at the pre- and post-grazing respectively (RN). This was compared with other grazing management aiming to maximize herbage mass accumulation and harvest efficiency through pre- and post-grazing sward height of 27 and 7 cm, respectively (RT; Savian et al., 2018; Schons et al., Unpublished).

3.2.2. Reconstitution of the diet apparently ingested in the grazing treatments

Two treatments that consisted of diets apparently consumed by animals under both grazing management were evaluated. In a previous study (2017), we elaborated a bite-code grid of *Lolium multiflorum* Lam., (Chapter IV; Figure 1) and determined the proportion that each bite accounted for the accumulated intake of animals during a continuous-bite monitoring experiment (Chapter V). The accumulated intake during the observation time (from sunrise to sunset) was calculated by multiplying the number of times each bite type occurred by its mass (dry matter basis); afterwards, the dry matter ingested per bite type was divided by the accumulated intake to obtain their relative proportion for the accumulated intake (Table 1).

In the following grazing season (2018), twenty-four Corriedale year-round lambs (45 kg ± 4 kg of BW; 2018) grazed an area of 1.86 ha, established in late May of 2018 (see Chapter IV for details of pasture an animal managements). Briefly, the area was divided equally in eight paddocks receiving the two grazing managements. In addition, a variable number of “regulator” animals were put or retired of paddocks to maintain treatment heights (Mott and Lucas, 1952). The sward height was measured daily during evaluations, with a “sward stick” (150 readings per paddock; Barthram, 1985) at the pre- and post-grazing. A strip-grazing was adopted, with strip change occurring between 14:00 and 15:00 h. After seeding, once strips of both treatments achieved the targeted pre-grazing height, an adaptation period of 15 days was considered to start, after of which the herbage sampling was conducted with pasture at full vegetative stage (period 1) and early shoot elongation (period 2).

On each period, 4-days of herbage hand-plucking were conducted to simulate each bite type of the bite-code grid on each of the eight experimental units (paddocks). For this, a trained person observed the biting activity of the three tester animals within a paddock during the main morning and afternoon grazing events. Afterwards, the observer simulated at least 20 times each bite of the bite-coding grid according to Bonnet et al. (2011). The simulation was performed in alternate fashion, completing one paddock of each treatment per day. At the fourth day of each period, the bite-coding grid was simulated once on all paddocks (four per treatment). Bite samples were put on a cooler with ice immediately after sampling, and within 4 hours stored at -20 °C until freeze-drying. Afterwards, all samples were ground (1 mm) and a 10 g sample of freeze-dried bites was compounded considering the relative proportion of each bite type contributed to the total accumulated intake of each treatment previously estimated (Table 1). The reconstituted samples were considered the diet apparently consumed by animals under both grazing managements (dietary treatments). The pre- and post-grazing sward height and chemical composition of the diet composited with the herbage hand-plucked from the pasture is presented in Table 2. The OM, CP, NDF, ADF, ADL, total soluble sugars and crude fat, in grams per kilogram of DM, were determined with NIRS (*sensu* Decruyenaere et al., 2009).

3.2.3 In vitro fermentation

Samples of the reconstituted diets of each paddock, used as replicated, for both observation periods and grazing managements were incubated in three separated runs with 96 bottles per run to stop fermentation after different incubation times. Hence, the experimental scheme was as follows:

2 treatments x 4 paddocks × 2 periods x 6 incubation times x 3 fermentation runs

+ 1 blank (no substrate, non-incubated) = 291 bottles

Rumen liquid (500 ml) was collected from three rumen-fistulated sheep, adapted to the sampling protocol, and fed with alfalfa hay and concentrate diet (50:50). Fresh access to water was allowed, except 1 h before collection. With the aid of a vacuum pump, rumen fluid was collected at 07:00 h, in a thermic bottle previously warmed at 39°C and saturated with CO_2, and carried to the laboratory. Temperature and anaerobic conditions were maintained until incubation. Fluid samples were filtered with a strainer before mixing with buffer solution containing (g/1000 ml of distilled water) 3.58, 1.55. 0.15, 8.7 and 1.71 of $Na_2HPO_4.12H_2O$, KH_2PO_4, $MgCl_2.6H_2O$, $NaHCO_3$ and NH_4HCO_3, respectively. The incubation medium (12.5 ml; 1500 ml of rumen liquid plus 394.7 ml of buffer) and herbage samples (substrate: 0.125 g) were anaerobically dispensed into 60 ml serum bottles for every feed and incubation time (2, 4, 6, 10, 24 and 48 h). Bottles were capped with a rubber stopper and held in a shaking incubator at 39°C.

3.2.3.1 Fermentation products

At each incubation time fermentation was stopped by quenching the flaks in iced water for 10 min. Afterwards, the gas produced was recorded using a pressure transducer

(Infield 7, UMS), and the headspace of the flask was inserted into a syringe adapted to a GC (FM, Dual Column GC 700; FM Scientific Corp., Avondale, PA, USA) and analyzed for CH_4 and H_2, according to Fievez et al. (2003). After gas sampling, the pH was scored (Mettler Toledo, AG), and an aliquot of 1.0 ml containing incubation medium was acidified with 100 µL of a solution containing 10 mg of 2-ethylbutyric acid per ml of formic acid, and centrifuged at 1500 rpm at 4°C during 15 min (MSE, Amsterdam, Netherlands). The supernatant was transferred to GC vials, and stored until analysis for SCFA using the procedures described in Fievez et al. (2003). The net production of SCFA was calculated by subtracting the concentration on the blank (non-incubated) and processed as previously described. The fermented organic matter (FOM; mg) was calculated from the molar proportion of acetate (C2), propionate (C3) and butyrate (C4), as (C2/2 + C3/2 + C4) × 162 (Goel et al., 2009). At all incubation times, an aliquot of rumen liquid (1.0 mL) was frozen at -20°C for spectrophotometric analysis of N-NH_3 according to Chaney and Marbach (1962). Briefly, samples were brought to room temperature and centrifuged at 17,000 g for 20 min. Afterwards 50 µl of a solution (formic acid + EBA 10 %) was added to the supernatant, into a 10 ml tube. To this, there were added 4.5 ml of each of two solutions and after samples remained under room condition for four hours, absorbance was red in a (equipment). Ammonia nitrogen was calculated as [Ammonia nitrogen] = [(NH_3 concentration) x 14.0067] / [14.0067 + 3(1.00797)]*1.1.

3.2.4. Statistical analysis

ANOVA, at 5 % of significance was performed for both diet and *in vitro* data, using a complete randomized design, with four replicates (paddocks as experimental unit), repeated over six incubation times and two herbage sampling periods. All statistical analyses were done using R 3.5.1 (lmer procedure, R Core Team, 2018). The used the model Y_{ijkl}= mean + T_i + Pa_j + Pe_k + IT_l +T_i x IT_k + Run_l + E_{ijkl}, where T is the dietary treatment, P is the period, IT incubation time, T x IT treatments per incubation time, Run and residual error. This model, considering the fixed effects of treatments and incubation time, and random effects of paddock, period and run, was selected as no interaction between treatment and incubation time was observed for chemical composition, and also by the likelihood ratio test using Akaike's information criterion (AIC). Means were compared using the least-squares mean linear hypothesis test adjusted for Tukey comparison.

3.3. Results

3.3.1 pH, SCFA and CH_4

The pH did not differed between treatments, with an average value of 6.68, remained stable between the 2 and 10 hours, decreased at 24 h, and scored the lowest value at 48 h (Figure 2a). There was a time-dependent increase in net production of SCFA, with higher amounts recorded at 6 (+6.3 %) and 10 h (+5.2 %) in the RN diet ($P<0.05$; Figure 2b). The total gas volume and CH_4 (Figure 2c and 2d) were not altered by the diet, but all increased with incubation time ($P<0.001$). There was no treatment x incubation time interaction for net production or proportion of any SCFA. However, the RN diet increased 2% the net production of C2 (539.5 vs 529.9 µmol; $P= 0.0206$) and in 6% the C4 (70.3 vs. 66.1 µmol; $P<0.0001$), but no effect over C3 was observed, with an average value of 309.5 µmol ($P>0.05$); all these SCFA increased with incubation time

(Figure 3). The C2C4/C3 ratio was similar between treatments ($P>0.05$), but affected by incubation time (Figure 3d). The proportion of C2 and C3 were similar between treatments, with mean values of 578.1 and 328.3 mmol/mol, respectively ($P>0.05$), and the C4 increased 3.7% in the RN diet (67.6 vs. 65 mmol/mol; $P<0.0001$); all these proportions where affected by the incubation time (Figure 5).

3.3.2. N-NH_3, BCFA and FOM

The N-NH_3 measured at 24 h was of 408 and 356 mg/l for RN and RT, respectively ($P<0.05$; Figure 4a). The RN diet also had higher net production (Figure 4b) and proportion (Figure 5d) of BCFA, both from 4 to 48 h of incubation, and valeric acid (data not shown) from 6 to 48 h of incubation. The diet fermentability, expressed as milligrams of FOM, was higher for the RN diet at 10 h (+ 5 %; $P=0.06$), and reached a maximum value of 70.5 mg (53 %), at 48 h, for both treatments.

3.4. Discussion

3.4.1. pH, SCFA and CH_4

We tested the potential of the RN management (maximize the short-term intake rate) to drive fermentation pathways reducing *in vitro* CH_4, hypothesized to occur owing the higher dietary supply of fermentable compounds, known to be less methanogenic (Dijkstra, 1994; Moe and Tyrrell, 1979; van Houtert, 1993), this, in turn, derived from setting conditions for animal to grass preferentially leaf lamina (Chapter IV). Nonetheless, the shift in the fermentation profile of dietary carbohydrates was minimal and unable to affect CH_4 production. The lower fibrousness of the RN diet slightly increased the total SCFA, C2 and C4, as a sign of higher diet fermentability, as observed in other reports with high-sugar forages, low in fiber (Berthiaume et al., 2010; Purcell et al., 2014). This was confirmed by the also slightly higher FOM recorded in the RN diet. However, the increase was negligible and unable to modify CH_4 production and yield, as a sign fermentation shifts towards H_2-sink pathways (Figure 1). In agreement, Alende (2016) found no difference in total SCFA concentration and proportion of the main SCFA, and despite not having measured CH_4, its production and yield were likely not affected by the high-sugar trait of the ryegrasses evaluated. With high-sugar ryegrasses, Purcell et al. (2011a) found a significant, but a biologically small 3.5 % decrease in CH_4 yield (24.6 vs 25.5 ml/g DM), with also no signs of propionic fermentation. The proportion of individual SCFA (Table 2), was within the range observed in other *in vitro* fermentation of good quality ryegrass (Purcell et al., 2011; Lee et al., 2003; Vibart et al., 2012), and coincides with the observed limited effect of chemical difference between ryegrasses (Purcell et al., 2011) or other high-quality temperate forages (Keim et al., 2014) over *in vitro* rumen fermentation variables and CH_4 production at 24 h of incubation. Others, however, recorded less CH_4 output, associated to propionic fermentation from ryegrasses with high sugar content (Amer et al., 2012; Berthiaume et al., 2010; Lovett et al., 2006) or by adding soluble sugars (+ 33 to 50 % in DM) in the *in vitro* environment (Purcell et al., 2014), as indication of growth of microbes competing with methanogens for H_2 (Jalč et al., 2002; Li et al., 2010). In Lee et al. (2003), despite not having measured CH_4 output, authors detected a less methanogenic fermentation profile, and Lovett et al. (2006) reported a strong negative association between sugar content and CH_4 production, and negative with NDF content for ryegrass cultivars; however, authors noted that some of them, with similar chemical composition and OM digestibility had different CH_4 output and suggested that rather than the sugar content, variable outputs can arise from plant intrinsic factors, such as the content of organic acids, which are known to differ within cultivars of ryegrasses. In this experiment though, such hypothesis is not valid as changes in nutritive value were driven solely by the grazing management.

Our results opposes to the theoretical negative relationship between the chemical composition of forages and CH_4 emissions (Blaxter and Clapperton, 1965; Hegarty, 2009; Johnson and Johnson, 1995; Shibata and Terada, 2010), but agree with studies that failed to associate chemical attributes of forages with CH_4 emissions (Hammond et al., 2013; Pinares-Patiño et al., 2003; C.S. Pinares-Patiño et al., 2007; Sun et al., 2011), with the study of (Jonker et al., 2017) who observed inconsistent reduction in CH_4 emissions not attributable to the high-sugar trait of ryegrass cultivars (offered indoors) or with Beauchemin et al. (2008) who suggested that effective CH_4 mitigation through feeding readily fermentable carbohydrates occurs only when these reach around 60 % of the DM intake; levels naturally not present on forages, even with improved grazing managements or plant breeding. In this *in vitro* experiment, we attempted to partially explain the mechanism underlying the 13% reduction in CH_4 per kilogram of OM intake previously observed *in vivo* with the RN grazing management Savian et al. (2018). However, Brown et al. (2002) studied the concordance between *in vitro* and *in vivo* rumen fermentation profile, and found significant differences between individual SCFA concentration and their proportions. The author considered this response in part due to the dynamic rumen environment, profoundly affected by the level and rate of intake, and feeding frequency (Boadi et al., 2004; Sutton et al., 1986), not accounted on *in vitro* approaches. For instance, Nozière et al. (2011), through modelling, reported that changes in SCFA are strongly associated with the level of intake. Accordingly, the CH_4 differences between the *in vivo* study of Savian et al. (2018) and this *in vitro* trial, might be related first, to the 18% higher OM intake of RN animals, and arguably, to the highly contrasting intake rate recorded between the RN and RT grazing managements (40 % higher in the RN; unpublished data). Clearly, fed-restricted animals increase the intake rate (DOUGHERTY et al., 1989; Patterson et al., 1998; Soca et al., 2014), especially of the first meal bout following the restriction, which is likely occurring immediately after the daily strip-change in the RT management. At this initial moment, the sward height is on average of 27 cm, however, as the sward is depleted to 7 cm, the progressive depletion of the pasture structure reduce the intake rate during the following meal bouts. Differently from this, the pre- and post-grazing sward height of 18 and 11 cm in the RN management (for *Lolium multiflorum* pastures; Amaral et al., 2013), are within the range of heights in which animals have the opportunity to maximize the intake rate at any time while grazing. Thereby, the different rate at which feed reaches the rumen under such contrasting grazing managements, might trigger shifts in rumen fermentation, more than the chemical composition of the herbage *per se*, as observed by Costa et al. (2019) who found minor changes in some rumen variables and passage rate (liquid phase) among C3 and C4 forage species, all of them providing high quantity and quality material for grazing.

The passage rate is arguably the most important factor governing low-CH_4 fermentation pathways, such as propionic acid production (Janssen, 2010). This trait, highly variable within animals (Pinares-Patiño et al., 2003), might be significantly affected by the grazing management. For example, animals adopt different grazing strategies to increase the intake rate during the first meal following fed restriction, two of them are increasing the bite mass (Gibb et al., 1998; LACA et al., 1992) and reducing the time advocated to masticate (Newman et al., 1994). Larger feed particles reaching the rumen (e.g. during the first meal bout after strip change in RT management) might spend more time in the rumen before they are cleared out, conversely, feed particles rapidly cleared out of the rumen reduce the fermentation extend and thus CH_4 production (Mathison et al., 1998). The latter could likely occur in the RN management, as higher intake is associated with increased passage rates (Tedeschi et al., 2019). Moreover, a 20 % higher water content in feces of RN animals was also evident (Chapter IV), probably due to the leafy grazing of animals. In this regard, Pacheco et al. (2014) hypothesized upon the role of DM of forages on CH_4/kg DM intake. Authors showed that DM, more than NDF in the DM, seems to account for more variation in CH_4 per feed intake, and associated this response to a possible faster feed clearance out the rumen. Freeze-drying and mincing of forages before batch culture, cancel these source of variation affecting CH_4 emissions. Thereby, we recognize that the benefits of offering high-quality pastures are partially canceled when the grazing management does not allow animals to maximize their individual intake, and that grazing strategies can affect the timing of nutrients supply for rumen microbes and fermentation profile (Gregorini et al., 2008), effectively altering CH_4 emissions.

3.4.2. N-NH_3, BCFA and FOM

Differently from the slight shifts in the fermentation profile derived from carbohydrates, the by-products of protein degradation and fermentation were highly affected by the dietary treatment. The CP content of temperate species is normally in excess, surpassing the capacity of microbes to incorporate it into microbial protein

(Huntington and Archibeque, 2000). This results in high NH_3 in the rumen (Figure 3), and in plasma urea N and fecal N excretion (Chapter IV). In the RT diet, its concentration (derived from buffer solution and diet), are comparable to those reported by Berthiaume et al. (2010) in continuous cultures of alfalfa, or to those of Keim et al. (2014) with fertilized permanent pastures, both with similar CP content, and considered not to impair microbial growth (235 mg/L; Mehrez et al., 1977); nonetheless, in the RN it increases in 14%, thus it is demonstrated the exacerbation of N excess for microbes for this treatment. Increased *in vitro* ammonia yield or concentration (Vibart et al., 2012, 2019) were found in afternoon-allocated grazing, time at which intake of CP increases in response to daily grazing patterns. Nitrogen use efficiency in grazing animals is low, as its excretion in manure can be at rates of 81 % (Cheng et al., 2015; Zhao et al., 2016); thus, it is priority adopting strategies that efficiently capture most of the N by microbes and reduce N deposition in soil and environmental pollution (Dijkstra et al., 2013).

The increased net production and proportion of BCFA in the RN diet (Table 2) was probably due to the higher CP content in the diet, as shown by Purcell et al. (2011) with perennial ryegrass, or by Berthiaume et al. (2010) with alfalfa; therefore from the higher availability of valine and leucine for BCFA synthesis by bacteria. In this regard, Vibart et al. (2019) explain that lower microbial protein synthesis from the N-NH_3 concentrated at initial stages of fermentation, might have been the cause of the numerical increase in BCFA, through shifts of N-using microbial communities, which also produce less ATP. An eventual “futile” use of the more digestible carbohydrates provided by the RN diet, reinforce the need for energy supply for reducing rumen N-NH_3 for optimal microbial functioning. The synthesis of BCFA also diverts small amounts of H_2 from methanogenesis (reference), though at rates insufficient to shift fermentation towards less CH_4, as observed by their increase in 23 % in the rumen liquid but unaffected CH_4 yield.

The supplementation of BCFA has proved to be beneficial for productive purposes and milk quality traits. For example, in Liu et al. (2018) the supplementation with BCFA increased concentration of ruminal SCFA, C2 and C4, which stimulated milk yield and provided precursors for milk fat synthesis. The shift towards a lipogenic fermentation, is consistent with protein supplementation (Caton et al., 1988) and with the stimulatory effect of BCFA over cellulolytic bacteria populations and their enzymatic activity (Liu et al., 2014; Wang et al., 2015; Zhang et al., 2015) and by the BCFA regulation of genes related to milk fat synthesis (Liu et al., 2018). In this *in vitro* study, the production and proportion of C4 increases only marginally, thus, assessing the extent to which the RN management could drive changes milk-quality traits, as suggested by the observed increase in compounds conferring organoleptic properties to milk (e.g. indole and skatole), that accompany the augment in $N\text{-}NH_3$ and BCFA (Pacheco et al., 2006), warrants further research.

The strong and negative correlation with intake level and CH_4 yield reinforce the idea of promoting voluntary intake as the main CH_4 mitigation strategy, which for indoor forage-fed animals, it is highly affected by the physical-chemical attributes of the forage (Tedeschi et al., 2019), whereas for the grazing ruminant, pastures structural characteristics are thought to anticipate the physical-chemical forages attributes to constrain the intake (Carvalho et al., 2001; Da Silva and Carvalho, 2005); feed needs to be in the rumen to exert important physical and metabolic constrains. Offering animals the opportunity to maximize the intake rate at any time during the grazing-down, opens the opportunity for they to maximize the daily nutrients intake, with the additional consequence of increasing the nutritive value of the herbage ingested (preferential leaf lamina grazing; Chapter IV). This *in vitro*, and other *in vivo* studies failing to associate the nutritive value of forages with biologically strong shifts in fermentation profile (Costa et al., 2019) or to CH_4 emission, suggesting that the ‘quality’ trait, *per se*, does not necessarily brings benefits (e.g. mitigate CH_4 emissions), especially when high-quality forages are improperly managed. Furthermore, animal factors, such as passage rate, seems to be more important drivers of CH_4 emissions (Janssen, 2010; Pinares-Patiño et al., 2007b). The grazing management can affect both the nutritive value of the ingested herbage and fermentation dynamics, thus play an important role in the production and yield of CH_4 grazing animals.

3.5. Conclusion

The grazing management affects the *in vitro* fermentation profile of the herbage apparently ingested by lambs grazing Italian ryegrass pastures. However, its effect over carbohydrates fermentation is minimal and unable to create a low-CH_4 rumen environment. Conversely, it shows a high potential of the RN management to increase the by-products of protein degradation and fermentation, such as N-NH_3, valeric and branched-chain fatty acids, whose absorption and use in mammary tissues are responsible of some desirable milk quality traits. As well, we highlight the necessity of adjusting the RN management for reducing the excessive N-NH_3 concentration in the rumen. Finally, the CH_4 mitigation potential of the RN management demonstrated previously with sheep (Savian et al., 2018) seems most likely related to the higher level of intake of animals and other animal-related factors, rather than by the improved nutritive value of the ingested herbage.

Acknowledgments

To the CONACYT (Mexico), for providing the scholarship (CV: 171912). To the technical personnel at LANUPRO (Ghent University) and at Agro-Bio Tech (Liége University). All the graduation students that assisted in the data collection during the field experiment. To Fernanda Moojen and Mariluh Fernandes and for the artwork.

Conflict of interest

None

3.6. Consulted literature

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

Alende, M., 2016. Evaluation of high sugar ryegrass varieties. PhD Thesis. Clemson University, South Carolina, USA.

Barthram, G. T. 1985. Experimental techniques: the HFRO sward stick. In: the Hill Farming Research Organization Biennial Report, 1984/1985. HFRO, Penicuik, pp.29-30.

Amer, S., Hassanat, F., Berthiaume, R., Seguin, P., Mustafa, A.F., 2012. Effects of water soluble carbohydrate content on ensiling characteristics, chemical composition and in vitro gas production of forage millet and forage sorghum silages. Anim. Feed Sci. Technol. 177, 23–29.

https://doi.org/10.1016/j.anifeedsci.2012.07.024

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

Beauchemin, K.A., McGinn, S.M., 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Can. J. Anim. Sci. 86, 401–408. https://doi.org/10.4141/A06-021

Berndt, A., Tomkins, N.W., 2013. Measurement and mitigation of methane emissions from beef cattle in tropical grazing systems: a perspective from Australia and Brazil. animal 7, 363–372. https://doi.org/10.1017/S1751731113000670

Berthiaume, R., Benchaar, C., Chaves, A.V., Tremblay, G.F., Castonguay, Y., Bertrand, A., Bélanger, G., Michaud, R., Lafrenière, C., McAllister, T.A., Brito, A.F., 2010. Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture. J. Dairy Sci. 93, 693–700. https://doi.org/10.3168/jds.2009-2399

Blaxter, K.L., Clapperton, J.L., 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19, 511–522. https://doi.org/10.1079/BJN19650046

Boadi, D., Benchaar, C., Chiquette, J., Massé, D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84, 319–335. https://doi.org/10.4141/A03-109

Bonnet, O., Hagenah, N., Hebbelmann, L., Meuret, M., Shrader, A.M., 2011. Is Hand Plucking an Accurate Method of Estimating Bite Mass and Instantaneous Intake of Grazing Herbivores? Rangel. Ecol. Manag. 64, 366–374. https://doi.org/10.2111/REM-D-10-00186.1

Carvalho, P. C. F., Ribeiro-Filho, E. M. N., Poli, C. H. C. E., de Moraes, A., Delagarde, R. 2001. Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo. *In*: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 38, Piracicaba. **Anais** [...]. Piracicaba: ESALQ, 2001. p. 853–871. Available in: http://www.ufrgs.br/gpep/documents/capitulos/Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo.pdf. Access in: 22 Sept. 2016.

Carvalho, P.C.D.F., 2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? Trop. Grasslands - Forrajes Trop. 1, 137. https://doi.org/10.17138/TGFT(1)137-155

Caton, J.S., Freeman, A.S., Galyean, M.L., 1988. Influence of Protein Supplementation on Forage Intake, in Situ Forage Disappearance, Ruminal Fermentation and Digesta Passage Rates in Steers Grazing Dormant Blue Grama Rangeland. J. Anim. Sci. 66, 2262. https://doi.org/10.2527/jas1988.6692262x

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8, 130–132.

Cheng, L., McCormick, J., Hussein, A.N., Fraslin, C., Moonsan, Y., Logan, C., Grabot, J., Edwards, G.R., 2015. Urinary nitrogen excretion, grazing and urination behaviour of dairy heifers grazing pasture, chicory and plantain in autumn, in: Proceedings of New Zealand Society of Animal Production. pp. 70–73.

Clark, H., 2013. Nutritional and host effects on methanogenesis in the grazing ruminant. animal 7, 41–48. https://doi.org/10.1017/S1751731112001875

Costa, D.F.A., Quigley, S.P., Isherwood, P., McLennan, S.R., Sun, X.Q., Gibbs, S.J., Poppi, D.P., 2019. Small differences in biohydrogenation resulted from the similar retention times of fluid in the rumen of cattle grazing wet season C3 and C4 forage species. Anim. Feed Sci. Technol. 253, 101–112. https://doi.org/10.1016/j.anifeedsci.2019.05.010

Czerkawski, J.W., Blaxter, K.L., Wainman, F.W., 1966. The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20, 349–362. https://doi.org/10.1079/BJN19660035

Da Silva, S.C., Carvalho, P.C. de F., 2005. Foraging behaviour and herbage intake in the favourable tropics/sub-tropics. Grassl. a Glob. Resour. 1, 81–95.

de Souza Filho, W., Nunes, P.A. de A., Barro, R.S., Kunrath, T.R., de Almeida, G.M., Genro, T.C.M., Bayer, C., de Faccio Carvalho, P.C., 2019. Mitigation of enteric methane emissions through pasture management in integrated crop-livestock systems: Trade-offs between animal performance and environmental impacts. J. Clean. Prod. 213, 968–975. https://doi.org/10.1016/j.jclepro.2018.12.245

Decruyenaere, V., Lecomte, P., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D., Buldgen, A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim. Feed Sci. Technol. 148, 138–156. https://doi.org/10.1016/j.anifeedsci.2008.03.007

Dijkstra, J., 1994. Production and absorption of volatile fatty acids in the rumen. Livest. Prod. Sci. 39, 61–69. https://doi.org/10.1016/0301-6226(94)90154-6

Dijkstra, J., Oenema, O., van Groenigen, J.W., Spek, J.W., van Vuuren, A.M., Bannink, A., 2013. Diet effects on urine composition of cattle and N 2 O emissions. animal 7, 292–302. https://doi.org/10.1017/S1751731113000578

DOUGHERTY, C.T., BRADLEY, N.W., CORNELIUS, P.L., LAURIAULT, L.M., 1989. Short-term fasts and the ingestive behaviour of grazing cattle. Grass Forage Sci. 44, 295–302. https://doi.org/10.1111/j.1365-2494.1989.tb02167.x

Fievez, V., Dohme, F., Danneels, M., Raes, K., Demeyer, D., 2003. Fish oils as potent rumen methane inhibitors and associated effects on rumen fermentation in vitro and in vivo. Anim. Feed Sci. Technol. 104, 41–58. https://doi.org/10.1016/S0377-8401(02)00330-9

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G., 2013. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Gibb, Huckle, Nuthall, 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass Forage Sci. 53, 41–46. https://doi.org/10.1046/j.1365-2494.1998.00102.x

Goel, G., Arvidsson, K., Vlaeminck, B., Bruggeman, G., Deschepper, K., Fievez, V., 2009. Effects of capric acid on rumen methanogenesis and biohydrogenation of linoleic and α-linolenic acid. animal 3, 810–816. https://doi.org/10.1017/S1751731109004352

Grainger, C., Beauchemin, K.A., 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol. 166–167, 308–320. https://doi.org/10.1016/j.anifeedsci.2011.04.021

Gregorini, P., Gunter, S.A., Beck, P.A., 2008. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: Timing of herbage and fasting allocation1. J. Anim. Sci. 86, 1006–1020. https://doi.org/10.2527/jas.2007-0432

Hammond, K.J., Burke, J.L., Koolaard, J.P., Muetzel, S., Pinares-Patiño, C.S., Waghorn, G.C., 2013. Effects of feed intake on enteric methane emissions from sheep fed fresh white clover (Trifolium repens) and perennial ryegrass (Lolium perenne) forages. Anim. Feed Sci. Technol. 179, 121–132. https://doi.org/10.1016/j.anifeedsci.2012.11.004

Herrero, M., Havlik, P., Valin, H., Notenbaert, A., Rufino, M.C., Thornton, P.K., Blummel, M., Weiss, F., Grace, D., Obersteiner, M., 2013. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. Proc. Natl. Acad. Sci. 110, 20888–20893. https://doi.org/10.1073/pnas.1308149110

Hristov, A.N., Kebreab, E., Niu, M., Oh, J., Bannink, A., Bayat, A.R., Boland, T.M., Brito, A.F., Casper, D.P., Crompton, L.A., Dijkstra, J., Eugène, M., Garnsworthy, P.C., Haque, N., Hellwing, A.L.F., Huhtanen, P., Kreuzer, M., Kuhla, B., Lund, P., Madsen, J., Martin, C., Moate, P.J., Muetzel, S., Muñoz, C., Peiren, N., Powell, J.M., Reynolds, C.K., Schwarm, A., Shingfield, K.J., Storlien, T.M., Weisbjerg, M.R., Yáñez-Ruiz, D.R., Yu, Z., 2018. Symposium review: Uncertainties in enteric methane inventories, measurement techniques, and prediction models. J. Dairy Sci. 101, 6655–6674. https://doi.org/10.3168/jds.2017-13536

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M., 2013. SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options1. J. Anim. Sci. 91, 5045–5069. https://doi.org/10.2527/jas.2013-6583

Huntington, G.B., Archibeque, S.L., 2000. Practical aspects of urea and ammonia metabolism in ruminants. J. Anim. Sci. 77, 1. https://doi.org/10.2527/jas2000.77E-Suppl1y

Jalč, D., Kišidayová, S., Nerud, F., 2002. Effect of plant oils and organic acids on rumen fermentationin Vitro. Folia Microbiol. (Praha). 47, 171–177. https://doi.org/10.1007/BF02817677

Janssen, P.H., 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160, 1–22. https://doi.org/10.1016/j.anifeedsci.2010.07.002

Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492. https://doi.org/10.2527/1995.7382483x

Jonker, A., Molano, G., Koolaard, J., Muetzel, S., 2017. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. Anim. Prod. Sci. 57, 643. https://doi.org/10.1071/AN15656

Keim, J.P., López, I.F., Berthiaume, R., 2014. Nutritive value, in vitro fermentation and methane production of perennial pastures as affected by botanical composition over a growing season in the south of Chile. Anim. Prod. Sci. 54, 598. https://doi.org/10.1071/AN13026

Kumar, S., Choudhury, P.K., Carro, M.D., Griffith, G.W., Dagar, S.S., Puniya, M., Calabro, S., Ravella, S.R., Dhewa, T., Upadhyay, R.C., Sirohi, S.K., Kundu, S.S., Wanapat, M., Puniya, A.K., 2014. New aspects and strategies for methane mitigation from ruminants. Appl. Microbiol. Biotechnol. 98, 31–44. https://doi.org/10.1007/s00253-013-5365-0

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

Lee, M.R.., Merry, R.., Davies, D.., Moorby, J.., Humphreys, M.., Theodorou, M.., MacRae, J.., Scollan, N.., 2003. Effect of increasing availability of water-soluble carbohydrates on in vitro rumen fermentation. Anim. Feed Sci. Technol. 104, 59–70. https://doi.org/10.1016/S0377-8401(02)00319-X

Leng, R.A., 2018. Unravelling methanogenesis in ruminants, horses and kangaroos: the links between gut anatomy, microbial biofilms and host immunity. Anim. Prod. Sci. 58, 1175–1191. https://doi.org/10.1071/AN15710

Leng, R.A., 2014. Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. Anim. Prod. Sci. 54, 519. https://doi.org/10.1071/AN13381

Li, X.Z., Long, R.J., Yan, C.G., Choi, S.H., Jin, G.L., Song, M.K., 2010. Rumen microbial responses in fermentation characteristics and production of CLA and methane to linoleic acid in associated with malate or fumarate. Anim. Feed Sci. Technol. 155, 132–139. https://doi.org/10.1016/j.anifeedsci.2009.11.002

Liu, Q., Wang, C., Guo, G., Huo, W.J., Zhang, S.L., Pei, C.X., Zhang, Y.L., Wang, H., 2018. Effects of branched-chain volatile fatty acids on lactation performance and mRNA expression of genes related to fatty acid synthesis in mammary gland of dairy cows. animal 12, 2071–2079. https://doi.org/10.1017/S1751731118000113

Liu, Q., Wang, C., Pei, C.X., Li, H.Y., Wang, Y.X., Zhang, S.L., Zhang, Y.L., He, J.P., Wang, H., Yang, W.Z., Bai, Y.S., Shi, Z.G., Liu, X.N., 2014. Effects of isovalerate supplementation on microbial status and rumen enzyme profile in steers fed on corn stover based diet. Livest. Sci. 161, 60–68. https://doi.org/10.1016/j.livsci.2013.12.034

Lovett, D.K., McGilloway, D., Bortolozzo, A., Hawkins, M., Callan, J., Flynn, B., O'Mara, F.P., 2006. In vitro fermentation patterns and methane production as influenced by cultivar and season of harvest of Lolium perenne L. Grass Forage Sci. 61, 9–21. https://doi.org/10.1111/j.1365-2494.2006.00500.x

Mathison, G.W., Okine, E.K., McAllister, T.A., Dong, Y., Galbraith, J., Dmytruk, O.I.N., 1998. Reducing Methane Emissions from Ruminant Animals. J. Appl. Anim. Res. 14, 1–28. https://doi.org/10.1080/09712119.1998.9706212

Mehrez, A.Z., Ørskov, E.R., McDonald, I., 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38, 437–443. https://doi.org/10.1079/BJN19770108

Moe, P.W., Tyrrell, H.F., 1979. Methane Production in Dairy Cows. J. Dairy Sci. 62, 1583–1586. https://doi.org/10.3168/jds.S0022-0302(79)83465-7

MOTT, G.O., LUCAS, H.L., 1952. The design, conduct and interpretation of grazing trials on cultivated and improved pastures, in: International Grassland Congress. Pensylvania, pp. 1380–1395.

Nozière, P., Glasser, F., Sauvant, D., 2011. In vivo production and molar percentages of volatile fatty acids in the rumen: a quantitative review by an empirical approach. animal 5, 403–414. https://doi.org/10.1017/S1751731110002016

Oenema, O., de Klein, C., Alfaro, M., 2014. Intensification of grassland and forage use: driving forces and constraints. Crop Pasture Sci. 65, 524. https://doi.org/10.1071/CP14001

Pacheco, D., Waghorn, G., Janssen, P.H., 2014. Decreasing methane emissions from ruminants grazing forages: a fit with productive and financial realities? Anim. Prod. Sci. 54, 1141. https://doi.org/10.1071/AN14437

PACHECO, M.H.T.D., LANE, G.A., FRASER, K., DEATH, A.F., BURKE, J.L., HICKEY, M.J., COSGROVE, G.P., 2006. The effects of ryegrass varieties differing in soluble sugar content on the rumen fermentation of amino acids and consequences for milk flavour chemistry, in: Proceedings of the New Zealand Grassland Association. pp. 261–265.

Patterson, D.M., McGilloway, D.A., Cushnahan, A., Mayne, C.S., Laidlaw, A.S., 1998. Effect of duration of fasting period on short-term intake rates of lactating dairy cows. Anim. Sci. 66, 299–305. https://doi.org/10.1017/S1357729800009425

Pinares-Patiño, C.S., Baumont, R., Martin, C., 2003. Methane emissions by Charolais cows grazing a monospecific pasture of timothy at four stages of maturity. Can. J. Anim. Sci. 83, 769–777. https://doi.org/10.4141/A03-034

Pinares-Patiño, C.S., D’Hour, P., Jouany, J.-P., Martin, C., 2007. Effects of stocking rate on methane and carbon dioxide emissions from grazing cattle. Agric. Ecosyst. Environ. 121, 30–46. https://doi.org/10.1016/j.agee.2006.03.024

Pinares-Patiño, C S, Waghorn, G.C., Machmüller, A., Vlaming, B., Molano, G., Cavanagh, A., Clark, H., 2007. Methane emissions and digestive physiology of non-lactating dairy cows fed pasture forage. Can. J. Anim. Sci. 87, 601–613. https://doi.org/10.4141/CJAS06023

Purcell, P.J., Boland, T.M., O’Kiely, P., 2014. The effect of water-soluble carbohydrate concentration and type on in vitro rumen methane output of perennial ryegrass determined using a 24-hour batch-culture gas production technique. Irish J. Agric. Food Res. 21–36.

Purcell, P.J., O’Brien, M., Boland, T.M., O’Donovan, M., O’Kiely, P., 2011. Impacts of herbage mass and sward allowance of perennial ryegrass sampled throughout the growing season on in vitro rumen methane production. Anim. Feed Sci. Technol. 166–167, 405–411. https://doi.org/10.1016/j.anifeedsci.2011.04.073

RAO, I., PETERS, M., CASTRO, A., SCHULTZE-KRAFT, R., WHITE, D., FISHER, M., MILES, J., LASCANO, C., BLUMMEL, M., BUNGENSTAB, D., TAPASCO, J., HYMAN, G., BOLLIGER, A., PAUL, B., VAN DER HOEK, R., MAASS, B., TIEMANN, T., CUCHILLO, M., DOUXCHAMPS, S., VILLANUEVA, C., RINCÓN, Á., AYARZA, M., ROSENSTOCK, T., SUBBARAO, G., ARANGO, J., CARDOSO, J., WORTHINGTON, M., CHIRINDA, N., NOTENBAERT, A., JENET, A., SCHMIDT, A., VIVAS, N., LEFROY, R., FAHRNEY, K., GUIMARÃES, E., TOHME, J., COOK, S., HERRERO, M., CHACÓN, M., SEARCHINGER, T., RUDEL, T., 2015. LivestockPlus - The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics. Trop. Grasslands - Forrajes Trop. 3, 59. https://doi.org/10.17138/TGFT(3)59-82

Richmond, A.S., Wylie, A.R.G., Laidlaw, A.S., Lively, F.O., 2015. Methane emissions from beef cattle grazing on semi-natural upland and improved lowland grasslands. Animal 9, 130–137. https://doi.org/10.1017/S1751731114002067

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

SHIBATA, M., TERADA, F., 2010. Factors affecting methane production and mitigation in ruminants. Anim. Sci. J. 81, 2–10. https://doi.org/10.1111/j.1740-0929.2009.00687.x

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith, J., 2008. Greenhouse gas mitigation in agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 789–813. https://doi.org/10.1098/rstb.2007.2184

Soca, P., González, H., Manterola, H., Bruni, M., Mattiauda, D., Chilibroste, P., Gregorini, P., 2014. Effect of restricting time at pasture and concentrate supplementation on herbage intake, grazing behaviour and performance of lactating dairy cows. Livest. Sci. 170, 35–42. https://doi.org/10.1016/j.livsci.2014.07.011

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V., Rosales, M., Rosales, M., de Haan, C., 2006. Livestock's long shadow: environmental issues and options. Food & Agriculture Org.

Sun, X.Z., Hoskin, S.O., Muetzel, S., Molano, G., Clark, H., 2011. Effects of forage chicory (Cichorium intybus) and perennial ryegrass (Lolium perenne) on methane emissions in vitro and from sheep. Anim. Feed Sci. Technol. 166–167, 391–397. https://doi.org/10.1016/j.anifeedsci.2011.04.027

Sutton, J.D., Hart, I.C., Brosters, W.H., Elliott, R.J., Schuller, E., 1986. Feeding frequency for lactating cows: effects on rumen fermentation and blood metabolites and hormones. Br. J. Nutr. 56, 181–192. https://doi.org/10.1079/BJN19860098

Tedeschi, L.O., Molle, G., Menendez, H.M., Cannas, A., Fonseca, M.A., 2019. The assessment of supplementation requirements of grazing ruminants using nutrition models. Transl. Anim. Sci. 3, 811–828. https://doi.org/10.1093/tas/txy140

van Gastelen, S., Dijkstra, J., Bannink, A., 2019. Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? J. Dairy Sci. 102, 6109–6130. https://doi.org/10.3168/jds.2018-15785

van Houtert, M.F.J., 1993. The production and metabolism of volatile fatty acids by ruminants fed roughages: A review. Anim. Feed Sci. Technol. 43, 189–225.

https://doi.org/10.1016/0377-8401(93)90078-X

Vibart, R., Tavendale, M., Burke, J., Pacheco, D., 2012. In vitro fermentation of [15 N-] ryegrass and ruminal digesta of sheep grazing a ryegrass-based pasture in the morning or in the afternoon, in: Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, pp. 100–105.

Vibart, R.E., Ganesh, S., Kirk, M.R., Kittelmann, S., Leahy, S.C., Janssen, P.H., Pacheco, D., 2019. Temporal fermentation and microbial community dynamics in rumens of sheep grazing a ryegrass-based pasture offered either in the morning or in the afternoon. animal 13, 2242–2251. https://doi.org/10.1017/S1751731119000168

Wang, C., Liu, Q., Zhang, Y.L., Pei, C.X., Zhang, S.L., Wang, Y.X., Yang, W.Z., Bai, Y.S., Shi, Z.G., Liu, X.N., 2015. Effects of isobutyrate supplementation on ruminal microflora, rumen enzyme activities and methane emissions in Simmental steers. J. Anim. Physiol. Anim. Nutr. (Berl). 99, 123–131. https://doi.org/10.1111/jpn.12191

Zhang, Y.L., Liu, Q., Wang, C., Pei, C.X., Li, H.Y., Wang, Y.X., Yang, W.Z., Bai, Y.S., Shi, Z.G., Liu, X.N., 2015. Effects of supplementation of Simmental steers with 2-methylbutyrate on rumen microflora, enzyme activities and methane production. Anim. Feed Sci. Technol. 199, 84–92. https://doi.org/10.1016/j.anifeedsci.2014.11.003

Zhao, Y.G., Gordon, A.W., O'Connell, N.E., Yan, T., 2016. Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep offered fresh perennial ryegrass (Lolium perenne)1. J. Anim. Sci. 94, 5321–5331. https://doi.org/10.2527/jas.2016-0541

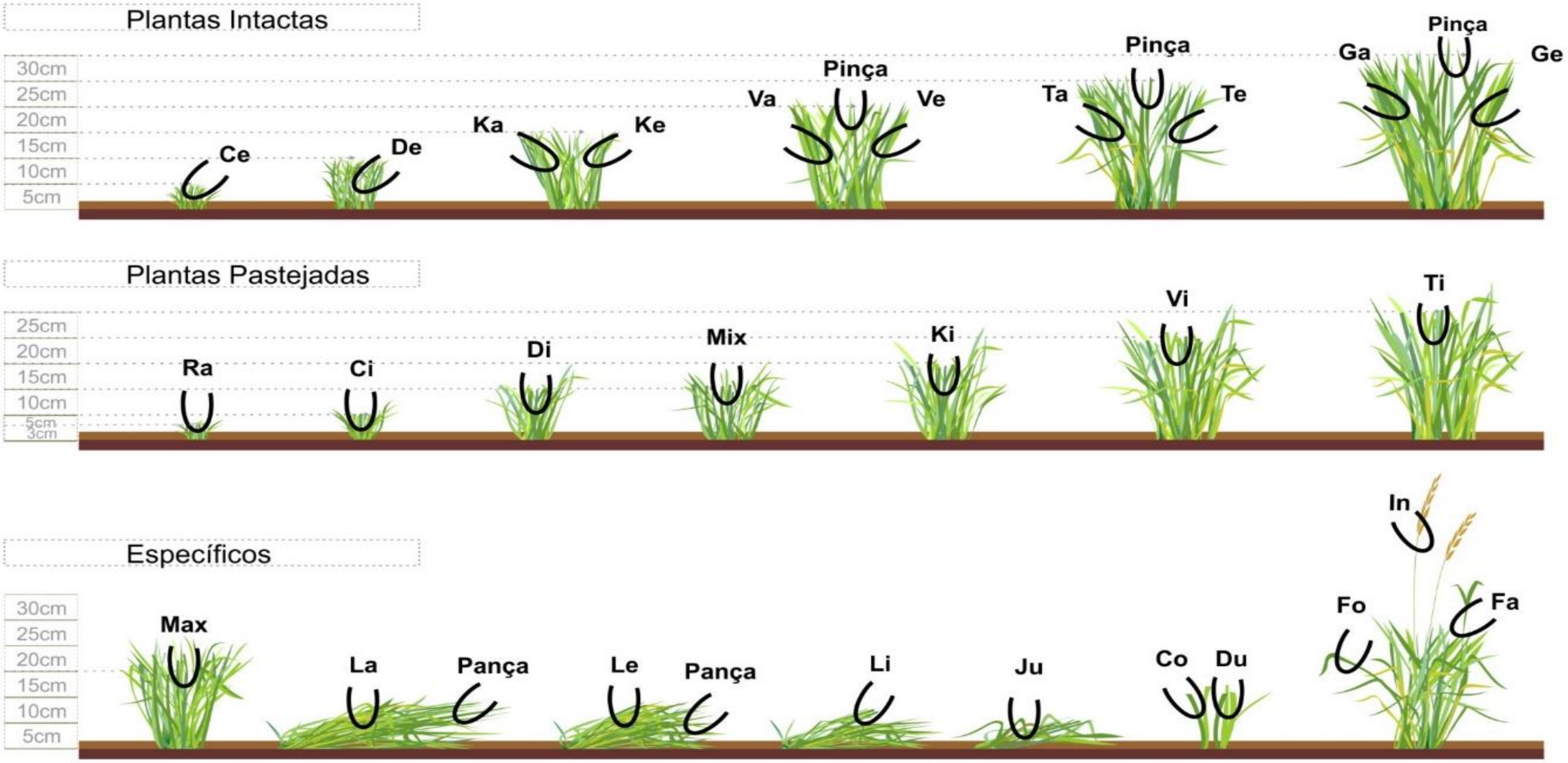


Figure 1. Bite-code grid of *Lolium multiflorum* Lam., performed by sheep grazing under contrasting grazing managements in rotational stocking. The pictogram illustrates the “ideal bite” for each bite category. The consonants “C, D, K, V, T and G” indicates, respectively, plants of 5, 10, 15, 20, 25 and ≥ 25 cm (± 2 cm, except for “Ga” and “Ge” with a deviation of +5 cm). Otherwise indicated, the vowels “a” and “e” after the consonants indicate bites performed on “intact” leafs, with “a” also indicating dense bites, while “e” being indicative of less dense bites. The vowel “i” indicates “grazed” leafs with the presence or not of stems. The bite “Ra” is a bite performed on plants ≤3cm with minimum with no leafs. The “Max” and “Mix” are bites allocated on grazed/non-grazed leafs, with the presence or not of stem, at sward height ≥ or ≤ 15 cm, respectively. The “Pinza” is a bite allocated at the tip (superficial) of a single or no more than two leafs, intact or grazed, and at any sward height; the “Panca” is the same bite but on a lying plants. The “La” and “Le” are bites on lying leafs ≥ or ≤ 20 cm, respectively, with average density of 2 to 3 leafs. The “Li” is a bite on 2 to 3 grazed lying leafs of any horizontal length. The “Co” and “Du” are bites performed on a single or on two defoliated stems, respectively, at sward height between 5 and 15 cm. The “Fo” is a bite performed on a single leaf within the canopy, whereas the “Fa” is a bite performed on the “flag” leaf below the inflorescence. The “In” is a bite on inflorescence. The “Ju” is a bite given on a trampled plant, in which animals manipulate and gather between 2 and 4 leafs. From Chapter III.

Table 1. Contribution of byte types (%) to the DM incubated (125 mg), representing the biting behavior of sheep grazing Italian ryegrass pastures under two grazing managements with contrasting pre- and post-grazing SSH. From Chapter III.

Bite Type	[1]Period 1		[2]Period 2	
	RN	RT	RN	RT
Ce	0.91	4.48	0.25	3.86
Ci	4.48	12.82	1.61	11.77
Co	0.01	0.28	0.01	0.48
De	3.86	2.50	1.72	2.68
Di	8.12	13.47	5.31	13.55
Du	0.00	0.06	0.00	0.16
Fa	0.23	0.10	0.16	0.34
Fo	0.08	0.14	0.08	0.17
Ga	0.26	0.84	2.23	0.81
Ge	2.05	2.25	5.50	2.95
In	0.02	0.00	0.00	0.01
Jun	0.00	1.10	0.00	1.92
Ka	0.24	0.06	1.00	0.01
Ke	13.79	4.77	8.32	6.55
Ki	6.03	3.89	5.27	7.21
La	0.00	1.78	0.08	1.85
Le	0.17	3.98	0.93	4.84
Li	0.01	4.03	0.23	4.36
Max	4.08	3.72	9.12	5.89
Mix	11.29	1.01	10.67	13.30
Panza	0.00	0.03	0.00	0.38
Pinza	0.40	0.46	0.28	0.24
Ra	0.40	13.22	0.27	2.90
Ta	1.15	1.08	2.89	0.34
Te	10.65	4.98	12.31	3.84
Ti	0.51	0.33	1.36	1.70
Va	2.67	0.80	4.26	0.43
Ve	25.59	6.72	22.27	6.13
Vi	2.70	1.89	3.89	4.01

RN = Rotatinuous stocking; RT = Traditional rotational stocking. [1]Diet composed with bites hand-plucked in 2018 during the vegetative stage of growth (period 1). [2]Diet composed with bites hand-plucked in 2018 during the early shoot elongation stage of growth (period 2).

Table 2. Sward surface height (cm), sward height depletion (%) and chemical composition (g/kg DM) of the herbage apparently ingested by sheep grazing Italian ryegrass pastures under two grazing managements with contrasting pre- and post-grazing SSH.

Variable	RN	RT	SD	P-Value
Sward surface height, cm				
Pre-grazing	19.2	27.5	4.36	< 0.0001
Post-grazing	12.4	7.5	3.03	< 0.0001
Sward height depletion	37.8	73.0	0.20	<0.0001
Chemical composition, g/kg DM				
OM	896	883	13.7	0.036
CP	254	220	21.8	<0.0001
NDF	358	367	38.9	0.4086
ADF	259	321	55.9	0.009
ADL	23	33	10.8	0.027
TSS	144	126	12.3	0.0086
CF	41	37	5.8	0.056

RN = Rotatinuous stocking; RT = Traditional rotational stocking. OM= organic matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignine, TSS= total soluble carbohydrates, CF= crude fat.

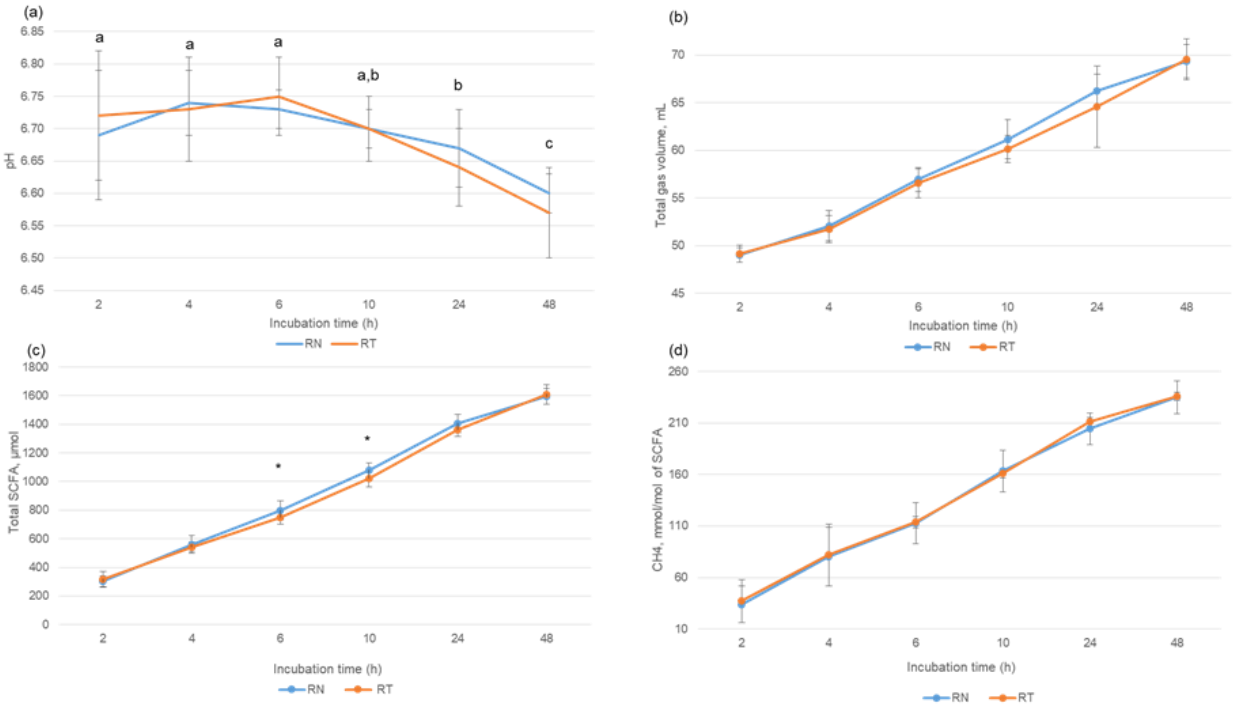


Figure 2. pH (a), total gas volume (mL; b), SCFA net production (µmol; c), CH_4 yield (mmol/mol SCFA; d) on rumen liquor (LS-means ± SD) resulting from the *in vitro* fermentation of the herbage apparently ingested by sheep grazing Italian ryegrass pastures under contrasting grazing managements. Different lowercase literals indicates effect of incubation time. * indicates effect of treatment within incubation time.

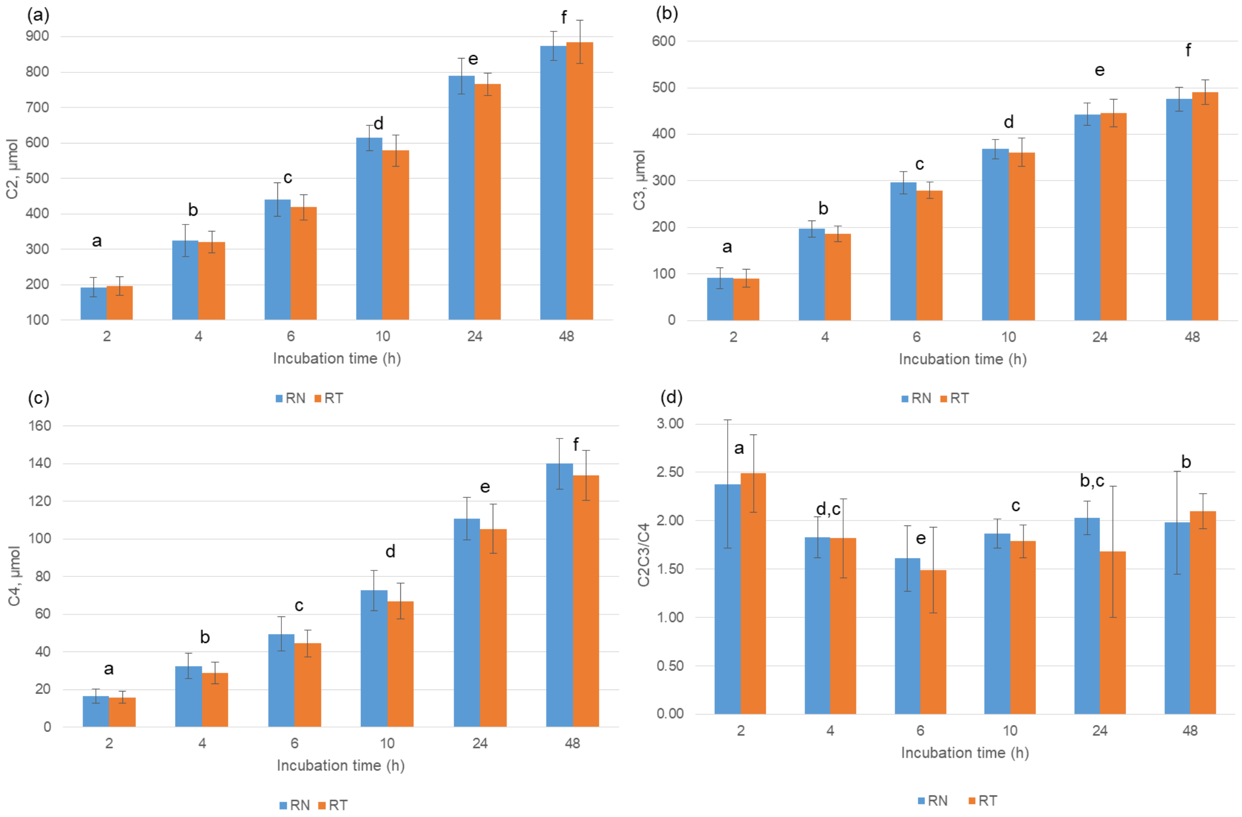


Figure 3. Net production of SCFA and C2C4/C3 ratio (mean values ± SD) resulting from the *in vitro* fermentation of the herbage apparently ingested by sheep grazing Italian ryegrass pastures under contrasting grazing managements. Different literals indicate difference between incubation time (P<0.05). Lowercase literals indicates effect of treatment within incubation time.

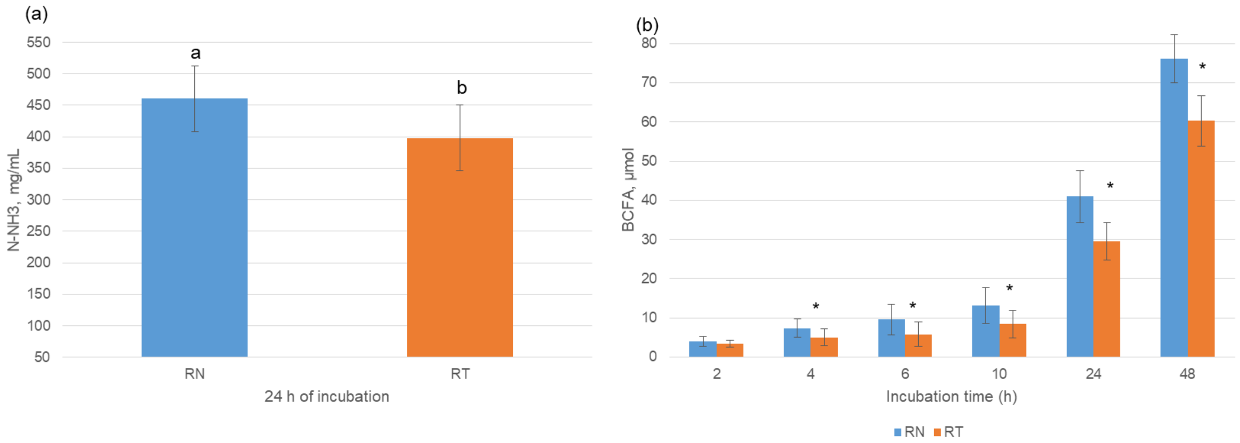


Figure 4. Concentration of N-NH_3 (a) and net production of BCFA (b), measured on rumen liquor (LS-mean ± SD) resulting from the *in vitro* fermentation of the herbage apparently ingested by sheep grazing Italian ryegrass pastures under contrasting grazing managements. Different lowercase literal indicates effect of treatment. * indicates effect of treatment within incubation time.

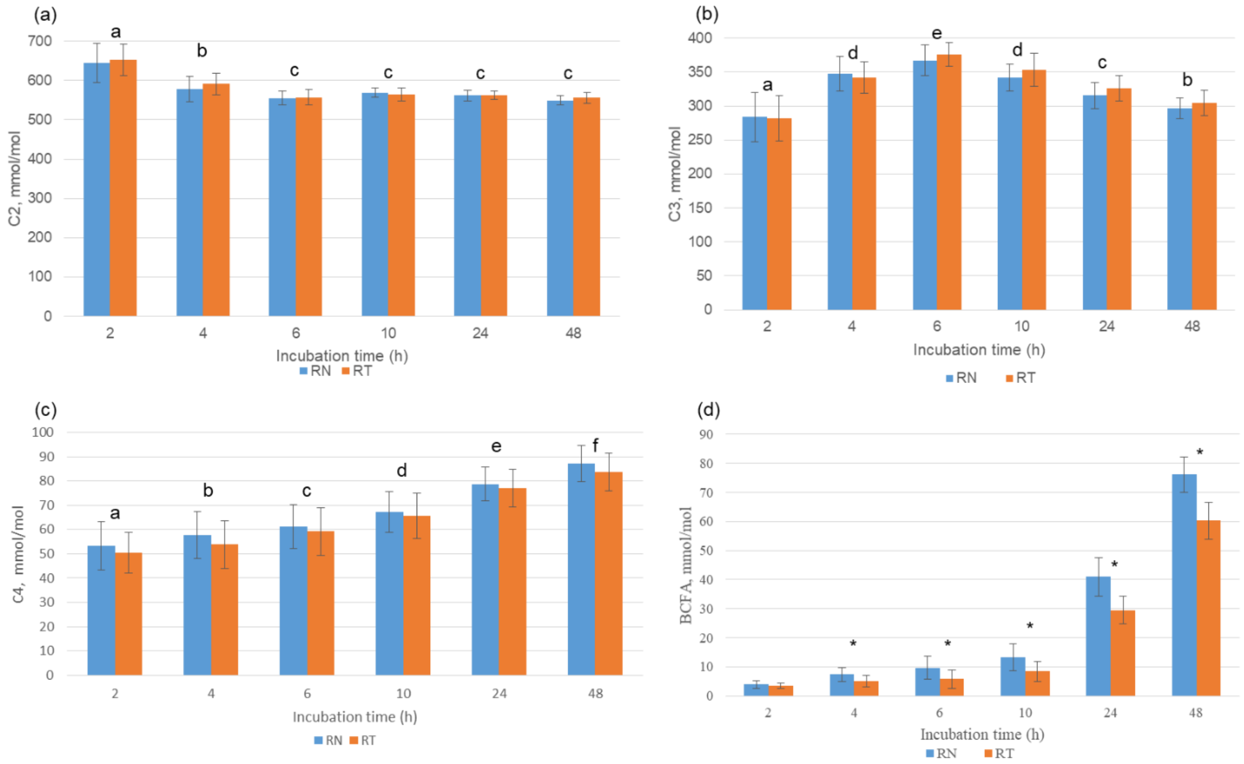


Figure 5. Relative proportion (%) of SCFA and BCFA on rumen liquor (mean values ± SD) resulting from the *in vitro* fermentation of the herbage apparently ingested by sheep grazing Italian ryegrass pastures under contrasting grazing managements. Different literals indicate difference between incubation time (P<0.05). * indicates effect of treatment within incubation time.

4. CHAPTER IV[1].

---

[1] Manuscript prepared in accordance with Science fo the Total Environment (Appendix 2)

Does grazing management provides opportunities to mitigate methane emissions by grazing ruminants in pastoral ecosystems?[1]

Abstract

Methane from enteric fermentation accounts for most of livestock's carbon footprint. Most of global livestock is reared in grasslands under suboptimal feeding conditions, usually resulting in low dry matter intake and LW gain. Thus, the CH_4 quota of maintenance dilutes in low levels of animal outputs, increasing the emission intensity (g CH_4 per kg of animal output) of pastoral ecosystems. Thus increasing the ingestion capacity and performance of a large number of innefficient grazing ruminants represents a great mitigation potential. We show evidence that emissions from animals grazing tropical species can equal those of temperate grasses, thus the generalized idea relating tropical pastures with low nutritive value and intrinsically higher CH_4 emissions is challenged. We demonstrate the medium to high mitigation potential of some plant-oriented grazing managements, most of them associating the nutritive value of the herbage on offer to intake and CH_4 emission of grazing ruminants. As an alternative approach, we stress the predominant influence of pasture structural attributes over animal behavioral responses driving the ingestion process of grazers, thus over their emissions. From this ecological perspective, we introduce a grazing management concept aiming to offer the best pasture structure that allow animals to optimize the nutrients intake per unit of grazing time, thus that opens the window for animals to maximize the daily intake. Finally, we show the trade-off between grazing intensity and CH_4 emissions, stressing that mitigation can be substantially increased when sound grazing management is adopted.

We highlight the evidence suggesting the key role of pasture structure over CH_4 emission from grazing animals. Finally, it is concluded that optimizing secondary productivity of grazing ruminants to around 43 to 57% of their gain observed in feedlot, would dramatically reduce CH_4 emission intensity to around 0.2 kg/ LW gain, as observed from intense feeding systems.

Keywords: pasture structure, intake rate, sward height, grazing animal, emission intensity

4.1. Introduction

Food production, especially protein from ruminants, is an important driver of global environmental changes, including the increase of greenhouse gases (GHG) in the atmosphere (Aiking, 2014; Ramankutty et al., 2018; Rockström et al., 2009). The atmospheric methane (CH_4), with a global warming potential 28 times higher than CO_2 (IPCC, 2014), has increased by a factor of 2.5 since preindustrial times, from 720 ppb in 1750 (Etheridge et al., 1998) to 1,850 ppb in 2017 (Nisbet et al., 2019). As a byproduct of the microbial fermentation of feed in the rumen (Hill et al., 2016; Hook et al., 2010; Janssen, 2010), it largely contributes to the carbon footprint of livestock (Gerber et al., 2013; Herrero et al., 2011; Steinfeld et al., 2006). Globally, CH_4 accounts for 65% of livestock non-CO_2 GHG emissions (Herrero et al., 2013), and of all anthropic CH_4 from enteric fermentation, around 77% comes from cattle (Gerber et al., 2013).

While current emissions ascribed to livestock are large, there is a significant potential to reduce its carbon footprint, especially from pasture-based systems (Gerssen-Gondelach et al., 2017). Mitigation strategies are mostly nutritional-oriented (Beauchemin et al., 2008; Berndt and Tomkins, 2013; Clark, 2013; Hristov et al., 2018, 2013; Kumar et al., 2014; Makkar, 2016; Patra, 2016; SHIBATA and TERADA, 2010; Smith et al., 2008), developed for intensive managements systems in temperate climates (Knapp et al., 2014; Leng, 2014). Those were mostly tested on animals with large intake, high feed efficiency and low CH_4 yield (Hristov et al., 2013) and applicability to grazing systems still challenging given technical, economical, welfare or operational constraints.

Grasslands occupy 37% of ice-free land of earth. They are located especially in least- and sub-developed countries (Reid et al., 2004) and support most of domestic ruminants. These regions produce 57% and 45% of the total beef and milk production of the world, respectively (Outlook, 2011), with forages as the main source of feed for ruminants (O’Mara, 2012). Animals from pasture-based systems usually have higher CH_4 emissions intensity (Gerber et al., 2013; Herrero et al., 2016, 2013; Hristov et al., 2013; Oenema et al., 2014; Rao et al., 2015) compared to housed animals eating energy-dense diets. The underlying causes are multifactorial, but, overall, the high grazing intensities (Ren et al., 2016; Tang et al., 2019), thus suboptimal feeding conditions explains most of such inefficiencies (de Souza Filho et al., 2019). Given that most of ruminants are under poor nutritional conditions (Steinfeld et al., 2006), optimizing productivity per unit of feed intake is by far the most effective levering to lowering enteric CH_4 production. Moreover, increasing evidence shows that primary and/or secondary productivity from grasslands can substantially increase and the CH_4 emission per feed or animal input be equal, or even lower, to animals concentrate-based diets when improved pasture management practices are adopted (Amaral et al., 2016; Andrade et al., 2016; Cezimbra et al., unpublished; Congio et al., 2018; da Silveira Pontes et al., 2018; de Souza Filho et al., 2019; Dini et al., 2018; McCaughey et al., 1997; Savian et al., 2018, 2014).

In this review, we describe the basis from which some plant-related pasture management criteria are used to mitigate CH_4 emissions. We highlight the importance of sward structure in the foraging process by the grazing ruminant and its relevance for defining grazing management targets. Finally, we approach how a new concept in grazing management based on animal behavioral responses creates the conditions to maximize their short-term intake rate (Carvalho, 2013), enabling the maximization of their daily herbage and nutrient intake, with potential to mitigate CH_4 emissions and intensity (Savian et al., 2018).

4.2. CH_4 emissions from enteric fermentation in grazing systems

A wide variety of ruminant production systems are observed across the globe in response to differing agro-ecological conditions, as well as historical and present-day socio-economic orientations (Herrero et al., 2013; Latawiec et al., 2014; Martha et al., 2012; McManus et al., 2016). Pastoral systems vary in forage species and management, and this reflects on the quantity and nutritive value of the ingested diet, consequently on CH_4 emissions (Herrero et al., 2013; Lobato et al., 2014). Moreover, differences in herd composition, breed, health status, supplementation, reproductive efficiency within animal operations (McManus et al., 2016) make animal productivity and CH_4 emission intensity highly variable.

Studies addressing the effect of pasture management on herbage intake and CH_4 emissions from sheep and cattle under grazing-only conditions are shown in Table 1. The differences in animal categories, grazing conditions and overall experimental settings between studies resulted in a large range of intake, nutritive value of forages and CH_4 emissions. This becomes important because as CH_4 production increases with intake, CH_4 yield (g CH_4/kg DM intake) decreases (Hammond et al., 2013; Jonker et al., 2017). For both cattle and sheep, most values of dry matter intake ranged between 2 and 3% of LW, with some below 2 or higher than 3.8 %. Dry matter intake ≥ 3% of LW are observed in temperate pastures associated to high forage allowance and also to tropical pastures managed at heights showing to not restrict forage intake (e.g. 30 cm for Pearl millet; see Castro, 2002). This latter demonstrates the high ingestion capacity of animal grazing tropical pastures. Moreover, other evidence showing lactating dairy cows ingesting 4.1 (Cavanagh et al., 2008) to 4.5% of their LW when very high herbage allowance and highly digestible forage was provided (41 kg DM/cow/day; Stockdale, 1993), indicate a great gap between grazing conditions allowing a margin to optimize herbage intake. Correspondingly to intake levels, Table 1 shows the great variation in CH_4 production. Daily CH_4 production for grazing-only cattle varied from 102 to 372 g per animal (37 to 136 kg CH_4/year), and between 10.9 to 41.7 g for sheep (3.9 to 15.2 kg CH_4/year). These large ranges of DM intake and CH_4 emissions rates show different emission factors that can be applied for national GHG inventories, and the low efficiency of some grazing managements, thus the gap for improvement.

Theoretically, the high nutritive value of forages would result in low CH_4 emissions per unit of DM intake. Moe and Tyrrell, (1979) and Archimède et al. (2011), suggest that temperate grasses and tropical legumes are less methanogenic than tropical grasses, with no differences between temperate grasses and legumes because of the difference in cell wall composition and content. Clearly, feed digestibility, as a function of its chemical composition, relates negatively with CH_4 yield (g CH_4 kg of DM or OM intake) over some dietary conditions (Hegarty, 2009; Shibata and Terada, 2010). In this regard, cattle grazing perennial ryegrass (*Lolium perenne*) monocultures (Boland et al., 2013; Wims et al., 2010) or mixed pastures (Cavanagh et al., 2008; Dini et al., 2018) displayed emissions below 21 g CH_4/kg DM, considered to be the average for dairy cattle in Australia; unusual reposts give values of 11 g CH_4/kg DM intake (Chiavegato et al., 2015). However, beef cattle (Berça et al., 2019; Demarchi et al., 2016) and growing lambs (Amaral et al., 2016) also presented low emissions from well managed tropical pastures, with values between 14 and 17 g CH_4/kg DM intake. These reports coincides with data from stable-fed animals receiving tropical forages (Ku-Vera et al., 2018; Nascimento et al., 2015).

Strategic supplementation of grazing animals increase nutrients intake and animal output (Peyraud and Delagarde, 2013), and reduce CH_4 emissions (Hristov et al., 2013). Table 2 shows that the total DM intake of supplemented-grazing animals is similar to grazing-only animals, but in some cases supplementation resulted in increased total DM intake. Values ranged from 1.7% of LW from beef cattle grazing *Urochloa brizantha* plus 1.1 kg DM of a corn-silage based concentrate (Cota et al., 2014) to 4.6% of LW of dairy cattle supplemented with 8 kg DM of a grain-based concentrate (van Wyngaard et al., 2018). In the latter study, cows offered eight kilograms of concentrate had 18 and 22%

lower CH_4/kg DM intake and CH_4 per kg of milk yield, respectively, compared to control cows; however, unsupplemented animals of this study had similar emissions (20.6 g CH_4/kg/DM intake) respect cows offered two kilograms of concentrate in the study of Jiao et al. (2014), both working with temperate pastures. In this latter study though, authors observed 9.5 and 30% reduction in emissions per DM intake and per kg milk in cows receiving 8 kg/day of a high-grain concentrate respect those receiving only 2 kg/day. Nevertheless, silage- or grain-based supplementation is not always effective in reducing CH_4 emissions. For example, (Muñoz et al., 2015) observed average emissions of 19.2 g CH_4/kg DM intake and 13.5 g CH_4/kg milk yield from cows grazing high-quality pastures (herbage allowance; 25 to 29 kg DM/cow) and offered one or five kilograms of concentrate. Similarly, Lovett et al. (2005) observed no decrease in CH_4 emissions per unit of DM intake or milk yield when offered six kilograms of high fiber concentrate, with average values of 18.7 g CH_4/kg DM intake and 19.4 g CH_4/kg milk yield. Other studies show lower CH_4 yield from partial grazing plus TMR than form TMR-only diet (Cameron et al., 2018; Dall-Orsoletta et al., 2016), or from grazing-only compared to TMR diet (O'Neill et al., 2012). Nonetheless, in the previous study of O'Neill et al. (2011), differences became significant with less emission from grazing-only cows. This data suggest that reducing CH_4 yield by offering nutrient-dense diets to grazing animals is possible, but difficult to obtain, when baseline pasture is of high quality, although a different response could be obtained when the pasture management does not facilitates either intake while grazing or pastures of low nutritive value. It might be considered also that concentrate and TMR usually result in higher DM intake (sometimes in detriment of grass intake) and milk yield, which is an indirect way of mitigation. Nevertheless, if concentrate and TMR rations result in higher milk yields it is also an indirect way of mitigation.

Protein and energy supplementation are frequently used on animals grazing tropical species to compensate for their lower CP and higher fiber contents (Sollenberger and Burns, 2001) and obtain acceptable production levels (Poppi et al., 2018). In some cases, this results in reduced CH_4 emissions. For example, (Carvalho et al., 2017) reported unusual values from 6.6 to 9.9 g CH_4/kg DM intake and 2.5 to 3.4% of gross energy intake from beef steers grazing on *Urochloa brizantha* pastures when added oil (energy) supplementation at 1% of LW, while Cota et al. (2014) observed higher, but still low emissions (11.7 g CH_4/kg DM intake or 4.2% of GE intake) from Nelore cattle offered a corn-silage based supplement and grazing the same pasture. Canesin et al. (2014) reported 27 to 33 g CH_4/kg DM intake from cattle grazing similar pasture plus 10 g/kg of LW per day of a citrus-pulp-cottonseed meal based supplement. These values are lower, equivalent or little higher to those of beef cattle in feedlot supplemented with lipids or distillers grains (Eugène et al., 2011; Fiorentini et al., 2014; Hünerberg et al., 2013) or from finishing beef cattle offered silage-based diets (Mc Geough et al., 2010a, 2010b). Accordingly, Congio et al. (2018), with dairy cows grazing on Elephant grass and supplemented, reported values of 20 g CH_4/kg DM intake, whereas the average emission from supplemented cattle grazing on temperate pastures (Table 2) is 21.6 g CH_4/kg DM intake. From the grazing-only or supplemented data, the generalized idea of low-quality forage (Sollenberger and Burns, 2001) and high CH_4 emission levels (Archimède et al., 2011; Moe and Tyrrell, 1979) of tropical pastures is challenged, as it is remarkable that emissions per unit of feed input from tropical grasses can be equivalent, or even lower than those from temperate grasses alone or mixed with legumes when grazing management allows high levels of intake of a good quality herbage. For this to happen, several grazing management practices are adopted, showing variable mitigation responses.

4.3. Grazing management practices to mitigate CH_4 emissions

The location, timing, duration and intensity of grazing, traditionally set at the farm level by the stocking rate and stocking method, and in experimentation by other grazing managerial decisions such as forage allowance and herbage mass, strongly affect the productivity of pastoral ecosystems. Efficient pasture-based systems are those in which the production and intake of forage rich in metabolizable energy are maximized (Hodgson, 1990; Boval and Dixon, 2012). While the grazing intensity influences the herbage growth (Lemaire et al., 2009) and its nutritive value (Da Trindade et al., 2016; Echeverria et al., 2016), the ingestion process of the grazing animal is greatly affected by the forage allowance (daily scale; Stobbs et al., 1975; Hodgson, 1999; Sollenberger and Vanzant, 2011) and sward structure (short-term scale; Allden and McDWhittaker, 1970; Da Silva and Carvalho, 2005; Laca et al., 1992). Thus, if minimizing carbon footprint of grazing livestock production becomes the target, the grazing management must provide pasture conditions to maximize herbage intake, its growth and nutritive value, and animal performance, balancing forage demand with supply, at a moderate grazing intensity (Boval and Dixon, 2012; Da Silva and Carvalho, 2005; Ren et al., 2016).

The relationship between grazing intensity, herbage intake and production efficiency is well established (Maraschin, 2001; Mott, 1960). Overall, intensive defoliation increases the herbage harvesting efficiency (kg DM ingested per kg DM in offer), reduces per-animal intake and performance (Da Trindade et al., 2016), but increases herd intake and per-area yield (Glindemann et al., 2009; McCarthy et al., 2011; Peyraud and Delagarde, 2013). At grazing efficiency of 50%, for example, individual intake is 0.75 times its capacity, decreasing abruptly from this point on. Conversely, maximum intake occurs when grazing efficiency decreases to 30% (Delagarde et al., 2001). Carvalho, (2013) considers this incompatibility of maximizing both the individual intake and pasture utilization as the apparent trade-off of pasture management. Romera and Doole, (2015) demonstrated through modelling of the grazing process that maximum pasture intake per cow was only possible at low levels of intake per hectare. Contrariwise, maximum intake per hectare was achieved only at relatively low intake per-cow. This is of utmost importance from a CH_4 mitigation perspective, since the level of intake is what affects the most the LW gain and enteric CH_4 production, thus CH_4 emission intensity.

The impact of such a trade-off of pasture management upon enteric CH_4 emissions have not been extensively documented. Nevertheless, most of reports advocate inducing animals to eat most of what is on offer to maximize grazing efficiency and avoid “waste” (Boland et al., 2013; DeRamus, 2004; Fariña and Chilibroste, 2019; Peyraud and Delagarde, 2013; Wims et al., 2010). It is argued that frequent and intense grazing would increase the herbage nutritive value (Da Trindade et al., 2016; DeRamus, 2004), by promoting new tillers regrowth (McEvoy et al., 2009), higher leaf-to-steam-ratio and less senescence (Curran et al., 2010; Hoogendoorn et al., 1992), and increase OM digestibility (McEvoy et al., 2009). This, in turn, is hypothesized to stimulate the intake of herbage with higher nutritive value, and animal performance, while reducing CH_4 yield. Most studies aiming to reduce CH_4 emissions through pasture management were conducted under this theoretical basis while few considered the grazing process and pasture structure as important drivers of the daily intake, thus of CH_4 emissions (Table 3). Between these studies, it is also evident the different experimental settings, with some considering the individual animal (grazing in a group, on a single or more paddock) as the experimental unit, and others considering the paddock (with sampling animals) as the statistical replicate (Table 3). This becomes of utmost importance given the sensibility that grazers have to small variations in the grazing environment (e.g. sward structure), even between paddocks apparently homogeneous, and the influence that certain animals could have over the grazing behavior of others (interdependency among experimental units), thus over the amount and nutritive value of the herbage ingested individually, as discussed by (Rook, 2004).

Stocking rate

The stocking rate is a common managerial target used in pastoral farming. Nevertheless, stocking rate is poorly related to animal performance (McCarthy et al., 2011) since it only refers to the animal component (e.g. animals per area or animal weight per area) and brings no information on the pasture. A given stocking number could result in different levels of intake, CH_4 production and yields, thus comparisons across systems or outside of a specific study are limited. Few studies have assessed the influence of stocking rate on CH_4 emissions. Alcock and Hegarty (2006) observed a two-fold increase in emissions from sown pastures with high stocking rate (9 ewes/ha), but no change in CH_4 per animal output. Pinares-Patiño et al. (2007) stocked Holstein-Friesian heifers at

1.1 and 2.2 livestock units per hectare and reported contrasting values of OM intake, LW gain and CH_4 production between years. Across years, the stocking rate had no effect over absolute CH_4 production. Instead, emissions were consistently related to herbage intake rather than its digestibility. Accordingly, in (Chiavegato et al., 2015), different stocking rate and density did not affect the DM intake of lactating beef cows but inconsistently resulted in different CH_4 emissions (g/day and g/kg DM intake). The above was associated with selective grazing and similar quality of the ingested herbage, which probably means that the herbage allowance was adequate, a trait not predicted by the stocking rate. Overall, Tang et al. (2019) recommended low grazing intensity (< 2 sheep/ha/year for a grassland ecosystem in China) to not decrease grasslands' soils capacity to uptake atmospheric CH_4, thus to offset C emissions from CH_4 of enteric and feces.

Stocking method

The rotational stocking aids in controlling the moment, site and extent of grazing, the amount and nutritive value of the forage ingested and is considered an improvement in grazing management (DeRamus, 2004; Latawiec et al., 2014). DeRamus et al. (2003) imposed a rotational stocking to optimize the herbage nutritive value in respect to continuous stocking. The authors observed an average 17% higher daily CH_4 production, but 49% lower CH_4/kg LW gain, for cattle rotationally stocked in *Cynodon dactylon*, but no difference in CH_4 yield in *Paspalum notatun* pastures. However, no fair comparison of stocking methods can be made from DeRamus et al. (2003) provided the difference in herbage species composition, fertilization, herbage mass in offer and other non-reported sward structural features between managements that affect the amount and nutritive value of the herbage ingested.

McCaughey et al. (1997) observed no effect of the stocking method and stocking rate over voluntary intake and CH_4 production of steers grazing mixed alfalfa pastures, after adjusted for body weight. Conversely, Savian et al. (2014) showed that, when compared at low and moderate grazing intensity, the continuous stocking increased the individual animal LW gain without affecting the LW gain per hectare, and reduced the CH_4 emission intensity. Both the higher forage intake and average daily gain were attributed to the greater nutritive value of the forage ingested, as animals had greater opportunity for selection compared to rotational stocking (Briske et al., 2008). In turn, this opposes to the view of the continuous stocking as unimproved management (DeRamus, 2004; Latawiec et al., 2014). In respect this, Briske et al. (2008) argue that, under a wide range of conditions, both methods produce comparable outcomes and that is the grazing intensity what really determines the output of the stocking method. Interestingly, in Savian et al. (2014) there was no effect of the grazing intensity (moderate or low) over average daily gain, but the moderate intensity increased the LW gain per hectare and daily CH_4 emissions per hectare, without affecting the CH_4 yield. From these contrasting results, it is evident that further studies are needed to establish clearer associations between the stocking method and CH_4 emissions.

Herbage mass

The herbage mass as a grazing management target quantitatively measures the forage in offer and indirectly indicates its nutritive value; overall, greater biomass accumulation is a result of a longer regrowth period, thus representing a mature forage of reduced nutritive value. Thereby, for some (Hoogendoorn et al., 1992), low herbage masses are regarded to have better nutritive value. For example, low herbage masse increased animal outputs in 11.5% (Muñoz et al., 2016) and reduced CH_4 emission intensity by up to 17.6 to 21.6% in dairy (Muñoz et al., 2016; Wims et al., 2010) and by 22.6% in beef cattle (Boland et al., 2013). However, over some circumstance, the nutritive value fails to associate with CH_4 emissions (Hammond et al., 2013; Jonker et al., 2017; Pinares-Patiño et al., 2003; Richmond et al., 2015; Sun et al., 2011). Herbage nutritive value associated to herbage mass as a grazing target could not consistently predict mitigation benefits.

Light interception

Harvesting pastures at 95% of light interception has been proposed as a pasture management criteria to maximize herbage mass accumulation while keeping senescence low, thus maintaining adequate nutritive value (da Silva and Nascimento Jr. 2007; da Silva et al., 2015; da Silveira et al., 2016). Congio et al. (2018) observed no differences in daily CH_4 emissions but a 21% reduction in CH_4 per milk yield (62.2 to 53.8 kg milk per kg of CH_4 per hectare) in dairy cows grazing Elephant grass managed at 95% light interception and grazed down to half of its initial height compared to pastures managed at 100 % of light interception and similar grazing down level. Those were the result of a 13.2% higher milk yield and 18% lower CH_4 per kg of DM intake. With similar management, (Berça et al., 2019) observed low CH_4 per kg of DM intake from cattle grazing a tropical pasture, with no mitigation effect of fertilization or inclusion of legumes in mixed pastures.

Forage allowance

The forage allowance (e.g. kg DM/kg LW) is a pasture-to-animal relation (Allen et al., 2011) used as an indicator of intake, performance and forage nutritive value (Maraschin, 2001). Although some studies have assessed its effects on daily forage intake (Da Trindade et al., 2016), and set the potential primary and secondary production of grazing systems (Peyraud et al., 1996; Maraschin, 2001), evidence of its effect on CH_4 emissions is scarce. The best compromise between individual and per hectare gains in continuously-stocked native grasslands occurs at around 12 kg DM/100 kg LW of daily forage allowance (Maraschin et al., 2001), but increasing grazing pressure in the spring results improved forage utilization by manipulation of canopy characteristics resulting in greater animal performance (Soares et al., 2005). In the same long-term study, Cezimbra et al., (Unpublished) observed greater daily CH_4 emissions from animals at higher herbage allowance treatments, but an inverse relationship for CH_4 emission per hectare and an exponential increase of CH_4/kg LW gain as forage allowances reduced, from 16 to 4 kg DM/100 kg LW per day. The lower CH_4 emissions did not derive from pastures with the higher forage nutritive value (i.e. 4 kg DM/100 kg LW per day), because those were also the most limiting in forage quantity. In pastoral systems not always offering the best diet is the best strategy to mitigate methane (Cezimbra et al., Unpublished).

As grazing management practices the stocking rate and method, the herbage mass and forage allowance have undoubtedly contributed to the better use of pastures (Euclides et al., 2010; Latawiec et al., 2014; Rouquette, 2016). Nevertheless, these plant-oriented managements do not describe the grazing environment (Sollenberger et al., 2005; Carvalho et al., 2001), instead, they merely indicate the quantity and nutritive value of forage on offer, or the amount of animals per area. They do not describe how the forage is presented and distributed in time and space (Hodgson 1990). Thereby, a given stocking rate, herbage mass or forage allowance, for example, can occur at different plant structural arrangements, with different effects on the grazing behavior, intake and performance, and arguably over enteric CH_4 emissions. According to Carvalho et al. (2001) and Da Silva and Carvalho (2005), as structural attributes of the pasture strongly affect the ingestion process, intake and diet selection of the grazing animal, such pastures attributes must be considered in developing grazing management targets, in order to maximize individual intake of animals for productive purposes, and reasonably for CH_4 emissions mitigation.

4.3.1 The effect of sward structure on foraging behavior, intake and CH_4 emission of the grazing ruminant

The feeding behavior and ingestion capacity between the indoor-fed and grazing ruminant are notably different. The classical metabolic and physical constraints of intake (Allen, 1996; Forbes and Barrio, 1992; Merterns, 1994; Forbes, 2003; Illius and Jessop, 1996) may operate conjunctly, but hierarchically different under such contrasting feeding conditions (Allden and McDWhittaker, 1970; Tedeschi et al., 2019). For penned animals, the chemical composition of the diet is an important driver of intake (CSIRO, 2007), while for the grazing ruminant, the ‘nutritional factors’ are hypothesized to be less important in regulating herbage intake (Da Silva and Carvalho, 2005). For instance, (Poppi et al., 1987) suggest forage harvesting as main constrain of intake at the ascending part of the response curve relating intake with forage allowance and nutritional constraints at the plateau of the response curve. Accordingly, Carvalho et al. (2001) suggest the predominant role of the pasture structure and behavioral factors upon the ingestion process by grazers and highlights the importance of the variable ‘time’ on it (Carvalho, 2013).

As grazers find the food resources distributed heterogeneously over time and space (Shipley, 2007), they adapted accordingly to built-up their daily intake from a series of behavioural decisions occurring in temporal-space scales (Carvalho, 2013). Laca and Ortega (1996) considered the ‘bite’ the leading cause of the daily herbage intake. This is because its characteristics, such as the bite rate and bite mass, directly affect the short-term intake rate (g DM/min), and indirectly the time a grazer advocates to foraging each day (Shipley, 2007). Ultimately, the daily intake is a function of the grazing time and the intake per unit of time while grazing (Allden and McDWhittaker, 1970). This way, the reduced intake of the grazing ruminant is primarily due to the low intake rate and constraints on the total daily time available for grazing (Gibb et al., 1997). The bite characteristics defining the intake rate, especially the bite mass, are altered by the sward structure (Allden and McDWhittaker, 1970; Benvenutti et al., 2016; Chacon and Stobbs, 1976; Guzatti et al., 2017; LACA et al., 1992), particularly the sward surface height (Hodgson, 1990; ; Mezzalira et al., 2017, 2014). Thereby, the intake rate responds quadratically to sward height, while the bite rate, also keeps a quadratic but negative relation (Allden and McDWhittaker, 1970; Amaral et al., 2013; Fonseca et al., 2013, 2012; Laca et al., 1992; Mezzalira et al., 2017, 2014).

The sward surface height is thus an indicator of both the herbage mass and its structure, this is an important factor affecting forage intake (Allden and McDWhittaker, 1970; GIBB et al., 1997; Laca et al., 1992) and animal performance (Carvalho et al., 2006; Euclides et al., 2018; Kunrath et al., 2020). De Souza Filho et al. (2019) recently reported its association with CH_4 emissions. The authors observed a linear decrease in CH_4 emission intensity, from 155 to 250 g CH_4/kg LW gain, as sward height increased from 10 to 40 cm, with the best compromise between performance and CH_4 yield occurring with swards managed at a light-to-moderate grazing intensity, between 23 and 30 cm; the improved pasture management resulted in a two-fold decrease in the fattening-to-slaughter emissions, as time to slaughter reduced by 63 days. Authors argued that by large-scale adoption of appropriate sward height managements, Brazil has the potential to achieve 14 and 25 % of the mitigations pledges in the Paris Agreements of GHG (whole agriculture) and enteric CH_4 (livestock), respectively.

The works relating the sward height with ingestion capacity of animals suggest, in general, that tall swards provide large bites, but reduce the intake rate as bite rate decreases; conversely, short swards allow grazers to take small bites at an increased bite rate, which also reduces the intake rate (Allden and McDWhittaker, 1970; Gibb et al., 1997; Laca et al., 1992). The grazing animal responds to reduced bite mass and intake rates by increasing the daily grazing time (Chilibroste et al., 2015; GIBB et al., 1997). However, there is a maximum grazing time available to an animal on a daily basis (usually less than 10 hours; Hodgson, 1990), and the consequence of a severe restriction of bite-size (i.e. short sward, heavy grazing), is frequently a reduction in intake. This is especially important for the dairy cow, as grazing activity competes with other time-consuming behaviors (i.e. ruminating, idling, socializing, displacement) or human interventions (i.e. milking). Therefore, if the intake rate and daily grazing time operate as important constrainers of the daily intake, grazing management targets must be oriented to optimize the amount of nutrient ingested per unit of time (Carvalho, 2013).

Some works fueled studies aiming to determine the sward height that allows animals to maximize the intake rate for some temperate and tropical species (see Carvalho, 2013). Those suggest a 40% grazing-down limit of optimal sward height, for allowing animals to sustain high intake rate. This conceptual approach was integrated to propose a grazing management concept that sets the pre- and post-grazing sward heights that offers to animals the possibility to maximize short-term intake rate at any time while grazing; this is, an animal-oriented grazing management concept. For Italian ryegrass pastures, it results in pre- and post-grazing sward height of 18 and 11 cm, respectively and can potentially reduce enteric CH_4 emission from pastoral systems. For instance, Savian et al. (2018) compared it with a traditional rotational stocking applied to growing lambs grazing on Italian ryegrass pastures (pre and post-grazing sward height of 27 and 7 cm, respectively). Authors observed increased CH_4 production (24.8 vs 22.2 g/animal/day), provided the higher OM intake (801 vs 653 kg OM/day), but significantly less CH_4 per unit of feed input (39.5 vs 5.7 g kg digestible OM intake) for the animal-oriented management. Moreover, the small differences in CH_4 production, but higher animal performance in the RN, led to a reduced CH_4 emission yield (217 vs 586 g kg LW gain). The daily emission per unit of area is 0.61 of the traditional rotational stocking (645 vs 1056 g ha d^{-1}) which demonstrate the great mitigation potential of CH_4 emissions. Interestingly, this management showed to optimize both the daily intake (Savian et al., 2018) and performance, concomitantly with herbage production (Schons et al. Unpublished), overcoming the classical trade-off between individual intake/performance and production per area.

Other grazing management practices

Other grazing management strategies manipulate the time of pasture allocation to better match animal requirements with nutrient supply (Gregorini et al., 2008). This could improve nutrient uptake by rumen microorganisms, reduce N excretion, improve microbial protein synthesis and shift rumen fermentation pathways towards low CH_4 production. While in theory this is sound, in practice is elusive (Hall and Huntington, 2008). Current evidence show changes in rumen fermentation as affected by time of pasture allocation (Ribeiro Filho et al., 2012), yet without efficiently reducing CH_4 emissions, as observed by Kidane et al. (2018) setting 7-day rotational grazing, daily strip grazing or daily forward grazing, as a mean to alter the nutritive value of the forage. Overall, it seems that when pasture is of high quality, matching nutrient supply does not bring important benefits for CH_4 mitigation. For instance, the higher soluble carbohydrates and lower fiber of pastures observed in the afternoon could reduce CH_4 emissions if afternoon strip-change is adopted in rotational stocking (Gregorini, 2012).

Nitrogen fertilization

Despite this is not a grazing management *per se*, it affects the herbage production, nutritive value and stocking rate. It has proven to indirectly abate GHG emissions per output unit (Hauggaard-Nielsen et al., 2016). Amaral et al. (2016) reported a 35% increase in both LW gain per hectare and CH_4 per hectare with sheep grazing tropical pastures as fertilization increased from 50 to 400 kg N/ha, mostly as a consequence of the two-fold increase in stocking rate. Similarly, Berndt et al. (2014) estimated that one hectare of an intensively managed pasture, with irrigation and 600 kg N/ha/year, can produce 7.0 times more meat with only 4.5 times the CH_4 than a degraded pasture, or produce the same amount of meat in a smaller area with lower emissions. Nitrogen fertilization offers medium to long-term benefits of N fertilization in terms of GHG emissions. Allard et al. (2007) showed that both intensively (high stocking rate and N fertilization) and extensively managed pastures (half stocking rate without fertilized) are net carbon sinks, but when taking into account the CO_2, N_2O and CH_4 from the soil, the intensive management maintained the carbon sink capacity, while the extensively manage pasture saw a reduction in its capacity to fix C in the soil after three years. Moreover, sowing cultivated species and fertilizing native grasslands can contribute to their preservation, as demonstrated by Vasconcelos et al. (2018) in the Pampa biome, in Southern Brazil.

4.4. Well-managed pastures have low CH_4 emission intensities

Grazing systems have high CH_4 emission intensity (EI; g CH_4 per kg of animal output) when DM intake, thus animal performance is low. Figure 1 shows the relationship of CH_4 EI and average daily gain pondered by the metabolic weight from published and unpublished experiments on pasture and feedlot. The negative exponential decline in CH_4 per animal output as animal performance increases was previously demonstrated with beef cattle fed high-grain diets (Kurihara et al., 1999) and pasture-based dairy cows (Watt et al., 2015), however, neither work integrates a broad range of production systems. When the animal gain is near zero, the maintenance quota associated to CH_4 is the highest, thus emission intensity is maximum. As animal performance improves to an average daily gain of 0.14 and 0.7 kg/day in sheep and beef steers, respectively, emission sharply decrease to 0.2 kg CH_4 kg LW gain. For both sheep and cattle, this performance represents around 43 to 57% of the growth rate in feedlot, reported to range from 1.3 to 1.5 kg/animal/day for cattle (Poppi et al., 2018), and from 0.245 to 0.312 kg/animal/day (Hernández et al., 2017; Rodríguez et al., 2007). From this breaking point on, additional weight gains do not lead to significant decreases in CH_4 emission intensity, as every unit of average daily gain leads to meaningless reductions of EI. Thus, the mitigation potential depends on the baseline production inefficiencies; therefore, the mitigation potential from high-yielding animals is already narrow. Conversely, grazing ruminants produce 75% of global ruminants CH_4 (FAO, 1999), therefore, the opportunity of mitigation, especially for ruminants grazing under sub-optimal feeding conditions, is large.

The prediction of CH_4 emissions from the chemical composition of forages is weaker than from intake (Hammond et al., 2013; Jonker et al., 2017; Sun et al., 2011), and altering the chemical composition of forages has limited scope for CH_4 mitigation (Buddle et al., 2011; Pacheco et al., 2014) over some feeding conditions (e.g. forages with high nutritive value). Some grazing studies support this assumption. For example, Pinares-Patiño et al. (2007, 2003) and Richmond et al. (2015) failed to find a strong relationship between forages chemical composition and CH_4 emissions, in Cezimbra et al. (Unpublished) and de Souza Filho et al. (2019), the lower CH_4 yield from grazing-only animals resulted from pastures allowing animals to optimize ingestion and not from pastures offering higher nutritive value. In the former study though, the higher forage quality was demonstrated afterwards by Da Trindade et al. (2016), working in the same 30-years experimental protocol. With tropical pastures, the nitrogen fertilization (150 kg N/ha) improved the nutritive values of the herbage apparently selected by dairy heifers, respect animals grazing non-fertilized or pastures mixed with legumes (14.2 % in hand-plucked herbage), but no reduction in CH_4 emissions (g/d, g kg/DM intake and % of GE intake) were evident (Berça et al., 2019). Thus, if high DM intake levels is warranted, the CH_4 emission intensity from grazing animals can be equal to those on intense-fed systems, independent on pasture source (Andrade et al., 2016; Poppi et al., 2018).

Performance levels from temperate and tropical pastures can range from 0.75 to 1.0 times those of feedlot (Poppi et al., 2018). For example, temperate cultivated pastures can support LW gains of 0.7 to 1.5 kg/day for beef cattle (Boland et al., 2013; Cox-O’Neill et al., 2017; Pinares-Patiño et al., 2007), whereas evidence for tropical forages indicate LW gains from 0.7 to 0.97 kg/day (Andrade et al., 2016). At those high performance levels, emission intensity for grazing systems drops below 180 kg CH_4/kg $LW^{0.75}$, similar to levels obtained for intensive-fed animals (Table 1 and 2; Figure 1; supplementary material 1). Data from sheep grazing temperate species (Orr et al., 2019), at moderate grazing intensity (Glindemann et al., 2009; Savian et al., 2014) or in rotational stocking (Schons et al., Unpublished), or tropical forages (Castro, 2002) show LW gains from 0.12 to 0.28

kg/day, this is, values near or above the limit 0.14 kg/day, for reduced CH_4 EI. Ultimately, improved individual animal weight gains from pastoral systems would allow finishing more animals in the same time required to finish animals at decreased growing rates, with a concomitant decrease in the CH_4 emitted per kg of live weight gain.

Rather nutritive value, the driver of higher emission intensities of slow-growing animals grazing high-nutritive value pastures can be better explained by a lowered herbage conversion efficiency (kg DM intake per kg of LW gain; Schons et al., Unpublished) and higher energy wastes (Carvalho et al., 2004; Glindemann et al., 2009; Osuji, 1974) associated to grazing management strategies. Reducing the individual intake and performance at higher grazing intensities leads to increased enteric CH_4 per LW gain and per hectare (Tang et al., 2019). Despite this evidence, from most of the studies reviewed in Table 3 indicating improvement in herbage nutritive value by imposing severe grazing treat it as the central issue for enteric CH_4 mitigation from pasture-based systems. Conversely, we suggest that for the grazing animals, optimizing individual intake through canopy structure management, first, and then the chemical composition of herbage, is especially important for CH_4 mitigation, and that the latter should be an effect, not a cause, of increasing the ingestion capacity of grazing animals (Cezimbra et al., unpublished; de Souza Filho et al., 2019; Savian et al., 2018). The evidence from grazing-supplementation or indoor-fed animals, failing to reduce CH_4 emissions with highly nutritive diets (e.g. temperate pastures), support this idea.

Overall, secondary production from pasture-based systems can increase above the limit that result in low CH_4 EI. For example, in Brazil, for example, Días-Filho (2004) estimated that 75% of the 200 million hectares of pastures are moderately to highly degraded, which results in productivity levels of 34% of its potential (Strassburg et al., 2014). Similarly, Nascimento Jr. et al. (2003) indicate that the average dairy and beef cattle production is around 0.8 to 1.0 t milk/ha/year and 60 to 100 kg LW gain/ha/year, respectively, compared to the 25 to 30 t milk/ha/year and 1000 to 1600 kg LW gain/ha/year that can be achieved with strategic supplementation, adjustment of stocking rate and adequate soil fertilization (Corsi et al., 2001). De Souza Filho et al. (2019) argued that large-scale adoption of moderate grazing intensity in Southern Brazil has the potential to achieve 13 to 14% of the mitigation target for the GHG emissions from agriculture, and 22 to 25% of the target of CH_4 emission from livestock pledged by the Brazilian government in the COP 21 Paris Agreement. Therefore, it is clear that overcoming production inefficiencies in grassland-based ruminant production systems are valuable options to hit two birds with one stone: increase carbon sequestration, reduce CH_4 emissions and increase food supply.

4.5. Final considerations

Grasslands provide numerous ecosystem services when properly managed, but when primary and secondary production is inefficient, the environmental impact in CH_4 EI is large. Adopting sound livestock management practices, at moderate grazing intensities can lower emissions by “diluting” the baseline emission in higher animal outputs, by offsetting emission through carbon sequestration, and by the land-savings effect of producing more in less area. Furthermore, improving animal performance can reduce age at slaughtering, which has the potential to reduce animal stocks. Such pathways are not mutually exclusive, as multiple mitigation pathways occur concomitantly, yet in a sustained fashion, this way addressing different specific issues and adapt to local circumstances. As many of the proposed strategies are knowledge- instead of input-based, their applicability on pastoral systems is more plausible. Since the DM intake strongly correlates positively with LW gain, and this latter does it negatively with CH_4 emission intensity, it is reasonable to think that for mitigating purposes, optimizing the ingestion capacity of grazing animals is of utmost importance. If this is the target, creating sward structures facilitating the ingestion process, maximizes the chances for animals to increase the daily intake directly from pasture. Finally, optimizing secondary productivity of grazing ruminants to around 43 to 57% of their gain observed in feedlot, would dramatically reduce CH_4 EI to levels comparable to more intense-fed animals.

Conflict of interest

None

Acknowledgments

We thank the National Council of Science and Technology (CONACYT) from Mexico, for the PhD scholarship (CV: 171912) provided. We also thanks all the graduate students that participated in the field data collection.

4.6. Consulted literature

Aiking, H., 2014. Protein production: planet, profit, plus people? Am. J. Clin. Nutr. 100, 483S-489S. https://doi.org/10.3945/ajcn.113.071209

Alcock, D. and Hegarty, R.S., 2006, July. Effects of pasture improvement on productivity, gross margin and methane emissions of a grazing sheep enterprise. In *International congress series* (Vol. 1293, pp. 103-106). Elsevier.

Allard, V., Soussana, J.-F., Falcimagne, R., Berbigier, P., Bonnefond, J.M., Ceschia, E., D'hour, P., Hénault, C., Laville, P., Martin, C., Pinarès-Patino, C., 2007. The role of grazing management for the net biome productivity and greenhouse gas budget (CO2, N2O and CH4) of semi-natural grassland. Agric. Ecosyst. Environ. 121, 47–58. https://doi.org/10.1016/j.agee.2006.12.004

Allden, W., McDWhittaker, I., 1970 The determinants of herbage intake by grazing sheep: The interrelationship of factors influencing herbage intake and availability. Aust. J. Agric. Res. 21, 755. https://doi.org/10.1071/AR9700755

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74, 3063. https://doi.org/10.2527/1996.74123063x

Allen, V.G., Batello, C., Berretta, E.J., Hodgson, J., Kothmann, M., Li, X., McIvor, J., Milne, J., Morris, C., Peeters, A., Sanderson, M., 2011. An international terminology for grazing lands and grazing animals. Grass Forage Sci. 66, 2–28. https://doi.org/10.1111/j.1365-2494.2010.00780.x

Amaral, G.A., David, D.B., Gere, J.I., Savian, J.V., Kohmann, M.M., Nadin, L.B., Chopa, F.S., Bayer, C., Carvalho, P.C.F., 2016. Methane emissions from sheep grazing pearl millet (Penissetum americanum (L.) Leeke) swards fertilized with increasing nitrogen levels. Small Rumin. Res. 141, 118–123. https://doi.org/10.1016/j.smallrumres.2016.07.011

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

Andrade, E.A., Almeida, E.X., Raupp, G.T., Miguel, M.F., de Liz, D.M., Carvalho, P.C.F., Bayer, C., Ribeiro-Filho, H.M.N., 2016. Herbage intake, methane emissions and animal performance of steers grazing dwarf elephant grass v. dwarf elephant grass and peanut pastures. animal 10, 1684–1688. https://doi.org/10.1017/S1751731116000628

Archimède, H., Eugène, M., Marie Magdeleine, C., Boval, M., Martin, C., Morgavi, D.P., Lecomte, P., Doreau, M., 2011. Comparison of methane production between C3 and C4 grasses and legumes. Anim. Feed Sci. Technol. 166–167, 59–64. https://doi.org/10.1016/j.anifeedsci.2011.04.003

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

Benrdt, A., Lemes, A., Sakamoto, L. and Oliveira, P., 2014. The impact of Brazilian livestock production on global warming. In *Embrapa Pecuária Sudeste-Artigo em anais de congresso (ALICE)*. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 51. 2014, Barra dos Coqueiros. A produção animal frente às mudanças climáticas e tecnológicas-palestra. Barra dos coqueiros: SBZ, 2014. 1 CD-ROM.

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

Berça, A.S., Cardoso, A. da S., Longhini, V.Z., Tedeschi, L.O., Boddey, R.M., Berndt, A., Reis, R.A., Ruggieri, A.C., 2019. Methane production and nitrogen balance of dairy heifers grazing palisade grass cv. Marandu alone or with forage peanut. J. Anim. Sci. 97, 4625–4634. https://doi.org/10.1093/jas/skz310

Berndt, A., Tomkins, N.W., 2013. Measurement and mitigation of methane emissions from beef cattle in tropical grazing systems: a perspective from Australia and Brazil. animal 7, 363–372. https://doi.org/10.1017/S1751731113000670

Boland, T.M., Quinlan, C., Pierce, K.M., Lynch, M.B., Kenny, D.A., Kelly, A.K., Purcell, P.J., 2013. The effect of pasture pregrazing herbage mass on methane emissions, ruminal fermentation, and average daily gain of grazing beef heifers1. J. Anim. Sci. 91, 3867–3874. https://doi.org/10.2527/jas.2013-5900

Boval, M., Dixon, R.M., 2012. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. animal 6, 748–762. https://doi.org/10.1017/S1751731112000304

Briske, D.D., Derner, J.D., Brown, J.R., Fuhlendorf, S.D., Teague, W.R., Havstad, K.M., Gillen, R.L., Ash, A.J., Willms, W.D., 2008. Rotational Grazing on Rangelands: Reconciliation of Perception and Experimental Evidence. Rangel. Ecol. Manag. 61, 3–17. https://doi.org/10.2111/06-159R.1

Buddle, B.M., Denis, M., Attwood, G.T., Altermann, E., Janssen, P.H., Ronimus, R.S., Pinares-Patiño, C.S., Muetzel, S., Neil Wedlock, D., 2011. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. Vet. J. 188, 11–17. https://doi.org/10.1016/j.tvjl.2010.02.019

Cameron, L., Chagunda, M.G.G., Roberts, D.J., Lee, M.A., 2018. A comparison of milk yields and methane production from three contrasting high-yielding dairy cattle feeding regimes: Cut-and-carry, partial grazing and total mixed ration. Grass Forage Sci. 73, 789–797. https://doi.org/10.1111/gfs.12353

Canesin, R.C., Berchielli, T.T., Messana, J.D., Baldi, F., Pires, A.V., Frighetto, R.T.S., Fiorentini, G., Reis, R.A., 2014. Effects of supplementation frequency on the ruminal fermentation and enteric methane production of beef cattle grazing in tropical pastures. Rev. Bras. Zootec. 43, 590–600. https://doi.org/10.1590/S1516-

35982014001100005

Carvalho, I.P.C. de, Fiorentini, G., Castagnino, P. de S., Jesus, R.B. de, Messana, J.D., Granja-Salcedo, Y.T., Detmann, E., Padmanabha, J., McSweeney, C.S., Berchielli, T.T., 2017. Supplementation with lipid sources alters the ruminal fermentation and duodenal flow of fatty acids in grazing Nellore steers. Anim. Feed Sci. Technol. 227, 142–153. https://doi.org/10.1016/j.anifeedsci.2017.02.017

Carvalho, P.C.D.F., Oliveira, J.O.R., Pontes, L.D.S., Silveira, E.O.D., Poli, C.H.E.C., Rübensam, J.M. and Santos, R.J.D., 2006. Características de carcaça de cordeiros em pastagem de azevém manejada em diferentes alturas. *Pesquisa Agropecuária Brasileira*, *41*(7), pp.1193-1198.

Carvalho, P.C.D.F., 2013. Harry Stobbs Memorial Lecture: Can grazing behavior support innovations in grassland management? Trop. Grasslands - Forrajes Trop. 1, 137. https://doi.org/10.17138/TGFT(1)137-155

Carvalho, P.D.F., Ribeiro Filho, H.M.N., Poli, C.H.E.C., MORAES, A.D., and Delagarde, R., 2001. Importância da estrutura da pastagem na ingestão e seleção de dietas pelo animal em pastejo. *Reunião Anual da Sociedade Brasileira de Zootecnia*, *38*(2001), p.871.

Castro, C.R.D.C., 2002. Relações planta-animal em pastagem de milheto (Pennisetum americanum (L.) Leeke.) manejada em diferentes alturas com ovinos. Dissertação de Mestrado, Universidade Federal do Rio Grande do Sol, Brasil.

Cavanagh, A., McNaughton, L., Clark, H., Greaves, C., Gowan, J.M., Pinares-Patino, C., Dalley, D., Vlaming, B., Molano, G., 2008. Methane emissions from grazing Jersey×Friesian dairy cows in mid lactation. Aust. J. Exp. Agric. 48, 230. https://doi.org/10.1071/EA07277

Chacon, E., Stobbs, Th., 1976. Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. Aust. J. Agric. Res. 27, 709. https://doi.org/10.1071/AR9760709

Chiavegato, M.B., Rowntree, J.E., Carmichael, D., Powers, W.J., 2015. Enteric methane from lactating beef cows managed with high- and low-input grazing systems. J. Anim. Sci. 93, 1365. https://doi.org/10.2527/jas.2014-8128

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

Clark, H., 2013. Nutritional and host effects on methanogenesis in the grazing ruminant. animal 7, 41–48. https://doi.org/10.1017/S1751731112001875

Congio, G.F.S., Batalha, C.D.A., Chiavegato, M.B., Berndt, A., Oliveira, P.P.A., Frighetto, R.T.S., Maxwell, T.M.R., Gregorini, P., Da Silva, S.C., 2018. Strategic grazing management towards sustainable intensification at tropical pasture-based dairy systems. Sci. Total Environ. 636, 872–880. https://doi.org/10.1016/j.scitotenv.2018.04.301

Corsi, M., Martha Jr, G.B., Nascimento Jr, D., Balsalobre, M.A.A., 2001. Impact of grazing management on productivity of tropical grasslands, in: XIX International Grassland Congress, Brazil. pp. 801–805.

Cota, O.L., de Figueiredo, D.M., Branco, R.H., Magnani, E., Do Nascimento, C.F., de Oliveira, L.F., Mercadante, M.E.Z., 2014. Methane emission by Nellore cattle subjected to different nutritional plans. Trop. Anim. Health Prod. 46, 1229–1234. https://doi.org/10.1007/s11250-014-0632-3

Cox-O'Neill, J.L., Hales, K.E., Ulmer, K.M., Rasby, R.J., Parsons, J., Shackelford, S.D., Freetly, H.C., Drewnoski, M.E., 2017. The effects of backgrounding system on growing and finishing performance and carcass characteristics of beef steers1. J. Anim. Sci. 95, 5309–5319. https://doi.org/10.2527/jas2017.1934

CSIRO, 2007. Nutrient requirements of domesticated ruminants. CSIRO publishing.

Curran, J., Delaby, L., Kennedy, E., Murphy, J.P., Boland, T.M., O'Donovan, M., 2010. Sward characteristics, grass dry matter intake and milk production performance are affected by pre-grazing herbage mass and pasture allowance. Livest. Sci. 127, 144–154. https://doi.org/10.1016/j.livsci.2009.09.004

da Silva, S., Sbrissia, A., Pereira, L., 2015. Ecophysiology of C4 Forage Grasses—Understanding Plant Growth for Optimising Their Use and Management. Agriculture 5, 598–625. https://doi.org/10.3390/agriculture5030598

Da Silva, S.C., Carvalho, P.C. de F., 2005. Foraging behaviour and herbage intake in the favourable tropics/sub-tropics. Grassl. a Glob. Resour. 1, 81–95.

da Silveira, M.C.T., Júnior, D. do N., Rodrigues, C.S., Pena, K. da S., Júnior, S.J. de S., Barbero, L.M., Limão, V.A., Euclides, V.P.B., da Silva, S.C., 2016. Forage sward structure of Mulato grass (Brachiaria hybrid ssp.) subjected to rotational stocking strategies. Aust. J. Crop Sci. 10, 864–873. https://doi.org/10.21475/ajcs.2016.10.06.p7568

da Silveira Pontes, L., Barro, R.S., Savian, J.V., Berndt, A., Moletta, J.L., Porfírio-da-Silva, V., Bayer, C., de Faccio Carvalho, P.C., 2018. Performance and methane emissions by beef heifer grazing in temperate pastures and in integrated crop-livestock systems: The effect of shade and nitrogen fertilization. Agric. Ecosyst. Environ. 253, 90–97. https://doi.org/10.1016/j.agee.2017.11.009

Da Trindade, J.K., Neves, F.P., Pinto, C.E., Bremm, C., Mezzalira, J.C., Nadin, L.B., Genro, T.C.M., Gonda, H.L., Carvalho, P.C.F., 2016. Daily Forage Intake by Cattle on Natural Grassland: Response to Forage Allowance and Sward Structure. Rangel. Ecol. Manag. 69, 59–67. https://doi.org/10.1016/j.rama.2015.10.002

Dall-Orsoletta, A.C., Almeida, J.G.R., Carvalho, P.C.F., Savian, J. V, Ribeiro-Filho, H.M.N., 2016. Ryegrass pasture combined with partial total mixed ration reduces enteric methane emissions and maintains the performance of dairy cows during mid to late lactation. J. Dairy Sci. 99, 4374–4383. https://doi.org/10.3168/jds.2015-10396

de Souza Filho, W., Nunes, P.A. de A., Barro, R.S., Kunrath, T.R., de Almeida, G.M., Genro, T.C.M., Bayer, C., de Faccio Carvalho, P.C., 2019. Mitigation of enteric methane emissions through pasture management in integrated crop-livestock systems: Trade-offs between animal performance and environmental impacts. J. Clean. Prod. 213, 968–975. https://doi.org/10.1016/j.jclepro.2018.12.245

Delagarde, R., Prache, S., D'hour, P. and Petit, M., 2001. Ingestion de l'herbe par les ruminants au pâturage. *Fourrages*, *166*(1), pp.189-212.

Demarchi, J.J.A.A., Manella, M.Q., Primavesi, O., Frighetto, R.T.S., Romero, L.A., Berndt, A., Lima, M.A., 2016. Effect of Seasons on Enteric Methane Emissions from Cattle Grazing Urochloa brizantha. J. Agric. Sci. 8, 106. https://doi.org/10.5539/jas.v8n4p106

DeRamus, H.A., 2004. Grazing Management of Ruminant Animals in Sustainable Agriculture. Outlook Agric. 33, 93–100. https://doi.org/10.5367/000000004773973073

DeRamus, H.A., Clement, T.C., Giampola, D.D., Dickison, P.C., 2003. Methane Emissions of Beef Cattle on Forages. J. Environ. Qual. 32, 269–277. https://doi.org/10.2134/jeq2003.2690

Dias-Filho, M.B., 2014. Diagnóstico das pastagens no Brasil. *Embrapa Amazônia Oriental-Documentos (INFOTECA-E)*.

Dini, Y., Gere, J.I., Cajarville, C., Ciganda, V.S., 2018. Using highly nutritious pastures to mitigate enteric methane emissions from cattle grazing systems in South America. Anim. Prod. Sci. 58, 2329. https://doi.org/10.1071/AN16803

Echeverria, J.R., Euclides, V.P.B., Sbrissia, A.F., Montagner, D.B., Barbosa, R.A., Nantes, N.N., 2016. Acúmulo de forragem e valor nutritivo do híbrido de Urochloa "BRS RB331 Ipyporã" sob pastejo intermitente. Pesqui. Agropecuária Bras. 51, 880–889. https://doi.org/10.1590/S0100-204X2016000700011

Etheridge, D.M., Steele, L.P., Francey, R.J., Langenfelds, R.L., 1998. Atmospheric methane between 1000 A.D. and present: Evidence of anthropogenic emissions and climatic variability. J. Geophys. Res. Atmos. 103, 15979–15993. https://doi.org/10.1029/98JD00923

Euclides, V.P.B., Carpejani, G.C., Montagner, D.B., Nascimento Junior, D., Barbosa, R.A., Difante, G.S., 2018. Maintaining post-grazing sward height of Panicum maximum (cv. Mombaça) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. Grass Forage Sci. 73, 174–182.

https://doi.org/10.1111/gfs.12292

Euclides, V.P.B., do Valle, C.B., Macedo, M.C.M., Almeida, R.G. de, Montagner, D.B., Barbosa, R.A., 2010. Brazilian scientific progress in pasture research during the first decade of XXI century. Rev. Bras. Zootec. 39, 151–168. https://doi.org/10.1590/S1516-35982010001300018

Eugène, M., Martin, C., Mialon, M.M., Krauss, D., Renand, G., Doreau, M., 2011. Dietary linseed and starch supplementation decreases methane production of fattening bulls. Anim. Feed Sci. Technol. 166–167, 330–337. https://doi.org/10.1016/j.anifeedsci.2011.04.023

Fariña, S.R., Chilibroste, P., 2019. Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. Agric. Syst. 176, 102631. https://doi.org/10.1016/j.agsy.2019.05.001

Food and Agriculture Organization of the United Nations 1999. Livestock and the environment. Meeting the challenge. Retrieved on 12 October 2018 from http:// www.fao.org/docrep/x5304e/x5304e00.htm

Fiorentini, G., Carvalho, I.P.C., Messana, J.D., Castagnino, P.S., Berndt, A., Canesin, R.C., Frighetto, R.T.S., Berchielli, T.T., 2014. Effect of lipid sources with different fatty acid profiles on the intake, performance, and methane emissions of feedlot Nellore steers1. J. Anim. Sci. 92, 1613–1620. https://doi.org/10.2527/jas.2013-6868

Fonseca, L., Carvalho, P.C. de F., Mezzalira, J.C., Bremm, C., Galli, J.R., Gregorini, P., 2013. Effect of sward surface height and level of herbage depletion on bite features of cattle grazing Sorghum bicolor swards. J. Anim. Sci. 91, 4357–4365. https://doi.org/10.2527/jas2013-5602

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

Forbes, J., Barrio, J., 1992. Abdominal chemo- and mechanosensitivity in ruminants and its role in the control of food intake. Exp. Physiol. 77, 27–50. https://doi.org/10.1113/expphysiol.1992.sp003581

Forbes, J.M., 2003. The multifactorial nature of food intake control. J. Anim. Sci. 81, E139–E144. https://doi.org/10.2527/2003.8114_suppl_2E139x

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G., 2013. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

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

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

Glindemann, T., Wang, C., Tas, B.M., Schiborra, A., Gierus, M., Taube, F., Susenbeth, A., 2009. Impact of grazing intensity on herbage intake, composition, and digestibility and on live weight gain of sheep on the Inner Mongolian steppe. Livest. Sci. 124, 142–147. https://doi.org/10.1016/j.livsci.2009.01.007

Gregorini, P., 2012. Diurnal grazing pattern: its physiological basis and strategic management. Anim. Prod. Sci. 52, 416. https://doi.org/10.1071/AN11250

Gregorini, P., Gunter, S.A., Beck, P.A., 2008. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: Timing of herbage and fasting allocation1. J. Anim. Sci. 86, 1006–1020. https://doi.org/10.2527/jas.2007-0432

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

Hall, M.B., Huntington, G.B., 2008. Nutrient synchrony: Sound in theory, elusive in practice1. J. Anim. Sci. 86, E287–E292. https://doi.org/10.2527/jas.2007-0516

Hammond, K.J., Burke, J.L., Koolaard, J.P., Muetzel, S., Pinares-Patiño, C.S., Waghorn, G.C., 2013. Effects of feed intake on enteric methane emissions from sheep fed fresh white clover (Trifolium repens) and perennial ryegrass (Lolium perenne) forages. Anim. Feed Sci. Technol. 179, 121–132. https://doi.org/10.1016/j.anifeedsci.2012.11.004

Hauggaard-Nielsen, H., Lachouani, P., Knudsen, M.T., Ambus, P., Boelt, B., Gislum, R., 2016. Productivity and carbon footprint of perennial grass–forage legume intercropping strategies with high or low nitrogen fertilizer input. Sci. Total Environ. 541, 1339–1347. https://doi.org/10.1016/j.scitotenv.2015.10.013

Hernández, P.A., Mendoza, G.D., Castro, A., Lara, A., Plata, F.X., Martínez, J.A., Ferraro, S., 2017. Effects of grain level on lamb performance, ruminal metabolism and leptin mRNA expression in perirenal adipose tissue. Anim. Prod. Sci. 57, 2001. https://doi.org/10.1071/AN15564

Herrero, M., Gerber, P., Vellinga, T., Garnett, T., Leip, A., Opio, C., Westhoek, H.J., Thornton, P.K., Olesen, J., Hutchings, N., Montgomery, H., Soussana, J.-F., Steinfeld, H., McAllister, T.A., 2011. Livestock and greenhouse gas emissions: The importance of getting the numbers right. Anim. Feed Sci. Technol. 166–167, 779–

782. https://doi.org/10.1016/j.anifeedsci.2011.04.083

Herrero, M., Havlik, P., Valin, H., Notenbaert, A., Rufino, M.C., Thornton, P.K., Blummel, M., Weiss, F., Grace, D., Obersteiner, M., 2013. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. Proc. Natl. Acad. Sci. 110, 20888–20893. https://doi.org/10.1073/pnas.1308149110

Herrero, M., Henderson, B., Havlík, P., Thornton, P.K., Conant, R.T., Smith, P., Wirsenius, S., Hristov, A.N., Gerber, P., Gill, M., Butterbach-Bahl, K., Valin, H., Garnett, T., Stehfest, E., 2016. Greenhouse gas mitigation potentials in the livestock sector. Nat. Clim. Chang. 6, 452–461. https://doi.org/10.1038/nclimate2925

Hill, J., McSweeney, C., Wright, A.-D.G., Bishop-Hurley, G., Kalantar-zadeh, K., 2016. Measuring Methane Production from Ruminants. Trends Biotechnol. 34, 26–35. https://doi.org/10.1016/j.tibtech.2015.10.004

Hodgson, J., 1990. *Grazing management. Science into practice*. Longman Group UK Ltd.

Hoogendoorn, C.J., HOLMES, C.W., CHU, A.C.P., 1992. Some effects of herbage composition, as influenced by previous grazing management, on milk production by cows grazing on ryegrass/white clover pastures. 2. Milk production in late spring/summer: effects of grazing intensity during the preceding spring per. Grass Forage Sci. 47, 316–325. https://doi.org/10.1111/j.1365-2494.1992.tb02277.x

Hook, S.E., Wright, A.-D.G., McBride, B.W., 2010. Methanogens: Methane Producers of the Rumen and Mitigation Strategies. Archaea 2010, 1–11. https://doi.org/10.1155/2010/945785

Hristov, A.N., Kebreab, E., Niu, M., Oh, J., Bannink, A., Bayat, A.R., Boland, T.M., Brito, A.F., Casper, D.P., Crompton, L.A., Dijkstra, J., Eugène, M., Garnsworthy, P.C., Haque, N., Hellwing, A.L.F., Huhtanen, P., Kreuzer, M., Kuhla, B., Lund, P., Madsen, J., Martin, C., Moate, P.J., Muetzel, S., Muñoz, C., Peiren, N., Powell, J.M., Reynolds, C.K., Schwarm, A., Shingfield, K.J., Storlien, T.M., Weisbjerg, M.R., Yáñez-Ruiz, D.R., Yu, Z., 2018. Symposium review: Uncertainties in enteric methane inventories, measurement techniques, and prediction models. J. Dairy Sci. 101, 6655–6674. https://doi.org/10.3168/jds.2017-13536

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M., 2013. SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options1. J. Anim. Sci. 91, 5045–5069. https://doi.org/10.2527/jas.2013-6583

Hünerberg, M., McGinn, S.M., Beauchemin, K.A., Okine, E.K., Harstad, O.M., McAllister, T.A., 2013. Effect of dried distillers grains plus solubles on enteric methane emissions and nitrogen excretion from growing beef cattle1. J. Anim. Sci. 91, 2846–2857. https://doi.org/10.2527/jas.2012-5564

Illius, A.W., Jessop, N.S., 1996. Metabolic constraints on voluntary intake in ruminants. J. Anim. Sci. 74, 3052. https://doi.org/10.2527/1996.74123052x

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Janssen, P.H., 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160, 1–22. https://doi.org/10.1016/j.anifeedsci.2010.07.002

Jiao, H.P., Dale, A.J., Carson, A.F., Murray, S., Gordon, A.W., Ferris, C.P., 2014. Effect of concentrate feed level on methane emissions from grazing dairy cows. J. Dairy Sci. 97, 7043–7053. https://doi.org/10.3168/jds.2014-7979

Jonker, A., Molano, G., Koolaard, J., Muetzel, S., 2017. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. Anim. Prod. Sci. 57, 643. https://doi.org/10.1071/AN15656

Kidane, A., Prestløkken, E., Zaralis, K., Steinshamn, H., 2018. Effects of three short-term pasture allocation methods on milk production, methane emission and grazing behaviour by dairy cows. Acta Agric. Scand. Sect. A — Anim. Sci. 68, 87–102. https://doi.org/10.1080/09064702.2019.1577912

Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico, J.M., 2014. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. J. Dairy Sci. 97, 3231–3261. https://doi.org/10.3168/jds.2013-7234

Ku-Vera, J.C., Valencia-Salazar, S.S., Piñeiro-Vázquez, A.T., Molina-Botero, I.C., Arroyave-Jaramillo, J., Montoya-Flores, M.D., Lazos-Balbuena, F.J., Canul-Solís, J.R., Arceo-Castillo, J.I., Ramírez-Cancino, L., Escobar-Restrepo, C.S., Alayón-Gamboa, J.A., Jiménez-Ferrer, G., Zavala-Escalante, L.M., Castelán-Ortega, O.A., Quintana-Owen, P., Ayala-Burgos, A.J., Aguilar-Pérez, C.F., Solorio-Sánchez, F.J., 2018. Determination of methane yield in cattle fed tropical grasses as measured in open-circuit respiration chambers. Agric. For. Meteorol. 258, 3–7. https://doi.org/10.1016/j.agrformet.2018.01.008

Kumar, S., Choudhury, P.K., Carro, M.D., Griffith, G.W., Dagar, S.S., Puniya, M., Calabro, S., Ravella, S.R., Dhewa, T., Upadhyay, R.C., Sirohi, S.K., Kundu, S.S., Wanapat, M., Puniya, A.K., 2014. New aspects and strategies for methane mitigation from ruminants. Appl. Microbiol. Biotechnol. 98, 31–44. https://doi.org/10.1007/s00253-013-5365-0

Kunrath, T.R., de Albuquerque Nunes, P.A., de Souza Filho, W., Cadenazzi, M., Bremm, C., Martins, A.P., de Faccio Carvalho, P.C., 2020. Sward height determines pasture production and animal performance in a long-term soybean-beef cattle integrated system. Agric. Syst. 177, 102716. https://doi.org/10.1016/j.agsy.2019.102716

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

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

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

Latawiec, A.E., Strassburg, B.B.N., Valentim, J.F., Ramos, F., Alves-Pinto, H.N., 2014. Intensification of cattle ranching production systems: socioeconomic and environmental synergies and risks in Brazil. Animal 8, 1255–1263. https://doi.org/10.1017/S1751731114001566

Lemaire, G., Da Silva, S.C., Agnusdei, M., Wade, M., Hodgson, J., 2009. Interactions between leaf lifespan and defoliation frequency in temperate and tropical pastures: a review. Grass Forage Sci. 64, 341–353. https://doi.org/10.1111/j.1365-2494.2009.00707.x

Leng, R.A., 2014. Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. Anim. Prod. Sci. 54, 519. https://doi.org/10.1071/AN13381

Lobato, J.F.P., Freitas, Ak., Devincenzi, T., Cardoso, L.L., Tarouco, J.U., Vieira, R.M., Dillenburg, D.R., Castro, I., 2014. Brazilian beef produced on pastures: Sustainable and healthy. Meat Sci. 98, 336–345. https://doi.org/10.1016/j.meatsci.2014.06.022

Lovett, D.K., Stack, L.J., Lovell, S., Callan, J., Flynn, B., Hawkins, M., O'Mara, F.P., 2005. Manipulating Enteric Methane Emissions and Animal Performance of Late-Lactation Dairy Cows Through Concentrate Supplementation at Pasture. J. Dairy Sci. 88, 2836–2842. https://doi.org/10.3168/jds.S0022-0302(05)72964-7

Makkar, H.P.S., 2016. Smart livestock feeding strategies for harvesting triple gain – the desired outcomes in planet, people and profit dimensions: a developing country perspective. Anim. Prod. Sci. 56, 519. https://doi.org/10.1071/AN15557

Maraschin, G.E., 2001. Production potential of South American grasslands, in: International Grassland Congress. pp. 5–18.

Martha, G.B., Alves, E., Contini, E., 2012. Land-saving approaches and beef production growth in Brazil. Agric. Syst. 110, 173–177. https://doi.org/10.1016/j.agsy.2012.03.001

Mc Geough, E.J., O'Kiely, P., Foley, P.A., Hart, K.J., Boland, T.M., Kenny, D.A., 2010a. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity1. J. Anim. Sci. 88, 1479–1491. https://doi.org/10.2527/jas.2009-2380

Mc Geough, E.J., O'Kiely, P., Hart, K.J., Moloney, A.P., Boland, T.M., Kenny, D.A., 2010b. Methane emissions, feed intake, performance, digestibility, and rumen fermentation of finishing beef cattle offered whole-crop wheat silages differing in grain content1. J. Anim. Sci. 88, 2703–2716. https://doi.org/10.2527/jas.2009-2750

McCarthy, B., Delaby, L., Pierce, K.M., Journot, F., Horan, B., 2011. Meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems. animal 5, 784–794. https://doi.org/10.1017/S1751731110002314

McCaughey, W.P., Wittenberg, K., Corrigan, D., 1997. Methane production by steers on pasture. Can. J. Anim. Sci. 77, 519–524. https://doi.org/10.4141/A96-137

McEvoy, M., O'Donovan, M., Kennedy, E., Murphy, J.P., Delaby, L., Boland, T.M., 2009. Effect of pregrazing herbage mass and pasture allowance on the lactation performance of Holstein-Friesian dairy cows. J. Dairy Sci. 92, 414–422. https://doi.org/10.3168/jds.2008-1313

McManus, C., Barcellos, J.O.J., Formenton, B.K., Hermuche, P.M., Carvalho, O.A. de, Guimarães, R., Gianezini, M., Dias, E.A., Lampert, V. do N., Zago, D., Neto, J.B., 2016. Dynamics of Cattle Production in Brazil. PLoS One 11, e0147138. https://doi.org/10.1371/journal.pone.0147138

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

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

Moe, P.W., Tyrrell, H.F., 1979. Methane Production in Dairy Cows. J. Dairy Sci. 62, 1583–1586. https://doi.org/10.3168/jds.S0022-0302(79)83465-7

Mott, G.O., 1960. Grazing pressure and the measurement of pasture production. Grazing Press. Meas. pasture Prod.

Muñoz, C., Hube, S., Morales, J.M., Yan, T., Ungerfeld, E.M., 2015. Effects of concentrate supplementation on enteric methane emissions and milk production of grazing dairy cows. Livest. Sci. 175, 37–46. https://doi.org/10.1016/j.livsci.2015.02.001

Muñoz, C., Letelier, P.A., Ungerfeld, E.M., Morales, J.M., Hube, S., Pérez-Prieto, L.A.,

2016. Effects of pregrazing herbage mass in late spring on enteric methane emissions, dry matter intake, and milk production of dairy cows. J. Dairy Sci. 99, 7945–7955. https://doi.org/10.3168/jds.2016-10919

Nascimento Jr, D., Barbosa, R.B., Marcelino, K.R.A., Garcez Neto, A.F., Difante, G.S. and Lopes, B.A., 2003. A produção animal em pastagens no Brasil: uso do conhecimento técnico e resultados. *PRODUÇÃO ANIMAL EM PASTAGENS*, *20*, pp.1-82.

Nascimento, C.F.M., Berndt, A., Romero Solorzano, L.A., Meyer, P.M., Frighetto, R.T.S., Demarchi, J.J.A.A., Rodrigues, P.H.M., 2015. Methane Emission of Cattle Fed Urochloa brizantha Hay Harvested at Different Stages. J. Agric. Sci. 8, 163. https://doi.org/10.5539/jas.v8n1p163

Nisbet, E.G., Manning, M.R., Dlugokencky, E.J., Fisher, R.E., Lowry, D., Michel, S.E., Myhre, C.L., Platt, S.M., Allen, G., Bousquet, P., Brownlow, R., Cain, M., France, J.L., Hermansen, O., Hossaini, R., Jones, A.E., Levin, I., Manning, A.C., Myhre, G., Pyle, J.A., Vaughn, B.H., Warwick, N.J., White, J.W.C., 2019. Very Strong Atmospheric Methane Growth in the 4 Years 2014–2017: Implications for the Paris Agreement. Global Biogeochem. Cycles 33, 318–342. https://doi.org/10.1029/2018GB006009

O'Mara, F.P., 2012. The role of grasslands in food security and climate change. Ann. Bot. 110, 1263–1270. https://doi.org/10.1093/aob/mcs209

O'Neill, B.F., Deighton, M.H., O'Loughlin, B.M., Galvin, N., O'Donovan, M., Lewis, E., 2012. The effects of supplementing grazing dairy cows with partial mixed ration on enteric methane emissions and milk production during mid to late lactation. J. Dairy Sci. 95, 6582–6590. https://doi.org/10.3168/jds.2011-5257

O'Neill, B.F., Deighton, M.H., O'Loughlin, B.M., Mulligan, F.J., Boland, T.M., O'Donovan, M., Lewis, E., 2011. Effects of a perennial ryegrass diet or total mixed ration diet offered to spring-calving Holstein-Friesian dairy cows on methane emissions, dry matter intake, and milk production. J. Dairy Sci. 94, 1941–1951. https://doi.org/10.3168/jds.2010-3361

Oenema, O., de Klein, C., Alfaro, M., 2014. Intensification of grassland and forage use: driving forces and constraints. Crop Pasture Sci. 65, 524. https://doi.org/10.1071/CP14001

Orr, R., Griffith, B., Rivero, M., Lee, M., 2019. Livestock Performance for Sheep and Cattle Grazing Lowland Permanent Pasture: Benchmarking Potential of Forage-Based Systems. Agronomy 9, 101. https://doi.org/10.3390/agronomy9020101

Osuji, P.O., 1974. The Physiology of Eating and the Energy Expenditure of the Ruminant at Pasture. J. Range Manag. 27, 437. https://doi.org/10.2307/3896717

Outlook, O.-F.A., 2011. OECD-FAO Agricultural Outlook 2011, Food Price Volatility. Avaialble on, OECD-FAO Agricultural Outlook. OECD. https://doi.org/10.1787/agr_outlook-2011-en

Pacheco, D., Waghorn, G., Janssen, P.H., 2014. Decreasing methane emissions from ruminants grazing forages: a fit with productive and financial realities? Anim. Prod. Sci. 54, 1141. https://doi.org/10.1071/AN14437

Patra, A.K., 2016. Recent Advances in Measurement and Dietary Mitigation of Enteric Methane Emissions in Ruminants. Front. Vet. Sci. 3, 39. https://doi.org/10.3389/fvets.2016.00039

Peyraud, J.L., Delagarde, R., 2013. Managing variations in dairy cow nutrient supply under grazing. animal 7, 57–67. https://doi.org/10.1017/S1751731111002394

Pinares-Patiño, C.S., Baumont, R., Martin, C., 2003. Methane emissions by Charolais cows grazing a monospecific pasture of timothy at four stages of maturity. Can. J. Anim. Sci. 83, 769–777. https://doi.org/10.4141/A03-034

Pinares-Patiño, C.S., D'Hour, P., Jouany, J.-P., Martin, C., 2007. Effects of stocking rate on methane and carbon dioxide emissions from grazing cattle. Agric. Ecosyst. Environ. 121, 30–46. https://doi.org/10.1016/j.agee.2006.03.024

Poppi, D.P., Hughes, T.P., L'huillier, P.J., 1987. Intake of pasture by grazing ruminants. Livest. Feed. pasture. Hamilt. New Zeal. Soc. Anim. Prod. 7, 55–64.

Poppi, D.P., Quigley, S.P., Silva, T.A.C.C. da, McLennan, S.R., 2018. Challenges of beef cattle production from tropical pastures. Rev. Bras. Zootec. 47. https://doi.org/10.1590/rbz4720160419

Ramankutty, N., Mehrabi, Z., Waha, K., Jarvis, L., Kremen, C., Herrero, M., Rieseberg, L.H., 2018. Trends in Global Agricultural Land Use: Implications for Environmental Health and Food Security. Annu. Rev. Plant Biol. 69, 789–815. https://doi.org/10.1146/annurev-arplant-042817-040256

RAO, I., PETERS, M., CASTRO, A., SCHULTZE-KRAFT, R., WHITE, D., FISHER, M., MILES, J., LASCANO, C., BLUMMEL, M., BUNGENSTAB, D., TAPASCO, J., HYMAN, G., BOLLIGER, A., PAUL, B., VAN DER HOEK, R., MAASS, B., TIEMANN, T., CUCHILLO, M., DOUXCHAMPS, S., VILLANUEVA, C., RINCÓN, Á., AYARZA, M., ROSENSTOCK, T., SUBBARAO, G., ARANGO, J., CARDOSO, J., WORTHINGTON, M., CHIRINDA, N., NOTENBAERT, A., JENET, A., SCHMIDT, A., VIVAS, N., LEFROY, R., FAHRNEY, K., GUIMARÃES, E., TOHME, J., COOK, S., HERRERO, M., CHACÓN, M., SEARCHINGER, T., RUDEL, T., 2015. LivestockPlus - The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics. Trop. Grasslands - Forrajes Trop. 3, 59. https://doi.org/10.17138/TGFT(3)59-82

Reid, R.S., Thornton, P.K., McCrabb, G.J., Kruska, R.L., Atieno, F., Jones, P.G., 2004. Is it possible to mitigate greenhouse gas emissions in pastoral ecosystems of the tropics? Environ. Dev. Sustain. 6, 91–109. https://doi.org/10.1023/B:ENVI.0000003631.43271.6b

Ren, W., Hou, X., Wang, Y., Badgery, W., Li, X., Ding, Y., Guo, H., Wu, Z., Hu, N., Kong, L., Chang, C., Jiang, C., Zhang, J., 2016. Overgrazing induces alterations in the hepatic proteome of sheep (Ovis aries): an iTRAQ-based quantitative proteomic analysis. Proteome Sci. 15, 2. https://doi.org/10.1186/s12953-016-0111-z

Ribeiro Filho, H.M.N., Peyraud, J.-L., Delagarde, R., 2012. Foraging behavior and ruminal fermentation of dairy cows grazing ryegrass pasture alone or with white clover. Pesqui. Agropecuária Bras. 47, 458–465. https://doi.org/10.1590/S0100-204X2012000300019

Richmond, A.S., Wylie, A.R.G., Laidlaw, A.S., Lively, F.O., 2015. Methane emissions from beef cattle grazing on semi-natural upland and improved lowland grasslands. Animal 9, 130–137. https://doi.org/10.1017/S1751731114002067

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E.F., Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J.A., 2009. A safe operating space for humanity. Nature 461, 472–475. https://doi.org/10.1038/461472a

Rodríguez, A.B., Bodas, R., Fernández, B., López-Campos, O., Mantecón, A.R., Giráldez, F.J., 2007. Feed intake and performance of growing lambs raised on concentrate-based diets under cafeteria feeding systems. animal 1, 459–466. https://doi.org/10.1017/S1751731107683803

Romera, A.J., Doole, G.J., 2015. Optimising the interrelationship between intake per cow and intake per hectare. Anim. Prod. Sci. 55, 384. https://doi.org/10.1071/AN14424

Rook, A.J., 2004. Statistical considerations in the design of herbage intake studies. Herb. Intake Handbook. 2ed. Read. Br. Grassl. Soc. 177–184.

Rouquette, F.M., 2016. I nvited R eview : The roles of forage management, forage quality, and forage allowance in grazing research 1 1This review article is based on a presentation by the author in the symposium "Design, Analysis, and Execution of Quality Grazing Research" at t. Prof. Anim. Sci. 32, 10–18. https://doi.org/10.15232/pas.2015-01408

Savian, J.V., Neto, A.B., de David, D.B., Bremm, C., Schons, R.M.T., Genro, T.C.M., do Amaral, G.A., Gere, J., McManus, C.M., Bayer, C., de Faccio Carvalho, P.C., 2014. Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: Implications for integrated crop–livestock system. Agric. Ecosyst. Environ. 190, 112–119. https://doi.org/10.1016/j.agee.2014.02.008

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

https://doi.org/10.1016/j.jclepro.2018.03.162

SHIBATA, M., TERADA, F., 2010. Factors affecting methane production and mitigation in ruminants. Anim. Sci. J. 81, 2–10. https://doi.org/10.1111/j.1740-0929.2009.00687.x

Shipley, L.A., 2007. The influence of bite size on foraging at larger spatial and temporal scales by mammalian herbivores. Oikos 116, 1964–1974. https://doi.org/10.1111/j.2007.0030-1299.15974.x

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith, J., 2008. Greenhouse gas mitigation in agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 789–813. https://doi.org/10.1098/rstb.2007.2184

Sollenberger, L.E., Burns, J.C., 2001. Canopy characteristics, ingestive behaviour and herbage intake in cultivated tropical grasslands, in: International Grassland Congress. pp. 321–327.

Sollenberger, L.E., Moore, J.E., Allen, V.G., Pedreira, C.G.S., 2005. Reporting Forage Allowance in Grazing Experiments. Crop Sci. 45, 896–900. https://doi.org/10.2135/cropsci2004.0216

Sollenberger, L.E., Vanzant, E.S., 2011. Interrelationships among Forage Nutritive Value and Quantity and Individual Animal Performance. Crop Sci. 51, 420–432. https://doi.org/10.2135/cropsci2010.07.0408

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V., Rosales, M., Rosales, M., de Haan, C., 2006. Livestock's long shadow: environmental issues and options. Food & Agriculture Org.

Stockdale, C., 1993. The productivity of lactating dairy cows fed irrigated Persian clover (Trifolium resupinatum). Aust. J. Agric. Res. 44, 1591. https://doi.org/10.1071/AR9931591

Strassburg, B.B.N., Latawiec, A.E., Barioni, L.G., Nobre, C.A., da Silva, V.P., Valentim, J.F., Vianna, M., Assad, E.D., 2014. When enough should be enough: Improving the use of current agricultural lands could meet production demands and spare natural habitats in Brazil. Glob. Environ. Chang. 28, 84–97. https://doi.org/10.1016/j.gloenvcha.2014.06.001

Sun, X.Z., Hoskin, S.O., Muetzel, S., Molano, G., Clark, H., 2011. Effects of forage chicory (Cichorium intybus) and perennial ryegrass (Lolium perenne) on methane emissions in vitro and from sheep. Anim. Feed Sci. Technol. 166–167, 391–397. https://doi.org/10.1016/j.anifeedsci.2011.04.027

Tang, S., Ma, L., Wei, X., Tian, D., Wang, B., Li, Z., Zhang, Y., Shao, X., 2019. Methane emissions in grazing systems in grassland regions of China: A synthesis. Sci. Total

Environ. 654, 662–670. https://doi.org/10.1016/j.scitotenv.2018.11.102

Tedeschi, L.O., Molle, G., Menendez, H.M., Cannas, A., Fonseca, M.A., 2019. The assessment of supplementation requirements of grazing ruminants using nutrition models. Transl. Anim. Sci. 3, 811–828. https://doi.org/10.1093/tas/txy140

van Wyngaard, J.D.V., Meeske, R., Erasmus, L.J., 2018. Effect of concentrate feeding level on methane emissions, production performance and rumen fermentation of Jersey cows grazing ryegrass pasture during spring. Anim. Feed Sci. Technol. 241, 121–132. https://doi.org/10.1016/j.anifeedsci.2018.04.025

Vasconcelos, K., Farinha, M., Bernardo, L., do N. Lampert, V., Gianezini, M., da Costa, J.S., Filho, A.S., Genro, T.C.M., Ruviaro, C.F., 2018. Livestock-derived greenhouse gas emissions in a diversified grazing system in the endangered Pampa biome, Southern Brazil. Land use policy 75, 442–448. https://doi.org/10.1016/j.landusepol.2018.03.056

Watt, L.J., Clark, C.E.F., Krebs, G.L., Petzel, C.E., Nielsen, S., Utsumi, S.A., 2015. Differential rumination, intake, and enteric methane production of dairy cows in a pasture-based automatic milking system. J. Dairy Sci. 98, 7248–7263. https://doi.org/10.3168/jds.2015-9463

Wims, C.M., Deighton, M.H., Lewis, E., O’Loughlin, B., Delaby, L., Boland, T.M., O’Donovan, M., 2010. Effect of pregrazing herbage mass on methane production, dry matter intake, and milk production of grazing dairy cows during the mid-season period. J. Dairy Sci. 93, 4976–4985. https://doi.org/10.3168/jds.2010-3245

Table 1. Intake and enteric CH_4 emissions by ruminants under grazing-only conditions and different grazing management practices.

Study target	Animal category	Pasture[11]	Intake, kg DM or OM (*)	Intake, % of LW	CH_4 production and intensity					Diff. between control and best alternative, %		Reference	Country
					g/d	g/kg DMI or OMI	% GE	g kg LW gain, milk yield (*), FCM (**)	Calculated kg year	g CH_4 per feed input	g CH_4 per animal output		
					Sheep								
Pasture type	Sheep	Multi-grasses	1.389 to 1.704	2.4 to 4.1	19.3 to 35.2	12.9 to 21.1	3.9 to 6.3	-	7.04 to 12.8	38.86	-	Ulyatt et al. (2005)	New Zeeland
Grazing intensity and stocking method	Growing lambs	AR	0.907 to 1.369	-	20.7 to 24.5	19.3 to 19.5	5.5 to 6.0	159 to 285	7.5 to 8.99	1	44.2	Savian et al. (2014)	Brazil
	Lactating ewes		1.48 to 1.91	-	38.7 to 41.7	19.3 to 27.7	6.1 to 8.6	164 to 215	14.1 to 15.2	30.32	23.72		
Nitrogen fertilization	Growing lambs,	PM	0.836 to 1.043	4.0 to 4.4	10.93 to 15.47	12.9 to 15.3	4.58 to 5.55	197.6 to 255.2	3.98 to 5.64	16.09	22.57	Amaral et al. (2016)[1,3]	Brazil
Sward heights	Growing lambs	AR	0.738 to 0.915	2.5 to 2.9	22.2 to 24.8	27.1 to 30.1	7.6 to 8.3	217 to 586	8.1 to 9.1	12.29	62.96	Savian et al. (2018)[1,2]	Brazil
					Beef cattle								
Stocking method and rate	Steers	AL, BR and other species	13.20 to 14.94	3.3 to 3.8	173 to 219	12.6 to 16.58	4.1 to 5.2	147.9 to 161.5	63.1 to 79.9	24	8.4	McCaughey et al. (1997)[10]	Canada
Pasture type	Cows	Mixture AL/BR or BR	9.7 to 11.4	1.8 to 2.2	227 to 293	19.9 to 30.3	7.1 to 9.5	-	82.9 to 106.9	22.5	-	McCaughey et al. (1999)[10]	Canada
Stocking method	Cows	BH or BE, with forbs or with ryegrass	-	-	191 to 228	-	-	432 to 594	69.8 to 83.2	-	44.5	DeRamus et al. (2003)[8]	United States
	Heifers		-	-	128 to 151	-	-	271 to 515	46.7 to 55.1	-	21.6		
Plant maturity	Cattle	TY	9.74 to 12.03	1.3 to 1.6	204.4 to 273.3	20.9 to 22.7	5.9 to 6.7	-	74.6 to 99.7	7.9	-	Pinares-Patiño et al. (2003)	France
Stocking rate	Cattle	Temperate native grasses, CK and WC	8.75 to 9.85*	1.7 to 1.9	213.3 to 220.9	22.5 to 24.4	6.4 to 7.0	290 to 298	77.8 to 80.6	7.8	2.7	Pinares-Patiño et al. (2007)[5]	France
Herbage mass	Heifers	PR	6.44. to 6.5	-	122.5 to 129	19.3 to 21.1	5.6 to 6.1	143 to 173	43.8 to 49.6	8.5	17.4	Boland et al. (2013)[9]	Ireland

Continuation…

Study target	Animal category	Pasture^{11}	Intake, kg DM or OM (*)	Intake, % of LW	CH_4 production and intensity					Diff. between control and best alternative, %		Reference	Country
					g/d	g/kg DMI or OMI	% GE	g kg LW gain, milk yield (*), FCM (**)	Calculated kg year	g CH_4 per feed input	g CH_4 per animal output		
					Beef cattle								
Pasture quality	Cattle	Natural grassland alone or with legumes	8.68 to 9.55	-	176 to 202	20.7 to 21.6	6.0 to 6.4	197 to 261	64.2 to 73.7	4.16	24.5	Richmond et al. (2014)	United Kingdon
Stocking rate and density	Cows	Mixed pastures grass/legume	14.7 to 15.7	2.6 to 2.8	192.2 to 248.3	11.9 to 17.2	3.75 to 5.45	-	70.15 to 90.61	30.6	-	Chiavegato et al. (2015)	United States
Mixed pastures grass/legume	Steers	PP grass alone or mixed with AP	6.7 to 7.8	2.7 to 3.1	146 to 180	22.9 to 25.3	-	230 to 254	53.3 to 65.7	9.5	9.5	Andrade et al. (2016)	Brazil
Full-sun or silvopastoral systems	Steers	MM alone or with trees	5.09 to 6.69	2.6 to 3.31	156 to 163.9	22.6 to 24.5	6.83 to 7.69	474 to 750	56.9 to 59.8	7.75	36.7	Da Frota et al. (2017)^{7}	Brazil
Forage-quality	Heifers	Grass-predominant with little legume or legume-predominant	7.8 to 11.45	2.0 to 3.0	137 to 169	17.95 to 20.2	5.6 to 6.5	163 to 402	50.0 to 61.7	11.11	59.4	Dini et al. (2018)	Uruguay
Shade and nitrogen fertilization	Steers	AR/BO mixture	-	-	155 to 170	-	-	168 to 286	56.8 to 62.1	-	41.2	Pontes et al. (2018)	Brazil
Sward height	Steers	AR/BO mixture	5.98 to 7.31	1.9 to 2.1	171 to 227	25.2 to 30.6		176 to 210	62.4 to 82.8	17.64	16.2	De Souza-Filho et al. (2018)	Brazil
Pasture-quality	Cows	Native grassland or SR-based pasture	11.8 to 12.1	-	157.5 to 202.7	13.1 to 17.3	4.3 to 5.6	-	57.5 to 73.9	24.3	-	Gere et al. (2019)	Argentina
Forage allowance	Steers	Natural complex grasslands	-	-	107 to 210	-	-	607 to 2,743	39.0 to 76.6	-	70.7	Cezimbra et al. (Submitted)	Brazil

Continuation…

Study target	Animal category	Pasture[11]	Intake, kg DM or OM (*)	Intake, % of LW	CH$_4$ production and intensity					Diff. between control and best alternative, %		Reference	Country
					g/d	g/kg DMI or OMI	% GE	g kg LW gain, milk yield (*), FCM (**)	Calculated kg year	g CH$_4$ per feed input	g CH$_4$ per animal output		
					Dairy cattle								
Pre-grazing herbage mass	Cows	PR	15.0 to 15.7	2.9 to 3.2	282 to 303	18.1 to 20.5	5.9 to 6.85	14.7 to 16.75*	102.9 to 110.6	11.7	12.2	Wims et al. (2010)[6]	Ireland
Pastures rich or legumes or in grasses	Cows	Temperate pastures rich in legumes or in grasses	16.8 to 17.3	3.02 to 3.15	364 to 372	21.6 to 22.7	6.4 to 6.7	17.58 to 18.69**	132.8 to 135.8	4.8	5.93	Dini et al. (2012)	Uruguay
Mixed or diverse pastures	Cows	PR/WC or PR, WC, LU, CH	17.0 to 17.9	-	402 to 421	22.6 to 24.9	-	25.2 to 26.1*	147 to 154	9.2	4.8	Jonket et al. (2018)	New Zeeland
Grass fertilized or mixed with legumes	Heifers	UB alone or with AP	8.46 to 9.35	2.6 to 2.8	115 to 140	15.8 to 16.4	4.8 to 4.9	-	48.2 to 51.1	2.4	-	Berça et al. (2019)	Brazil

---

[1] Value of g CH$_4$/kg DM intake not reported in the publication and calculated from individual data of daily CH$_4$ production and OM content of the herbage.

[2] Value of CH$_4$ as % of GEI not reported, but available from authors.

[3] Value of g CH$_4$/kg LW gain not reported and calculated from individual data of daily CH$_4$ production and LW gain.

[4] Value of g CH$_4$/kg milk yield or daily LW gain, g CH$_4$/kg DM intake or of DM intake as % of LW were not reported and calculated from individual data of daily CH$_4$ production and milk yield or daily LW gain, and from DM intake and LW.

[5] Average values of two grazing seasons; values of CH$_4$/kg OM intake and per kg LW gain were calculated from individual data of daily CH$_4$ production, OM intake and LW gain.

[6] Average values of two measurement periods.

[7] Average values of two seasons (dry and wet).

[8] Values calculated and averaged from data of two grazing managements applied on different season and type of forages, and only when animals had positive weight gains.

[9] Value of g CH$_4$/kg DM intake, as % of GEI, and DM intake are from the second measurement period (data not available for the first); g CH$_4$/d and per kg LW gain are from the average of the two periods.

[10] Values calculated accounting that 1 L of CH$_4$ equals 0.71386 kg of CH$_4$

[11] Pastures: annual ryegrass (AR), perennial ryegrass (PR), pearl millet (PM), bromegrass (BR), alfalfa (AL), white clover (WC), *U. brizantha* (UB), *A. pintoi* (AP), bahiagrass (BH), bermudagrass (BE), timothy (TY), cocksfoot (CK), *P. purpureum* (PP), black oat (BO), sorghum (SR), Lucerne (LU), chicory (CH).

Table 2. Intake and enteric CH_4 emissions by grazing ruminants under different supplementation practices.

Study target	Animal category	Pasture[3]	Intake, kg DM or OM (*)	Intake, % of BW	CH_4 production and yield					Diff. between control and best alternative, %		Reference	Country
					g/d	g/kg DMI or OMI	% GE	g kg LW gain, milk yield(*), ECM (**) or FPCM (***)	Calculated kg year	g CH_4 per feed input	g CH_4 per animal output		
					Beef cattle								
Feedlot or supplementing grazing	Steers/heifers	UB	6.6 to 8.9	1.7 to 2.5	98 to 104	11.7 to 15.7	3.7 to 4.2	-	35.9 to 37.9	25.4	-	Cota et al., 2014	Brazil
Forage mixtures	Heifers	PR or PR with WC or RC, or flowers	8.7 to 10.0	2.6 to 2.9	159 to 204	18.1 to 23.2	-	133 to 170	58.6 to 74	21.9	22	Hammond et al., 2014[1]	United Kingdom
Lipids supplementation	Steers	UB	11.0 to 12.8	-	70 to 114	6.6 to 9.9	2.3 to 3.4	140 to 240	25.5 to 41.6	33.3	41.6	De Carvalho et al., 2017	Brazil
Supplementation frequency	Steers	UB	7.7 to 7.87	-	226 to 253	28.4 to 33.4	9.9 to 11.7	-	82 to 92	14.9	-	Canesin et al., 2014	Brazil
Lipid supplementation	Steers	Native Tallgrasse, forbs, shrubs	6.5 to 7.3	-	175 to 202	24.5 to 31.2	7.1 to 9.7	168 to 466	63.8 to 73.7	12.5	64	Beck et al., 2019[2]	, United States
					Dairy cattle								
Light interception	Cows	PP	15.9 to 18.2	-	296.1 to 297.8	20.2 to 24.7	-	16.2 to 20.5	108.1 to 108.7	18.21	20.9	Congio et al., 2018	Brazil
Grass only or grass/clover	Cows	PR or PR/ clover	15.0 to 16.5	2.8 to 3.2	353.6 to 360.5	21.5 to 24.5	-	26.1 to 26.5	129.1 to 131.5	12.24	1.8	Enriquez-Hidalgo et al., 2014	Ireland
Pre-grazing herbage mass	Cows	PR-dominant	13.9 to 15.5	-	321 to 323	21.3 to 23.2	6.8 to 7.5	13.6 to 15.3	117.2 to 117.9	8.18	11.1	Muñoz et al., 2016	Chile
Concentrate level	Cows	PR	18.5 to 19.5	-	336 to 373.5	18.7 to 19.8	6.05 to 6.55	13.4 to 13.7	123 to 136	5.5	2.1	Muñoz et al., 2015	Chile
Concentrate level	Cows	PR	14.2 to 15.5	2.5 to 2.7	272 to 287	17.7 to 20.0	5.3 to 5.9	10.8 to 15.4	99.1 to 105	11.5	29.8	Jiao et al., 2014	United Kingdom

Continuation…

					CH$_4$ production and yield					Diff. between control and best alternative, %			
Study target	Animal category	Pasture[3]	Intake, kg DM or OM (*) intake	Intake, % of BW	g/d	g/kg DMI or OMI	% GE	g kg LW gain, milk yield(*), ECM (**) or FPCM (***)	Calculated kg year	g CH$_4$ per feed input	g CH$_4$ per animal output	Reference	Country
					Dairy cattle								
Concentrate level	Cows	PR, other grasses, WC	17.74 to 21.51	-	346 to 399	17.83 to 19.6	-	17.7 to 21.0	126 to 146	9	15.7	Lovett et al., 2005	Ireland
Grazing-only or Total Mixed Ratio	Cows	PR	14.3 to 19.7	2.98 to 3.6	251 to 397	18.1 to 20.3	5.74 to 6.47	174 to 200**	91.6 to 144.9	11.03	13	O'Neil et al., 2011	Ireland
Supplementing with Partial Mixed Ratio	Cows	PR	13.9 to 16.5	-	349 to 406	25.0 to 26.1	7.7 to 7.98	23.9 to 24.9	127.4 to 148.2	4.2	4	O'Neil et al., 2012	Ireland
Grazing-only or Total Mixed Ratio	Cows	AR	15.7 to 16.4	-	503 to 656	31.2 to 41.7	8.6 to 11.3	25.3 to 34.2	183. 6 to 239.4	25.17	26.02	Dall-Orsoletta et al., 2016	Brazil
Levels of supplementation	Cows	PR-dominant pastures	16.4 to 18.0	4.1 to 4.6	258 to 321	16.9 to 19.6	5.3 to 6.12	15.9 to 19.8	110.2 to 117.2	13.7	19.7	Van Wyngaard et al., 2018	South Africa
Type of concentrate	Cows	Temperate mixed pastures	-	-	221 to 251	-	-	8.1 to 9.0**	81 to 92		10.0	Storlien et al., 2015	Norway
Pasture allocation	Dairy	TY with temperate forages	17.5 to 18.4	3.1 to 3.3	285 to 301	16.0 to 16.9	-	12.1 to 12.7**	104 to 111	5.3	4.5	Kidane et al., 2018[1]	Norway

[1] Value of g CH_4/kg milk yield or LW gain, g CH_4/kg DM intake or of DM intake as % of LW were not reported and calculated from individual data of daily CH_4 production and milk yield or LW gain, and from DM intake and LW.

[2] Enteric CH_4 estimated with automated head chamber system

[3] Pastures: annual ryegrass (AR), perennial ryegrass (PR), white clover (WC), U. brizantha (UB), timothy (TY), *P. purpureum* (PP), black oat (BO)

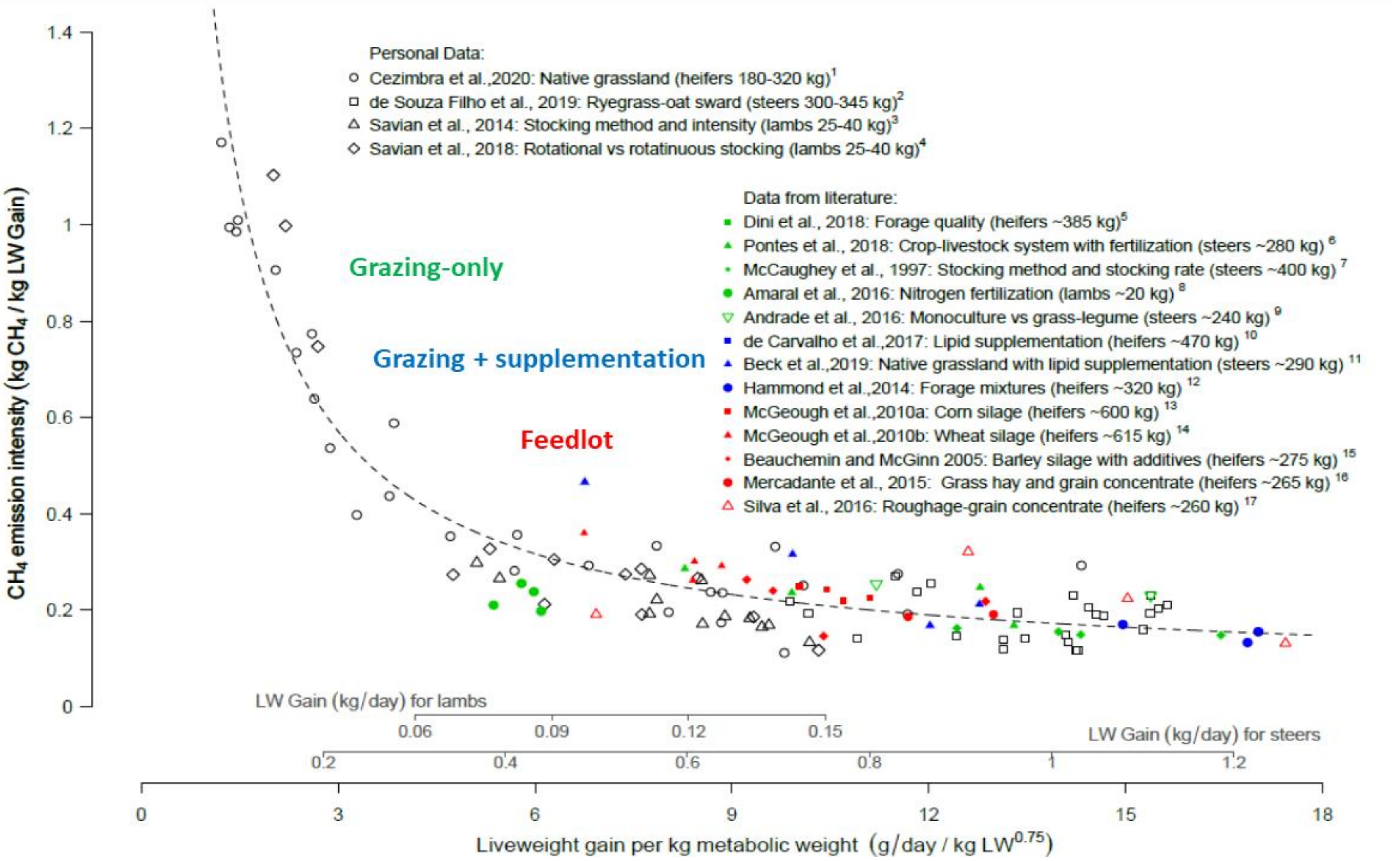


Figure 1. Relationship between CH_4 emission per kg of LW gain and daily LW gain per kg of metabolic weight. The broken line represent the regression model ($Y= 1.52 / X + 0.062$; $r^2= 0.88$), considering cattle under grazing-only, grazing plus supplementation and feedlot. Secondary horizontal axis is plotted to visualize LW gain of reference animals. Elaborated from data in supplementary material 1.

Table 3. Grazing management strategies and their implications over forage quality and enteric methane emissions.

Pasture management	Rationale of enteric CH_4 mitigation from grazing-only experiments	Experimental setting		Reference
		Experimental unit	Paddocks per treatment	
	Intake-oriented			
Pre- and post-grazing sward height	Allowing animals to maximize the short-term intake rate by managing the pre- and post-grazing sward surface heights, facilitates the ingestion process and opens the chance for animals to increase their individual DM intake, thus potentially reduce enteric CH_4 emissions	Paddock	Four paddocks of 0.22 ha each	Savian et al., 2018
Sward height	Managing the pasture at a sward surface height that allow animals to maximize intake and performance potentially reduce enteric CH_4 emissions from grazing cattle	Paddock	Three paddocks varying from 0.9 to 3.6 ha each	Souza-Filho et al., 2018
Forage allowance	Forage allowances that allow animals to increase intake and daily weight gains reduces enteric CH_4 emissions	Paddock	Two paddocks varying from 2.88 to 5.66	Cezimbra et al., unpublished
	Nutritional-oriented			
Stocking method and intensity	Animals under continuous stocking select forage of better quality, which might explain the lower CH_4 yield than animals under rotational management	Paddock	Three paddocks varying from 0.23 to 0.31 ha each	Savian et al., 2014
Herbage mass	Providing herbage of high nutritional quality through low herbage masses is a mean to increase animal performance and reduce CH_4 emission intensity	Animal	Single paddock with no specified dimensions (total experimental area of 2.01 ha)	Boland et al., 2013
Stocking rate	The stocking rate had no effect over daily CH_4 emissions, however, the low stocking tended to have greater emission per unit of digestible feed intake. Methane emissions was consistently related to herbage intake rather than herbage digestibility	Animal	Single paddock of 3.35 ha each	Pinares-Patiño et al., 2007

Continuation…

Pasture management	Rationale of enteric CH_4 mitigation from grazing-only experiments	Experimental setting		Reference
		Experimental unit	Paddocks per treatment	
	Nutritional-oriented			
Pre-grazing sward mass	Low pre-grazing herbage mass optimize the intake of high quality grass, which in turn allows the reduction of CH_4 production and yield	Animal	Single paddock of 8.2 ha divided in two; each half with 6 sub-divisions	Wims et al., 2010
Intensive rotational stocking	Intensive rotational stocking improves pasture quality and increases animal productivity, which in turn decreases the obligatory CH_4 associated to maintenance	Animal	Single paddock with 24 subdivisions of 0.5 ha each or a single paddock with no specified dimensions	DeRamus et al., 2003
Stocking method and stocking rate	The voluntary intake, thus CH_4 production, is higher in high quality pastures (legume-grass) than emissions from grass-dominant pastures. This results from the higher digestibility and passage rate of legumes than grasses	Paddock?	Two paddocks of 3.7 ha each	McCaughey et al., 1997
Low and high quality pastures	Enteric CH_4 emissions were significantly lower in animals that grazed on high-quality pasture, therefore, it is possible to use the quality of pastures as a mitigation strategy in grazing production systems	Animal	Single paddock of 0.7 or 0.15 ha	Dini et al., 2017
Mixed pastures rich in legumes or in grasses	At high herbage allowance, the quality of the diet selected by grazing cows did not differ between pastures rich in legumes or in grasses, and therefore there was no effect on milk or CH_4 production	Animal	No specified	Dini et al., 2012
Stocking rate and density	The stocking rate and density had no effect over DM intake and daily CH_4 emissions, probably because of the selective grazing, which allowed animals to eat forage with similar quality that met nutritional requirements with no difference in CH_4 emissions	Animal	Single paddock of 120 ha with subdivisions of 2 ha plus 3 subdivision of 0.7 ha or single paddock of 26 ha with 16 subdivisions of 1.6 ha	Chiavegato et al., 2015

Continuation…

Pasture management	Rationale of enteric CH_4 mitigation from grazing-only experiments	Experimental setting		Reference
		Experimental unit	Paddocks per treatment	
	Nutritional-oriented			
Light interception	Optimize plant growth, ruminant nutrition and their interface through grazing management is an effective practice to improve use efficiency of allocated resources and mitigate enteric CH_4	Animal	Six paddocks of 2,058 m^2 each	Congio et al., 2018
Pre-grazing sward mass	The low herbage mass, as a grazing management that favors maintaining high-quality pastures constitutes an effective strategy for moderately decreasing enteric CH_4 emissions by dairy cattle	Animal	Single paddock with six subdivisions of 0.5 ha each	Muñoz et al., 2016

Supplementary material 1. Figure 1 constructed from these data.

Treatment	Animal	Pasture	LW, kg	LW gain, kg/day	CH$_4$ emissions		Reference
					g/day	g/kg LW gain	
50 kg N ha	Lambs	Pearl millet	24.1	0.065	15.47	238	Amaral et al., 2016
100 kg N ha			22.6	0.06	15.31	255.2	
150 kg N ha			22.5	0.063	12.45	197.6	
200 kg N ha			20.7	0.052	10.93	210.2	
Continuous stocking/low grazing intensity	Lambs	Annual ryegrass	25.8	0.152	24.5	183	Savian et al., 2014[9]
Continuous stocking/moderate grazing intensity			22.8	0.148	22.7	159	
Rotational stocking/low grazing intensity			23.7	0.103	23.7	240	
Rotational stocking/moderate grazing intensity			20.8	0.76	20.7	285	
Pre- and post-grazing sward surface heights 18 and 11 cm	Lambs	Annual ryegrass	31.8	0.114	24.8	217	Savian et al., 2018[1,9]
Pre- and post-grazing sward surface heights 27 and 7 cm			29.1	0.038	22.2	586	
Sward surface height: 10 cm	Steers	Ryegrass/black oat mixture	321.5	0.818	171	210.1	De Souza-Filho et al., 2018[2,9]
Sward surface height: 20 cm			336.6	1.088	192	176.5	
Sward surface height: 30 cm			342.5	1.117	223	199.6	
Sward surface height: 40 cm			344.8	1.101	227	206.2	
Forage allowance (kg DM/100 kg BW): 4 %	Steers	Natural complex grasslands	224	0.039	107.0	2,743	Cezimbra et al., Unpublished[9]
Forage allowance (kg DM/100 kg BW): 8 %			265	0.169	118.0	698	
Forage allowance (kg DM/100 kg BW): 8-12 %			260	0.242	146.9	607	
Forage allowance (kg DM/100 kg BW): 12 %			267	0.213	151.4	710	
Forage allowance (kg DM/100 kg BW): 16 %			264	0.238	145.9	613	
Grazing fullsun dry period	Steers	*Megathyrsus maximum*	199	0.137	120.6	1324	Da Frota et al., 2017
Grazing fullsun rainy period			278	1.15	192.8	175.2	
Silvopatoral dry period		*Megathyrsus maximum* with *Attalea speciosa* trees	185.8	0.194	124.4	733.3	
Silvopastoral rainy period			253.9	0.958	203.3	214.8	

Continuation…

Treatment	Animal	Pasture	LW, kg	LW gain, kg/day	CH_4 emissions		Reference
					g/day	g/kg LW gain	
Crop-livestock with 90 kg N ha-1	Steers	Ryegrass/black oat mixture on an integrated crop-livestock	283.3	0.882	170	247	Pontes et al., 2018
Crop-livestock with 180 kg N ha-1			291.2	0.937	166	168	
Crop-livestock-tree with 90 kg N ha-1			279.7	0.567	165	286	
Crop-livestock-tree with 180 kg N ha-1			271.9	0.664	155	236	
Rotational stocking high stocking rate	Steers	Alfalfa (60 %), bromegrass (28.6 %) and other species	391.5	1.26	188.3	149.4	McCaughey et al., 1997
Rotational stocking low stocking rate			417.1	1.29	199.9	155	
Continuous stocking high stocking rate			380.1	1.07	172.9	161.5	
Continuous stocking low stocking rate			403	1.48	218.9	147.9	
Low quality pasture winter	Heifers	Grass-predominant (>60%) with little legume (<10%)	366.7	0.269	109	404	Dini et al., 2017[3, 4]
Low quality pasture spring			404	0.411	164	399	
High quality pasture winter		Legume-predominant (near 50 %)	360.7	0.8	160	200	
High quality pasture spring			400	1.404	177	126	
Monoculture	Steers	Elephant grass	248	0.7	146	254	Andrade et al., 2016[3]
Grass/legume mixture		Elephant grass/*Arachis pintoi*	251	0.97	180	230	
Monoculture plus 0.61 kg of concentrate	Heifers	Perennial ryegrass	346	1.2	204	170	Hammond et al., 2014
Grass/legume mixture plus 0.61 kg of concentrate		Perennial ryegrass/clover	324	1.3	202	155.4	
Grass/flowers mixture plus 0.61 kg of concentrate		Perennial ryegrass/wild flowers	295	1.2	159	132.5	
Grazing without additional fat	Steers	*Urochloa brizantha*	467	0.59	114	240	De Carvalho et al., 2017[4]
Grazing with palm oil (1 % BW)			456	0.57	112	240	
Grazing with linseed oil (1 % BW)			472	0.65	70.2	140	
Grazing with protected fat (1 % BW)			476	0.58	101	230	
Grazing with whole soybeans (1 % BW)			471	0.59	82.4	180	

Continuation...

Treatment	Animal	Pasture	LW, kg	LW gain, kg/day	CH_4 emissions		Reference
					g/day	g/kg LW gain	
Non-fat supplementation	Steers	Native Tallgrasses and forbs with small amount of shrubs	270	0.45	200	466	Beck et al., 2019[5]
Whole cottonseed supplement (1.4 kg DM/d)			264	0.65	175	316	
Soybean oil supplement (1.4 kg DM/d)			325	0.92	177	168	
Bypass oil supplement (1.4 kg DM/d)			304	0.93	202	212	
Corn silage harvested: September 13th plus 2.57 kg of concentrate	Steers	Feedlot	595.2	1.208	301	249.2	McGeough et al., 2010a[6, 7]
Corn silage harvested: September 28th plus 2.57 kg of concentrate			603.9	1.353	304	224.7	
Corn silage harvested October 9th plus 2.57 kg of concentrate			587.4	1.246	301	241.6	
Corn silage harvested: October 23th plus 2.57 kg of concentrate			601.1	1.298	284	218.8	
Wheat silage with grain:straw ratio: 11:89	Beef steers	Feedlot	602	0.82	295	359.8	McGeough et al., 2010b[6, 7]
Wheat silage with grain:straw ratio: 21:79			619.5	1.046	315	301.1	
Wheat silage with grain:straw ratio: 31:69			623	1.103	322	291.9	
Wheat silage with grain:straw ratio: 47:53			619	1.043	273	261.7	
Concentrate diet with no additive	Heifers	Feedlot	218.2	0.73	159.3	218.2	Beauchemin and McGinn et al., 2005[6]
Concentrate diet with fumaric acid			290.9	0.65	170.6	262.5	
Concentrate diet with essential oil			292.3	0.68	163.1	239.9	
Concentrate diet with canola oil			295.2	0.74	108	145.9	
Low residual feed intake animals fed concentrate diet (Urochloa brizantha hay:concentrate (44.5:55.5)	Steers	Feedlot	267.5	0.774	144	186.0	Mercadante et el., 2015[8]
High residual feed intake animals fed concentrate diet (Urochloa brizantha hay:concentrate (44.5:55.5)			264.5	0.853	163	191.1	

Continuation…

Treatment	Animal	Pasture	LW, kg	LW gain, kg/day	CH_4 emissions		Reference
					g/day	g/kg LW gain	
Sugarcane:concentrate (70:30)	Steers	Feedlot	237.5	0.42	165	321	Silva et al., 2016[8]
Sugarcane:concentrate (50:50)			265.5	0.83	186	224	
Corn silage:concentrate (70:30)			266	0.99	115	131	
Corn silage:concentrate (50:50)			282	1.2	185	156	

[1] Value of LW gain not reported, but available from authors

[2] Value of g CH_4/kg LW gain not reported, but available from authors

[3] Value of LW during CH_4 measurement not reported. But calculated from DM intake in relation to LW (%)

[4] Value of LW gain not reported, but calculated from g CH_4/d^{-1} and g CH_4/kg LW gain

[5] Value of LW during CH_4 measurement not reported, but calculated as the average between the initial and final LW (final LW calculated from initial LW, LW gain and days until CH_4 measurements).

[6] Value of LW during CH_4 measurement not reported, but calculated from initial LW, LW gain and days until CH_4 measurement

[7] Value of g CH_4/kg LW gain not reported, but calculated from g CH_4/d^{-1} and LW gain

[8] Value of LW nor reported, but calculated as the average between initial and final LW.

[9] The table includes the average values per treatments only. These values resulted from variable number of paddocks (experimental units), seasons and year of sampling of CH_4 emissions, which are excluded in the table but included in the Figure 1.

5. CHAPTER V.

5.1 GENERAL DISCUSSION AND FURTHER DIRECTIONS

The grazing management modifies the ingestive behavior of grazing ruminants at its smallest scale, the bite. This, in turn, affects the amount and nutritive value of the herbage ingested and triggers a series of events affecting the whole system. This thesis depicts biting behavioral features allowing animals to collect preferentially leaf lamina while grazing Italian ryegrass pastures with contrasting sward height management. This feature showed to be an important driver of the nutritive value of the ingested herbage and of blood biochemical-hematological indicators of nutrition and stress. Overall, the Rotatinuopus (RN) management, with pre- and post-grazing sward heights of 18 and 11cm, respectively, allowed animals to have a higher intake of a more nutritive herbage; the higher concentration of plasma urea nitrogen and albumin of lambs grazing Italian ryegrass pastures, and also the higher *in vitro* concentration of N-NH_3, valeric and branched-chain fatty acids, resulted from the increased CP content of ingested herbage. This confirms the exacerbation of the nitrogen imbalance of animals under the RN management and highlights the necessity of adopting grazing management strategies or nutritional practices improving nutrient use efficiency in the rumen and reducing nitrogen excretion and environment pollution. As well, the RN management might improve some milk quality traits, by both increased intake of CP and fatty acids as suggested by results from COPPA et al. (2015) and LIU et al. (2018). If this hypothesis is confirmed, more than a thousand dairy farms in Southern Brazil, nowadays applying the RN management with temperate and tropical forages, could push for an eventual transition towards milk quality payments schemes and beneficiate from it.

Among grazing strategies, the timing of the strip-change or grazing schedule, (GREGORINI; GUNTER; BECK, 2008), could be tested as alternatives to tackle the mentioned trade-off of increasing CP intake. Eventual shifts in the fermentation profile towards a more efficient rumen environment could drive other benefits such as a further reduction of enteric methane emissions (GREGORINI, 2012), most likely to occur with tropical forages. Respect nutritional options, the proper supplementation strategy that would assist in capturing the excessive rumen N-NH_3, but that also result in improved performance and reduce overall GHG emission remains to be defined. Complex natural grasslands could explore the benefits of the active compounds that the ingestion of native legumes could bring over nutrient digestion and metabolism, methane emission and animal welfare, under a RN management.

The benefits of grazing on animal welfare is well documented (BUROW et al., 2011; RADKOWSKA; HERBUT, 2014). Indeed, pasture-based systems are regarded as animal-friendly (LOBATO et al., 2014; PROVENZA; KRONBERG; GREGORINI, 2019). Nonetheless, as indicated in this thesis and in COSTA et al. (2015) by the N:L ratio, there is other evidence that grazing *per se* might not warrant this condition, when severe grazing is imposed (REN et al., 2016). Yet, it is worth noticing that the findings of this thesis do not represent high yielding animals, such as those in the transition period and specially the periparturient animal, whose nutritional deficit and immune system are much more challenged (BERTONI; MINUTI; TREVISI, 2015; COLLIER; RENQUIST; XIAO, 2017). Thereby, it is advisable that given the subjective nature of the welfare concept, no single biochemical, immunological or behavioral measurement is conclusive. Instead, complementary approaches such as assessing inflammatory responses and oxidative stress (BERTONI; MINUTI; TREVISI, 2015), studying hepatic enzymes through proteomics tools (REN et al., 2016) or performing behavioral tests denoting demotivation to eat and frustration for non-rewarded eating attempts (CATANESE et al., 2013), conjunctly, would be valuable indicators of well-being of animals submitted to sub-optimal grazing conditions.

As mentioned before, the preferential leaf lamina biting behavior of animals under RN management increased the intake of herbage with higher content of soluble compounds, known to be less methanogenic (ARCHIMÈDE et al., 2011; MOE; TYRRELL, 1979; VAN HOUTERT, 1993). However, the low-CH_4 *in vitro* rumen environment was not confirmed in the *in vitro* study. This support previous suggestions upon the secondary role that the chemical composition has over fermentation pathways driving CH_4 production (HAMMOND et al., 2011; PINARES-PATIÑO; BAUMONT; MARTIN, 2003; SUN et al., 2011), at least for high-nutritive temperate forages, and highlights the primary role that the individual intake has for mitigating purposes, especially for grazing ruminants. Alternatively, the rate at which the herbage is harvested reaches the fermentation pool and passes the rumen, as well as the forage particle size and ruminating behavior, as affected by the grazing managements, could be more important in shifting the rumen environment. Technological developments could assist, for instance, the conduction of real-time monitoring of the rumen environment (e.g. pH), or monitoring of the ruminating behavior, and establishing relationships with CH_4 emissions.

Several studies show other benefits that ingesting large amounts of fatty acids could bring for animals. Among these are the increase of beneficial Conjugates of Linoleic Acid (CLA) on animal products (COPPA et al., 2015; ELGERSMA, 2015; GREGORINI, 2012; LOURENÇO et al., 2007; VASTA et al., 2012), improve the reproductive performance (HERRERA-CAMACHO et al., 2011) and immune response of newborns (BERTONI; MINUTI; TREVISI, 2015; MAVANGIRA; SORDILLO, 2018; SORDILLO, 2016). These, however, have been demonstrated mostly on intense-fed animals supplemented with oils; thus, while it is reasonably to think that these benefits could be less apparent on grazers, it is also true that the intake of fatty acids under grazing is usually underrated, in part due to excessive sward depletion. Indeed, in Southern Brazil, farms applying the RN management under the PISA project (Producao Integrada de Sistemas Agropecuarios) have observed, at some extent, the occurrence of these benefits. Therefore, assessing the extent to which grazing managements promoting higher fatty acids intake drive above mentioned benefits to ruminants, especially those with high energy demands, warrants further research.

Finally, herbivory set the rate and extent of environment and natural resources deterioration when grazing is improperly conducted. Conversely, when sound grazing practices are adopted, it determines the magnitude of ecosystem services obtained from grasslands; when this happens, grazing ruminants are highly beneficial for human well-being. For instance, this thesis provides evidence of the opportunities that pastoral ecosystems have to reduce their CH_4 emissions intensity of animal commodities to levels comparable to those of intense-fed animal. It stresses the preponderant role that the sward structure has for accomplishing this by facilitating the herbage ingestion. It adds to the scientific literature showing other possible benefits beyond the economic or environmental perspective, as it is the animal welfare. Conjunctly, it highlights the “good side” of pasture-based systems. Although it is out of the scope of this thesis discussing about the criticism existing against pasture-based farming, it is worthy reflecting on the consequence of reducing/eliminating ruminant production from grasslands. For instance, in Brazil, SILVA et al. (2016) forecasted that reduced beef consumption would actually lead to less productive beef systems, associated with higher emissions intensities and total emissions, whereas increased production would lead to more efficient systems with boosted soil organic carbon stocks, reducing both per kilogram and total GHG emissions. In the UK, GREEN et al. (2015) predicted that a significant reduction of GHG emissions (40%) is unlikely without radically changing current consumption patterns and potentially reducing the nutritional value of diets. Thus, rather than discouraging the consumption of animal commodities coming from grasslands, the target must be finding ways of coupling grazing ruminants with the ecosystem (DUMONT; GROOT; TICHIT, 2018), especially on integrated crop-livestock systems, and fully exploit grasslands' potential to revert decades of natural resources depletion and environment deterioration on improperly managed grasslands… in "re-greening the earth", says JANZEN (2011).

REFERENCES

AIKING, H. Protein production: planet, profit, plus people? **The American Journal of Clinical Nutrition**, Oxford, v. 100, p. 483S-489S, 2014. Supl. 1. Available in: https://academic.oup.com/ajcn/article/100/suppl_1/483S/4576686. Access in: 2 Feb. 2015.

ALEXANDRATOS, N.; BRUINSMA, J. **World agriculture towards 2030/2050:** the 2012 revision. Rome: ESA, 2012. (ESA working paper, no. 12-03).

ALLARD, V. *et al*. The role of grazing management for the net biome productivity and greenhouse gas budget (CO2, N2O and CH4) of semi-natural grassland. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 121, n. 1/2, p. 47–58, 2007. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0167880906004324. Access in: 10 Mar. 2017.

ALLDEN, W. G.; MCDWHITTAKER, I. A. The determinants of herbage intake by grazing sheep: the interrelationship of factors influencing herbage intake and availability. **Australian Journal of Agricultural Research**, Melbourne, v. 21, n. 5, p. 755, 1970. Available in: http://www.publish.csiro.au/?paper=AR9700755. Access in: 18 June 2019.

AMARAL, G. A. *et al*. Methane emissions from sheep grazing pearl millet (Penissetumamericanum (L.) Leeke) swards fertilized with increasing nitrogen levels. **Small Ruminant Research**, Amsterdam, v. 141, p. 118–123, 2016. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0921448816301730. Access in: 7 Aug. 2017.

AMARAL, M. F. *et al*. Sward structure management for a maximum short-term intake rate in annual ryegrass. **Grass and Forage Science**, Oxford, v. 68, n. 2, p. 271–277, 2013. Available in: http://doi.wiley.com/10.1111/j.1365-2494.2012.00898.x. Access in: 23 Feb. 2018.

ANDRADE, B. O. *et al*. Grassland degradation and restoration: a conceptual framework of stages and thresholds illustrated by southern Brazilian grasslands. **Natureza & Conservação**, Rio de Janeiro, v. 13, n. 2, p. 95–104, 2015. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1679007315000341. Access in: 25 Feb. 2017.

ANDRADE, E. A. *et al*. Herbage intake, methane emissions and animal performance of steers grazing dwarf elephant grass v. dwarf elephant grass and peanut pastures. **Animal**, Cambridge, v. 10, n. 10, p. 1684–1688, 2016. Available in: https://www.cambridge.org/core/product/identifier/S1751731116000628/type/journal_article. Access in: 1º Jan. 2017.

ARCHIMÈDE, H. *et al*. Comparison of methane production between C3 and C4 grasses and legumes. **Animal Feed Science and Technology**, Amsterdam, v. 166–167, p. 59–64, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840111001222. Access in: 5 May 2015.

ARCHIMÈDE, H. *et al*. Potential of tannin-rich plants, Leucaenaleucocephala, Glyricidiasepium and Manihotesculenta, to reduce enteric methane emissions in sheep. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 100, n. 6, p. 1149–1158, 2016. Available in: http://doi.wiley.com/10.1111/jpn.12423. Access in: 15 Sept. 2018.

ARCHIMÈDE, H. *et al*. Intake, total-tract digestibility and methane emissions of Texel and Blackbelly sheep fed C4 and C3 grasses tested simultaneously in a temperate and a tropical area. **Journal of Cleaner Production**, Amsterdam, v. 185, p. 455–463, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0959652618307169. Access in: 29 Feb. 2015.

BARGO, F. *et al*. Invited Review: production and digestion of supplemented dairy cows on pasture. **Journal of Dairy Science**, Champaign, v. 86, n. 1, p. 1–42, 2003. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030203735814. Access in: 25 Dec. 2015.

BAUMONT, R. *et al*. A mechanistic model of intake and grazing behaviour in sheep integrating sward architecture and animal decisions. **Animal Feed Science and Technology**, Amsterdam, v. 112, n. 1/4, p. 5–28, 2004. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840103002967. Access in: 1º Dec. 2015.

BEAUCHEMIN, K. A. *et al*. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle1. **Journal of Animal Science**, Champaign, v. 85, n. 8, p. 1990–1996, 2007.
Available in: https://academic.oup.com/jas/article/85/8/1990/4778278. Access in: 22 Nov. 2015.

BEAUCHEMIN, K. A. *et al*. Nutritional management for enteric methane abatement: a review. **Australian Journal of Experimental Agriculture**, East Melbourne, v. 48, n. 2, p. 21, 2008. Available in: http://www.publish.csiro.au/?paper=EA07199. Access in: 22 Sept. 2015.

BEAUCHEMIN, K. A. *et al*. Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. **Animal**, Cambridge, v. 14, n. S1, p. s2–s16, 2020. Available in: https://www.cambridge.org/core/product/identifier/S1751731119003100/type/journal_article. Access in: 22 Mar. 2020.

BEAUCHEMIN, K. A.; MCGINN, Sean M. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. **Canadian Journal of Animal Science**, Ottawa, v. 86, n. 3, p. 401–408, 2006. Available in: http://www.nrcresearchpress.com/doi/10.4141/A06-021. Access in: 11 May 2016.

BELLAVER, C.; BELLAVER, I. H. Livestock production and quality of societies' life in transition economies. **Livestock Production Science**, Amsterdam, v. 59, n. 2/3, p. 125–135, 1999. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0301622699000214. Access in: 19 Feb. 2017.

BENCHAAR, C.; POMAR, C.; CHIQUETTE, J. Evaluation of dietary strategies to reduce methane production in ruminants: a modelling approach. **Canadian Journal of Animal Science**, Ottawa, v. 81, n. 4, p. 563–574, 2001. Available in: http://www.nrcresearchpress.com/doi/10.4141/A00-119. Access in: 11 Feb. 2016.

BENVENUTTI, M. A. *et al*. Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. **Grass and Forage Science**, Oxford, v. 71, n. 3, p. 424–436, 2016. Available in: http://doi.wiley.com/10.1111/gfs.12186. Access in: 21 Apr. 2017.

BERNDT, A.; TOMKINS, N. W. Measurement and mitigation of methane emissions from beef cattle in tropical grazing systems: a perspective from Australia and Brazil. **Animal**, Cambridge, v. 7, n. s2, p. 363–372, 2013. Available in: https://www.cambridge.org/core/product/identifier/S1751731113000670/type/journal_article. Access in: 18 July 2015.

BERTONI, G.; MINUTI, A.; TREVISI, Erminio. Immune system, inflammation and nutrition in dairy cattle. **Animal Production Science**, Melbourne, v. 55, n. 7, p. 943, 2015. Available in: http://www.publish.csiro.au/?paper=AN14863. Access in: 8 Oct. 2016.

BICKELL, S. *et al*. Rethinking the management of health and reproduction in small ruminants. *In*: WITTWER, F. *et al*. (ed.). **Updates on ruminant production and medicine**. Santiago, Chile: World Buiatrics Congress, 2010. p. 317-325.

BILENCA, D.; MIÑARRO, F. **Identificación de áreas valiosas de pastizal en las pampas y campos de Argentina, Uruguay y sur de Brasil**. Buenos Aires: Fundación de Vida Silvestre Argentina, 2004. p. 353.

BLAXTER, K. L.; CLAPPERTON, J. L. Prediction of the amount of methane produced by ruminants. **British Journal of Nutrition**, Wallingford, v. 19, n. 1, p. 511–522, 1965. Available in: https://www.cambridge.org/core/product/identifier/S0007114565000466/type/journal_article. Access in: 5 Jan. 2017.

BOND, W. J.; PARR, C. L. Beyond the forest edge: ecology, diversity and conservation of the grassy biomes. **Biological Conservation**, Montpellier, v. 143, n. 10, p. 2395–2404, 2010. Available in:

https://linkinghub.elsevier.com/retrieve/pii/S0006320709005205. Access in: 30 June 2017.

BOVAL, M.; DIXON, R. M. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. **Animal**, Cambridge, v. 6, n. 5, p. 748–762, 2012. Available in: http://www.journals.cambridge.org/abstract_S1751731112000304. Access in: 21 Mar. 2015.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Projeções do agronegócio:** Brasil 2016/16 a 2025/26. 7. ed. Brasília, DF: Ministério da Agricultura, Pecuária e Abastecimento. Assessoria de Gestão Estratégica, 2016.

BRISKE, D. D. *et al*. Rotational grazing on rangelands: reconciliation of perception and experimental evidence. **Rangeland Ecology & Management**, Denver, v. 61, n. 1, p. 3–17, 2008. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1550742408500078. Access in: 1º Mar. 2015.

BRUNETTI, H. B. *et al*. Sward structure, light interception and herbage accumulation in forage peanut cv. Belmonte subjected to strategies of intermittent grazing management. **Acta Scientiarum. Animal Sciences**, Maringá, v. 38, n. 4, p. 395, 2016. Available in: http://periodicos.uem.br/ojs/index.php/ActaSciAnimSci/article/view/32207. Access in: 5 Feb. 2017.

BUDDLE, B. M. *et al*. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. **The Veterinary Journal**, London, v. 188, n. 1, p. 11–17, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1090023310000821. Access in: 15 June 2015.

BUROW, E. *et al*. The effect of grazing on cow mortality in Danish dairy herds. **Preventive Veterinary Medicine**, New York, v. 100, n. 3/4, p. 237–241, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S016758771100095X. Access in: 30 Aug. 2019.

BUTLER, W. R. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. **Journal of Dairy Science,** Champaign, v. 81, n. 9, p. 2533–2539, 1998. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030298701468. Access in: 29 Oct. 2009.

CAETANO, M. *et al*. Efficacy of methane-reducing supplements in beef cattle rations. **Animal Production Science**, Melbourne, v. 56, n. 3, p. 276, 2016. Available in: http://www.publish.csiro.au/?paper=AN15601. Access in: 1º Feb. 2017.

CAPPER, J. L.; CADY, R. Al; BAUMAN, D. E. The environmental impact of dairy production: 1944 compared with 2007. **Journal of Animal Science**, Champaign, v. 87, n. 6, p. 2160–2167, 2009. Available in: https://academic.oup.com/jas/article/87/6/2160-2167/4731307. Access in: 31 Dec. 2015.

CARO, D.; KEBREAB, E.; MITLOEHNER, F. M. Mitigation of enteric methane emissions from global livestock systems through nutrition strategies. **Climatic Change**, Dordrecht, v. 137, n. 3/4, p. 467–480, 2016. Available in: http://link.springer.com/10.1007/s10584-016-1686-1. Access in: 30 Nov. 2017.

CARVALHO, P. C. F. *et al*. Can animal performance be predicted from short-term grazing processes? **Animal Production Science**, Melborune, v. 55, n. 3, p. 319, 2015. Available in: http://www.publish.csiro.au/?paper=AN14546. Access in: 29 Nov. 2015.

CARVALHO, P. C. F. Harry stobbs memorial lecture: can grazing behavior support innovations in grassland management? **Tropical Grasslands**, Brisbane, v. 1, n. 2, p. 137, 2013. Available in: http://www.tropicalgrasslands.info/index.php/tgft/article/view/81. Access in: 1º Nov. 2015.

CARVALHO, P. C. F.; BATELLO, C. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: the natural grasslands dilemma. **Livestock Science**, Amsterdam, v. 120, n. 1/2, p. 158–162, 2009. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1871141308001285. Access in: 1º Sept. 2015.

CATANESE, F. *et al*. The importance of diet choice on stress-related responses by lambs. **Applied Animal Behaviour Science**, Amsterdam, v. 148, n. 1/2, p. 37–45, 2013. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0168159113001792. Access in: 12 Dec. 2015.

CHACON, E.; STOBBS, T. Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. **Australian Journal of Agricultural Research**, East Melbourne, v. 27, n. 5, p. 709, 1976. Available in: http://www.publish.csiro.au/?paper=AR9760709. Access in: 19 Mar. 2000.

CHRISTIE, K. M. *et al*. Modelling enteric methane abatement from earlier mating of dairy heifers in subtropical Australia by improving diet quality. **Animal Production Science**, Melborune, v. 56, n. 3, p. 565, 2016. Available in: http://www.publish.csiro.au/?paper=AN15296. Access in: 16 May 2018.

CLARK, H. Nutritional and host effects on methanogenesis in the grazing ruminant. **Animal**, Cambridge, v. 7, n. s1, p. 41–48, 2013. Available in: https://www.cambridge.org/core/product/identifier/S1751731112001875/type/journal_article. Access in: 4 Feb. 2015.

COLLIER, R. J.; RENQUIST, B. J.; XIAO, Y. A 100-year review: stress physiology including heat stress. **Journal of Dairy Science**, Champaing, v. 100, n. 12, p. 10367–10380, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030217310330. Access in: 4 Nov. 2018.

CONGIO, G. F. S. *et al*. Strategic grazing management towards sustainable intensification at tropical pasture-based dairy systems. **Science of the Total Environment**, Amsterdam, v. 636, p. 872–880, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0048969718314797. Access in: 9 July 2019.

COPPA, M. *et al*. Frequent moving of grazing dairy cows to new paddocks increases the variability of milk fatty acid composition. **Animal**, Cambridge, v. 9, n. 4, p. 604–613, 2015. Available in: https://www.cambridge.org/core/product/identifier/S1751731114003000/type/journal_article. Access in: 1º July 2016.

COSTA, J. P. R. *et al*. The effect of grazing intensity and supplementation on performance, stress indicators and metabolic profiles of finishing lambs. **Semina: Ciências Agrárias**, Londrina, v. 36, n. 6, p. 3863, 2015. Available in: http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/19405. Access in: 1º Aug. 2017.

COTTLE, D. J.; NOLAN, John V; WIEDEMANN, S. G. Ruminant enteric methane mitigation: a review. **Animal Production Science**, Melbourne, v. 51, n. 6, p. 491, 2011. Available in: http://www.publish.csiro.au/?paper=AN10163. Access in: 1º Dec. 2015.

DELAGARDE, R. *et al*. Vertical distribution of biomass, chemical composition and pepsin—cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. **Animal Feed Science and Technology**, Amsterdam, v. 84, n. 1/2, p. 49–68, 2000. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840100001140. Access in: 1º Jan. 2020.

DHABHAR, F. S. *et al*. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. **The Journal of Immunology**, Rockville, v. 157, n. 4, p. 1638–1644, 1996.

DIAS-FILHO, M. B. **Diagnóstico das pastagens no Brasil**. Belém: Embrapa Amazônia Oriental, 2014. p. 38. Available in: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/986147/1/DOC402.pdf. Access in: 21 Mar. 2020.

DIAS, K. M. *et al*. Fatty acid profile in vertical strata of elephant grass subjected to intermittent stocking. **Anais da Academia Brasileira de Ciências**, Rio de Janeiro, v. 89, n. 3, p. 1707–1718, 2017. Available in: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652017000401707&tlng=en. Access in: 12 Apr. 2018.

DINI, Y. *et al*. Using highly nutritious pastures to mitigate enteric methane emissions from cattle grazing systems in South America. **Animal Production Science**, Melbourne, v. 58, n. 12, p. 2329, 2018. Available in: http://www.publish.csiro.au/?paper=AN16803. Access in: 10 Feb. 2019.

DOREAU, M. *et al*. Mitigation of enteric methane for French cattle: potential extent and cost of selected actions. **Animal Production Science**, Melbourne, v. 54, n. 9, p. 1417, 2014. Available in: http://www.publish.csiro.au/?paper=AN14207. Access in: 6 Nov. 2015.

DOVE, H. Balancing nutrient supply and nutrient requirements in grazing sheep. **Small Ruminant Research**, Amsterdam, v. 92, n. 1/3, p. 36–40, 2010. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0921448810000994. Access in: 9 Aug. 2015.

DU, Y. *et al*. A global strategy to mitigate the environmental impact of China's ruminant consumption boom. **Nature Communications**, London, v. 9, n. 1, p. 4133, 2018. Available in: http://www.nature.com/articles/s41467-018-06381-0. Access in: 3 Jan. 2019.

DUMONT, B.; GROOT, J. C. J.; TICHIT, M. Review: Make ruminants green again – how can sustainable intensification and agroecology converge for a better future? **Animal**, Cambridge, v. 12, n. s2, p. s210–s219, 2018. Available in: https://www.cambridge.org/core/product/identifier/S1751731118001350/type/journal_article. Access in: 1º Feb. 2019.

DURMIC, Z.; BLACHE, D. Bioactive plants and plant products: effects on animal function, health and welfare. **Animal Feed Science and Technology**, Amsterdam, v. 176, n. 1/4, p. 150–162, 2012. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840112002519. Access in: 1º Feb. 2015.

ELGERSMA, A. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives. **European Journal of Lipid Science and Technology**, Weinhein, v. 117, n. 9, p. 1345–1369, 2015. Available in: http://doi.wiley.com/10.1002/ejlt.201400469. Access in: 23 Nov. 2015.

ELLIS, J. L. *et al*. Effect of high-sugar grasses on methane emissions simulated using a dynamic model. **Journal of Dairy Science**, Champaign, v. 95, n. 1, p. 272–285, 2012. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030211006990. Access in: 1º Feb. 2015.

EUCLIDES FILHO, K. Supply chain approach to sustainable beef production from a Brazilian perspective. **Livestock Production Science**, Melbourne, v. 90, n. 1, p. 53–61, 2004. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0301622604001198. Access in: 1º Feb. 2008.

EUCLIDES, V. P. B. *et al*. Maintaining post-grazing sward height of Panicum maximum (cv. Mombaça) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. **Grass and Forage Science**, Oxford, v. 73, n. 1, p. 174–182, 2018. Available in: http://doi.wiley.com/10.1111/gfs.12292. Access in: 15 June 2019.

EUROPEAN UNION. Regulation EC No 1831/2003 of the European Parliament and Council of 22 September 2003 on additives for use in animal nutrition. (text with EEA relevance). **Official Journal of the European Union**, Luxembourg, p. L268, p. 29-43, 18 Oct. 2003. Available in: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1831&from=EN. Access in: 11 Mar. 2020.

FARIÑA, S. R.; CHILIBROSTE, P. Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. **Agricultural Systems**, Barking, v. 176, p. 102631, 2019. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0308521X18313787. Access in: 15 Mar. 2020.

FEARNSIDE, P. M. Deforestation in brazilian amazonia: history, rates, and consequences. **Conservation Biology**, Boston, v. 19, n. 3, p. 680–688, 2005.

FEDERATIVE Republic of Brazil. **Intended nationally determined contribution towards achieving the objective of the United Nations framework convention on climate change**. Brasília, DF: Ministry of Foreign Affairs, 2015.

FERRAZ, J. B. S.; FELÍCIO, P. E. Production systems – An example from Brazil. **Meat Science**, Illinois, v. 84, n. 2, p. 238–243, 2010. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0309174009001648. Access in: 1º Feb. 2015.

FISHER, J.; TOZER, P.; ABRECHT, D. Livestock in no-till cropping systems - a story of trade-offs. **Animal Production Science**, Melbourne, v. 52, n. 4, p. 197, 2012. Available in: http://www.publish.csiro.au/?paper=AN11123. Access in: 28 May 2015.

FONSECA, L. *et al*. Management targets for maximizing the short-term herbage intake rate of cattle grazing in Sorghum bicolor. **Livestock Science**, Amsterdam, v. 145, n. 1/3, p. 205–211, 2012.

GAO, Y.; GAO, X.; ZHANG, X. The 2 °C global temperature target and the evolution of the long-term goal of addressing climate change—from the United nations framework convention on climate change to the Paris agreement. **Engineering**, Beijing, v. 3, n. 2, p. 272–278, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S2095809917303077. Access in: 1º Sept. 2018.

GARNETT, T. Where are the best opportunities for reducing greenhouse gas emissions in the food system (including the food chain)? **Food Policy**, Guildford, v. 36, p. S23–S32, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0306919210001132. Access in: 24 Feb. 2013.

GARNETT, T. *et al*. **Grazed and confused?** Ruminating on cattle, grazing systems, methane, nitrous oxide, the soil carbon sequestration question-and what it all means for greenhouse gas emissions. Oxford: FCRN, 2017.

GASTAL, F.; LEMAIRE, G. Defoliation, shoot plasticity, sward structure and herbage utilization in pasture: review of the underlying ecophysiological processes. **Agriculture**, Basel, v. 5, n. 4, p. 1146–1171, 2015. Available in: http://www.mdpi.com/2077-0472/5/4/1146. Access in: 11 Dec. 2015.

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

GERE, J. I. *et al*. Methane emission factors for beef cows in Argentina: effect of diet quality. **New Zealand Journal of Agricultural Research**, Wellington, p. 1–9, 2019. Available in: https://www.tandfonline.com/doi/full/10.1080/00288233.2019.1621355. Access in: 1º Feb. 2020.

GODBER, O. F.; WALL, R. Livestock and food security: vulnerability to population growth and climate change. **Global Change Biology**, Oxford, v. 20, n. 10, p. 3092–3102, 2014. Available in: http://doi.wiley.com/10.1111/gcb.12589. Access in: 16 Sept. 2015.

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

GODFRAY, H. C. J. *et al*. Food security: the challenge of feeding 9 billion people. **Science**, Washington, DC, v. 327, n. 5967, p. 812–818, 2010b. Available in: https://www.sciencemag.org/lookup/doi/10.1126/science.1185383. Access in: 31 Dec. 2015.

GRASSINI, P.; ESKRIDGE, K. M.; CASSMAN, K. G. Distinguishing between yield advances and yield plateaus in historical crop production trends. **Nature Communications**, London, v. 4, n. 1, p. 2918, 2013. Available in: http://www.nature.com/articles/ncomms3918. Access in: 30 Jan. 2015.

GREEN, R. *et al*. The potential to reduce greenhouse gas emissions in the UK through healthy and realistic dietary change. **Climatic Change**, Dordretch, v. 129, n. 1/2, p. 253–265, 2015. Available in: http://link.springer.com/10.1007/s10584-015-1329-y. Access in: 21 Mar. 2018.

GREGORINI, P. Diurnal grazing pattern: its physiological basis and strategic management. **Animal Production Science**, Melborune, v. 52, n. 7, p. 416, 2012. Available in: http://www.publish.csiro.au/?paper=AN11250. Access in: 6 Nov. 2015.

GREGORINI, P.; GUNTER, S. A.; BECK, P. A. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: timing of herbage and fasting allocation. **Journal of Animal Science**, Champaing, v. 86, n. 4, p. 1006–1020, 2008. Available in: https://academic.oup.com/jas/article/86/4/1006/4789102. Access in: 29 June 2010.

GUZATTI, G. C. *et al*. Changes in the short-term intake rate of herbage by heifers grazing annual grasses throughout the growing season. **Grassland Science**, Oxford, v. 63, n. 4, p. 255–264, 2017. Available in: http://doi.wiley.com/10.1111/grs.12170. Access in: 16 July 2018.

HAMMOND, K. J. *et al*. Effects of feeding fresh white clover (Trifoliumrepens) or perennial ryegrass (Lolium perenne) on enteric methane emissions from sheep. **Animal Feed Science and Technology**, Amsterdam, v. 166/167, p. 398–404, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840111001477. Access in: 1º Feb. 2015.

HAMMOND, K. J. *et al*. Effects of feed intake on enteric methane emissions from sheep fed fresh white clover (Trifoliumrepens) and perennial ryegrass (Lolium perenne) forages. **Animal Feed Science and Technology**, Amsterdam, v. 179, n. 1/4, p. 121–132, 2013. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840112003884. Access in: 4 Feb. 2019.

HART, K. J. *et al*. Plant extracts to manipulate rumen fermentation. **Animal Feed Science and Technology**, Amsterdam, v. 147, n. 1/3, p. 8–35, 2008. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840107003550. Access in: 1º Dec. 2010.

HARTMANN, C.; SIEGRIST, M. Consumer perception and behaviour regarding sustainable protein consumption: a systematic review. **Trends in Food Science & Technology**, Cambridge, v. 61, p. 11–25, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0924224416302904. Access in: 23 Feb. 2018.

HASSANAT, F. *et al*. Replacing alfalfa silage with corn silage in dairy cow diets: Effects on enteric methane production, ruminal fermentation, digestion, N balance, and milk production. **Journal of Dairy Science**, Champaign, v. 96, n. 7, p. 4553–4567, 2013. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030213003743. Access in: 31 Dec. 2015.

HATEW, B. *et al*. Increasing harvest maturity of whole-plant corn silage reduces methane emission of lactating dairy cows. **Journal of Dairy Science**, Champaign, v. 99, n. 1, p. 354–368, 2016. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030215007663. Access in: 25 Jan. 2018.

HEGARTY, R. S. Nutritional management options to reduce enteric methane emissions from NSW beef and dairy herds. *In*: ANNUAL CONFERENCE OF THE GRASSLAND

SOCIETY OF NSW, 24., 2009, Orange. **Proceedings** […].Orange: Grassland Society of NSW, 2009. p. 40–45.

HENRY, Beverley; ECKARD, Richard. Greenhouse gas emissions in livestock production systems. **Tropical Grasslands**, Brisbane, v. 43, n. 4, p. 232, 2009.

HERRERA-CAMACHO, J. *et al*. Effect of fatty acids on reproductive performance of ruminants. *In*: MANAFI, M. **Artificial insemination in farm animals**. London: IntechOpen, 2011. p. 217-242.

HERRERO, M.; THORNTON, P. K. **Mitigating greenhouse gas emissions from livestock systems**. Washington, DC: International Food Policy Research Institute, 2009. v. 16.

HERRERO, M. *et al*. Livestock and greenhouse gas emissions: the importance of getting the numbers right. **Animal Feed Science and Technology**, Amsterdam, v. 166/167, p. 779–782, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840111002021. Access in: 24 Dec. 2015.

HERRERO, M. *et al*. Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. **Proceedings of the National Academy of Sciences**, Washington, DC, v. 110, n. 52, p. 20888–20893, 2013. Available in: http://www.pnas.org/cgi/doi/10.1073/pnas.1308149110. Access in: 16 Apr. 2015.

HERRERO, M. *et al*. Greenhouse gas mitigation potentials in the livestock sector. **Nature Climate Change**, London, v. 6, n. 5, p. 452–461, 2016. Available in: http://www.nature.com/articles/nclimate2925. Access in: 3 June 2017.

HILL, J. *et al*. Measuring methane production from ruminants. **Trends in Biotechnology**, Amsterdam, v. 34, n. 1, p. 26–35, 2016. Available in: https://linkinghub.elsevier.com/retrieve/pii/S016777991500222X. Access in: 1º Feb. 2018.

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

HOOK, S. E.; WRIGHT, A. D. G.; MCBRIDE, B. W. Methanogens: methane producers of the rumen and mitigation strategies. **Archaea**, London, v. 2010, p. 1–11, 2010. Available in: http://www.hindawi.com/journals/archaea/2010/945785/. Access in: 15 Mar. 2015.

HRISTOV, A. N. *et al*. Symposium review: uncertainties in enteric methane inventories, measurement techniques, and prediction models. **Journal of Dairy Science**, Champaign, v. 101, n. 7, p. 6655–6674, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030218303709. Access in: 29 Nov. 2019.

HRISTOV, A. N. *et al*. Special topics — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. **Journal of Animal Science**, Champaign, v. 91, n. 11, p. 5045–5069, 2013. Available in: https://academic.oup.com/jas/article/91/11/5045/4731308. Access in: 20 Aug. 2015.

HUBER, K. Invited review: resource allocation mismatch as pathway to disproportionate growth in farm animals – prerequisite for a disturbed health. **Animal**, Cambridge, v. 12, n. 3, p. 528–536, 2018. Available in: https://www.cambridge.org/core/product/identifier/S1751731117002051/type/journal_article. Access in: 1º Sept. 2019.

HULSHOF, R. B. A. *et al*. Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets1. **Journal of Animal Science**, Champaign, v. 90, n. 7, p. 2317–2323, 2012. Available in: https://academic.oup.com/jas/article/90/7/2317/4702068. Access in: 11 Feb. 2015.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATISTICA. Censo agropecuário. **Pesquisa da pecuária municipal**. Rio de Janeiro, 2017. Available in: https://brasilemsintese.ibge.gov.br/agropecuaria/efetivos-da-pecuaria.html. Access in: 20 Feb. 2020.

ILLIUS, A. W.; JESSOP, N. S. Metabolic constraints on voluntary intake in ruminants. **Journal of Animal Science**, Champaign, v. 74, n. 12, p. 3052, 1996. Available in: https://academic.oup.com/jas/article/74/12/3052-3062/4624806. Access in: 16 Jan. 2016.

INGVARTSEN, K. L.; MOYES, K. Nutrition, immune function and health of dairy cattle. **Animal**, Cambridge, v. 7, n. s1, p. 112–122, 2013. Available in: https://www.cambridge.org/core/product/identifier/S175173111200170X/type/journal_article. Access in: 16 Jan. 2015.

IPCC. **Climate Change 2014:** synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate. Geneva: IPCC, 2014. 151p.

IPCC. **Global warming of 1.5°C**: an IPCC special report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Geneva: IPCC, 2018.

JANZEN, H. H. What place for livestock on a re-greening earth? **Animal Feed Science and Technology**, Amsterdam, v. 166/167, p. 783–796, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S037784011100174X. Access in: 1º Feb. 2015.

JOHNSON, K. A.; JOHNSON, D. E. Methane emissions from cattle. **Journal of Animal Science**, Champaign, v. 73, n. 8, p. 2483–2492, 1995. Available in: https://academic.oup.com/jas/article/73/8/2483-2492/4632901. Access in: 21 July 2019.

JONKER, A. *et al*. Methane emissions from lactating and non-lactating dairy cows and growing cattle fed fresh pasture. **Animal Production Science**, Melbourne, v. 57, n. 4, p. 643, 2017. Available in: http://www.publish.csiro.au/?paper=AN15656. Access in: 30 June 2018.

KNAPP, J. R. *et al*. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. **Journal of Dairy Science**, Champaign, v. 97, n. 6, p. 3231–3261, 2014. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030214002896. Access in: 1º Feb. 2015.

KUMAR, S. *et al*. New aspects and strategies for methane mitigation from ruminants. **Applied Microbiology and Biotechnology**, Berlin, v. 98, n. 1, p. 31–44, 2014. Available in: http://link.springer.com/10.1007/s00253-013-5365-0. Access in: 26 Mar. 2015.

KURIHARA, M. *et al*. Methane production and energy partition of cattle in the tropics. **British Journal of Nutrition**, Wallingford, v. 81, n. 3, p. 227–234, 1999. Available in: https://www.cambridge.org/core/product/identifier/S0007114599000422/type/journal_article. Access in: 26 Mar. 2010.

LACA, E. A. *et al*. Effects of sward height and bulk density on bite dimensions of cattle grazing homogeneous swards. **Grass and Forage Science**, Oxford, v. 47, n. 1, p. 91–102, 1992. Available in: http://doi.wiley.com/10.1111/j.1365-2494.1992.tb02251.x. Access in: 30 Oct. 2015.

LEMAIRE, G. *et al*. Integrated crop–livestock systems: strategies to achieve synergy between agricultural production and environmental quality. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 190, p. 4–8, 2014.

LENG, R. A. Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. **Animal Production Science**, Melbourne, v. 54, n. 5, p. 519, 2014. Available in: http://www.publish.csiro.au/?paper=AN13381. Access in: 27 Aug. 2015.

LENG, R. A. Unravelling methanogenesis in ruminants, horses and kangaroos: the links between gut anatomy, microbial biofilms and host immunity. **Animal Production Science**, Melbourne, v. 58, n. 7, p. 1175–1191, 2018.

LI, P. J. Exponential growth, animal welfare, environmental and food safety impact: the case of China's livestock production. **Journal of Agricultural and Environmental Ethics**, Dordrecht, v. 22, n. 3, p. 217–240, 2009. Available in: http://link.springer.com/10.1007/s10806-008-9140-7. Access in: 27 Aug. 2014.

LIU, Q. *et al*. Effects of branched-chain volatile fatty acids on lactation performance and mRNA expression of genes related to fatty acid synthesis in mammary gland of dairy cows. **Animal**, Cambridge, v. 12, n. 10, p. 2071–2079, 2018. Available in: https://www.cambridge.org/core/product/identifier/S1751731118000113/type/journal_article. Access in: 9 June 2019.

LLONCH, P. *et al*. Current available strategies to mitigate greenhouse gas emissions in livestock systems: an animal welfare perspective. **Animal**, Cambridge, v. 11, n. 2, p. 274–284, 2017. Available in: https://www.cambridge.org/core/product/identifier/S1751731116001440/type/journal_article. Access in: 10 May 2018.

LOBATO, J. F. P. *et al*. Brazilian beef produced on pastures: sustainable and healthy. **Meat Science**, Illinois, v. 98, n. 3, p. 336–345, 2014. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0309174014001909. Access in: 23 May 2015.

LOURENÇO, M. *et al*. Effect of grazing pastures with different botanical composition by lambs on rumen fatty acid metabolism and fatty acid pattern of longissimus muscle and subcutaneous fat. **Animal**, Cambridge, v. 1, n. 4, p. 537–545, 2007. Available in: https://www.cambridge.org/core/product/identifier/S1751731107703531/type/journal_article. Access in: 24 Apr. 2015.

LYNCH, J. Availability of disaggregated greenhouse gas emissions from beef cattle production: a systematic review. **Environmental Impact Assessment Review**, New York, v. 76, p. 69–78, 2019. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0195925518303603. Access in: 1º Feb. 2015.

MARTHA, G. B.; ALVES, E.; CONTINI, E. Land-saving approaches and beef production growth in Brazil. **Agricultural Systems**, Barking, v. 110, p. 173–177, 2012. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0308521X12000340. Access in: 17 Jan. 2015.

MARTIN, C.; MORGAVI, D. P.; DOREAU, Michel. Methane mitigation in ruminants: from microbe to the farm scale. **Animal**, Cambridge, v. 4, n. 3, p. 351–365, 2010. Available in: http://www.journals.cambridge.org/abstract_S1751731109990620. Access in: 30 Oct. 2015.

MARTINELLI, L. A. *et al*. Agriculture in Brazil: impacts, costs, and opportunities for a sustainable future. **Current Opinion in Environmental Sustainability**, Amsterdam, v. 2, n. 5/6, p. 431–438, 2010. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1877343510001065. Access in: 10 Feb. 2015.

MATHISON, G. W. *et al*. Reducing methane emissions from ruminant animals. **Journal of Applied Animal Research**, Abingdon, v. 14, n. 1, p. 1–28, 1998. Available in: http://www.tandfonline.com/doi/abs/10.1080/09712119.1998.9706212. Access in: 2 Oct. 2015.

MAVANGIRA, V.; SORDILLO, Lorraine M. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. **Research in Veterinary Science**, London, v. 116, p. 4–14, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0034528817303016. Access in: 27 Dec. 2015.

MCGEOUGH, E. J. *et al*. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. **Journal of Animal Science**, Champaign, v. 88, n. 4, p. 1479–1491, 2010. Available in: https://academic.oup.com/jas/article/88/4/1479-1491/4745718. Access in: 28 Feb. 2015.

MCALLISTER, T. A.; NEWBOLD, C. J. Redirecting rumen fermentation to reduce methanogenesis. **Australian Journal of Experimental Agriculture**, East Melbourne, v. 48, n. 2, p. 7, 2008. Available in: http://www.publish.csiro.au/?paper=EA07218. Access in: 1º Feb. 2015.

MCARTHUR, J. W.; MCCORD, G. C. Fertilizing growth: agricultural inputs and their effects in economic development. **Journal of Development Economics**, Amsterdam, v. 127, p. 133–152, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0304387817300172. Access in: 14 Jan. 2016.

MCCAUGHEY, W. P.; WITTENBERG, K.; CORRIGAN, D. Methane production by steers on pasture. **Canadian Journal of Animal Science**, Ottawa, v. 77, n. 3, p. 519–524, 1997.

MCMANUS, C. *et al*. Dynamics of cattle production in Brazil. **PLOS ONE**, San Francisco, v. 11, n. 1, [art.] e0147138, 2016. Available in: https://dx.plos.org/10.1371/journal.pone.0147138. Access in: 1º Feb. 2017.

MEZZALIRA, J. C. *et al*. Mechanisms and implications of a type IV functional response for short-term intake rate of dry matter in large mammalian herbivores. **Journal of Animal Ecology**, London, v. 86, n. 5, p. 1159–1168, 2017.

MEZZALIRA, J. C. *et al*. Behavioural mechanisms of intake rate by heifers grazing swards of contrasting structures. **Applied Animal Behaviour Science**, Amsterdam, v. 153, p. 1–9, 2014. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0168159113003213. Access in: 24 Dec. 2015.

MOATE, P. J. *et al*. Reducing the carbon footprint of Australian milk production by mitigation of enteric methane emissions. **Animal Production Science**, Melbourne, v.

56, n. 7, p. 1017, 2016. Available in: http://www.publish.csiro.au/?paper=AN15222. Access in: 23 Feb. 2018.

MOE, P. W.; TYRRELL, H. F. Methane production in dairy cows. **Journal of Dairy Science**, Champaign, v. 62, n. 10, p. 1583–1586, 1979. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030279834657. Access in: 23 Feb. 2020.

MORAES, L. E. *et al*. Prediction of enteric methane emissions from cattle. **Global Change Biology**, Oxford, v. 20, n. 7, p. 2140–2148, 2014. Available in: http://doi.wiley.com/10.1111/gcb.12471. Access in: 13 June 2015.

MOTTET, A. *et al*. Livestock: on our plates or eating at our table? A new analysis of the feed/food debate. **Global Food Security**, Amsterdam, v. 14, p. 1–8, 2017. Available in: https://linkinghub.elsevier.com/retrieve/pii/S2211912416300013. Access in: 11 Jan. 2019.

NGUYEN, S. H.; BARNETT, M. C.; HEGARTY, R. S. Use of dietary nitrate to increase productivity and reduce methane production of defaunated and faunated lambs consuming protein-deficient chaff. **Animal Production Science**, Melbourne, v. 56, n. 3, p. 290, 2016. Available in: http://www.publish.csiro.au/?paper=AN15525. Access in: 1º Feb. 2019.

OECD-FAO. **OECD-FAO agricultural outlook 2011**. Rome: OECD/ FAO, 2011. Available in: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2011_agr_outlook-2011-en. Access in: 1º Feb. 2012.

OENEMA, O.; DE KLEIN, C.; ALFARO, M. Intensification of grassland and forage use: driving forces and constraints. **Crop and Pasture Science**, Collingwood, v. 65, n. 6, p. 524, 2014. Available in: http://www.publish.csiro.au/?paper=CP14001. Access in: 1º Feb. 2015.

O'MARA, F. P. The role of grasslands in food security and climate change. **Annals of Botany**, Oxford, v. 110, n. 6, p. 1263–1270, 2012. Available in: https://academic.oup.com/aob/article-lookup/doi/10.1093/aob/mcs209. Access in: 1º Feb. 2015.

ORR, R. J. *et al*. Changes in ingestive behaviour of yearling dairy heifers due to changes in sward state during grazing down of rotationally stocked ryegrass or white clover pastures. **Applied Animal Behaviour Science**, Amsterdam, v. 87, n. 3/4, p. 205–222, 2004. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0168159104000371. Access in: 3 Sept. 2015.

PACHECO, D.; WAGHORN, G.; JANSSEN, P. H. Decreasing methane emissions from ruminants grazing forages: a fit with productive and financial realities? **Animal Production Science**, Melbourne, v. 54, n. 9, p. 1141, 2014. Available in: http://www.publish.csiro.au/?paper=AN14437. Access in: 31 July 2015.

PATRA, A. K. Recent advances in measurement and dietary mitigation of enteric methane emissions in ruminants. **Frontiers in Veterinary Science**, Lausanne, v. 3, p. 39, 2016. Available in: http://journal.frontiersin.org/Article/10.3389/fvets.2016.00039/abstract. Access in: 27 Apr. 2017.

PETERSON, C. A. *et al*. Winter grazing does not affect soybean yield despite lower soil water content in a subtropical crop-livestock system. **Agronomy for Sustainable Development**, Les Ulis, v. 39, n. 2, p. 26, 2019. Available in: http://link.springer.com/10.1007/s13593-019-0573-3. Access in: 24 Oct. 2020.

PEYRAUD, J. L.; DELAGARDE, R. Managing variations in dairy cow nutrient supply under grazing. **Animal**, Cambridge, v. 7, n. s1, p. 57–67, 2013. Available in: https://www.cambridge.org/core/product/identifier/S1751731111002394/type/journal_article. Access in: 9 Nov. 2014.

PINARES-PATIÑO, C. S.; BAUMONT, R.; MARTIN, C. Methane emissions by Charolais cows grazing a monospecific pasture of timothy at four stages of maturity. **Canadian Journal of Animal Science**, Champaign, v. 83, n. 4, p. 769–777, 2003. Available in: http://www.nrcresearchpress.com/doi/10.4141/A03-034. Access in: 4 Jan. 2015.

PINGALI, P. L. Green revolution: impacts, limits, and the path ahead. **Proceedings of the National Academy of Sciences**, Washington, DC, v. 109, n. 31, p. 12302–12308, 2012. Available in: http://www.pnas.org/cgi/doi/10.1073/pnas.0912953109. Access in: 1º Feb. 2015.

PONTES, S. L. *et al*. Performance and methane emissions by beef heifer grazing in temperate pastures and in integrated crop-livestock systems: the effect of shade and nitrogen fertilization. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 253, p. 90–97, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0167880917304991. Access in: 1º Jan. 2020.

PROVENZA, F. D.; KRONBERG, S. L.; GREGORINI, P. Is grassfed meat and dairy better for human and environmental health? **Frontiers in Nutrition**, Chicago, v. 6, 2019. Available in: https://www.frontiersin.org/article/10.3389/fnut.2019.00026/full. Access in: 1º Jan. 2020.

RADKOWSKA, I.; HERBUT, E. Hematological and biochemical blood parameters in dairy cows depending on the management system. **Animal Science Papers & Reports**, Jastrzębiec, v. 32, n. 4, p. 317-325, 2014.

RAMANKUTTY, N. *et al*. Trends in global agricultural land use: implications for environmental health and food security. **Annual Review of Plant Biology**, Palo Alto, v. 69, n. 1, p. 789–815, 2018. Available in:

http://www.annualreviews.org/doi/10.1146/annurev-arplant-042817-040256. Access in: 19 June 2019.

RAMÍREZ-RESTREPO, C. A. *et al*. Effects of grazing willow fodder blocks upon methane production and blood composition in young sheep. **Animal Feed Science and Technology**, Amsterdam, v. 155, n. 1, p. 33–43, 2010. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840109003198. Access in: 24 June 2015.

RAO, I. *et al*. Livestock Plus - the sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics. **Tropical Grasslands**- ForrajesTropicales, Brisbane, v. 3, n. 2, p. 59, 2015. Available in: http://www.tropicalgrasslands.info/index.php/tgft/article/view/262. Access in: 14 July 2018.

RAY, D. K. *et al*. Recent patterns of crop yield growth and stagnation. **Nature Communications**, London, v. 3, n. 1, p. 1293, 2012. Available in: http://www.nature.com/articles/ncomms2296. Access in: 14 Sept. 2015.

REID, R. S. *et al*. Is it possible to mitigate greenhouse gas emissions in pastoral ecosystems of the tropics? **Environment, Development and Sustainability**, Dordrecht, v. 6, n. 1/2, p. 91–109, 2004. Available in: http://link.springer.com/10.1023/B:ENVI.0000003631.43271.6b. Access in: 30 Mar. 2015.

REN, W. *et al*. Overgrazing induces alterations in the hepatic proteome of sheep (Ovisaries): an iTRAQ-based quantitative proteomic analysis. **Proteome Science**, London, v. 15, n. 1, p. 2, 2016. Available in: http://proteomesci.biomedcentral.com/articles/10.1186/s12953-016-0111-z. Access in: 16 Oct. 2019.

RICHMOND, A. S. *et al*. Methane emissions from beef cattle grazing on semi-natural upland and improved lowland grasslands. **Animal**, Cambridge, v. 9, n. 1, p. 130–137, 2015. Available in: https://www.cambridge.org/core/product/identifier/S1751731114002067/type/journal_article. Access in: 1º Oct. 2016.

ROCKSTRÖM, J. *et al*. A safe operating space for humanity. **Nature**, London, v. 461, n. 7263, p. 472–475, 2009. Available in: http://www.nature.com/articles/461472a. Access in: 14 Apr. 2018.

ROSEGRANT, M. W. *et al*. Looking into the future for agriculture and AKST. *In*: MCINTYRE, B. D. *et al*. (ed.). **International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD):** agriculture at a crossroads, global report. Washington, DC: Island Press, 2009. p. 307-376.

RUSSELL, J. R.; BISINGER, J. J. Forages and pastures symposium: Improving soil health and productivity on grasslands using managed grazing of livestock. **Journal of Animal Science**, Champaign, v. 93, n. 6, p. 2626–2640, 2015. Available in: https://academic.oup.com/jas/article/93/6/2626/4703397. Access in: 1º Apr. 2017.

SAVIAN, J. V. *et al*. Grazing intensity and stocking methods on animal production and methane emission by grazing sheep: Implications for integrated crop–livestock system. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 190, p. 112–119, 2014. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0167880914000887. Access in: 1º Mar. 2015.

SAVIAN, J. V. *et al*. Rotatinuous stocking: a grazing management innovation that has high potential to mitigate methane emissions by sheep. **Journal of Cleaner Production**, Amsterdam, v. 186, p. 602–608, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0959652618308291. Access in: 1º Aug. 2019.

SEEG - SISTEMA DE ESTIMATIVAS DE GASES DE EFEITO ESTUFA. [**Dados obtidos da página**]. 2019. Available in: http://seeg.eco.br/. Access in: 31 May 2020.

SHIBATA, M.; TERADA, Fuminori. Factors affecting methane production and mitigation in ruminants. **Animal Science Journal**, Tokyo, v. 81, n. 1, p. 2–10, 2010. Available in: http://doi.wiley.com/10.1111/j.1740-0929.2009.00687.x. Access in: 24 Feb. 2015.

SILVA, R. O. *et al*. Increasing beef production could lower greenhouse gas emissions in Brazil if decoupled from deforestation. **Nature Climate Change**, London, v. 6, n. 5, p. 493–497, 2016. Available in: http://www.nature.com/articles/nclimate2916. Access in: 14 June 2017.

SMITH, P. *et al*. Greenhouse gas mitigation in agriculture. **Philosophical Transactions of the Royal Society B: Biological Sciences**, London, v. 363, n. 1492, p. 789–813, 2008. Available in: https://royalsocietypublishing.org/doi/10.1098/rstb.2007.2184. Access in: 22 Dec. 2015.

SORDILLO, L. M. Nutritional strategies to optimize dairy cattle immunity. **Journal of Dairy Science**, Champaign, v. 99, n. 6, p. 4967–4982, 2016. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030216001004. Access in: 8 Oct. 2017.

SOUZA FILHO, W. *et al*. Mitigation of enteric methane emissions through pasture management in integrated crop-livestock systems: trade-offs between animal performance and environmental impacts. **Journal of Cleaner Production**, Amsterdam, v. 213, p. 968–975, 2019. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0959652618339672. Access in: 1º Dec. 2019.

STEINFELD, H. *et al*. **Livestock's long shadow:** environmental issues and options. Rome: FAO, 2006.

SUN, X. Z. *et al*. Effects of forage chicory (Cichoriumintybus) and perennial ryegrass (Lolium perenne) on methane emissions in vitro and from sheep. **Animal Feed Science and Technology**, Amsterdam, v. 166/167, p. 391–397, 2011. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840111001465. Access in: 10 Nov. 2015.

SUN, X. *et al*. Lambs fed fresh winter forage rape (Brassica napus L.) emit less methane than those fed perennial ryegrass (Lolium perenne L.), and possible mechanisms behind the difference. **PLOS ONE**, San Francisco, v. 10, n. 3, [art.] e0119697, 2015. Available in: https://dx.plos.org/10.1371/journal.pone.0119697. Access in: 1º Nov. 2016.

TAMMINGA, S. A review on environmental impacts of nutritional strategies in ruminants. **Journal of Animal Science**, Champaign, v. 74, n. 12, p. 3112, 1996. Available in: https://academic.oup.com/jas/article/74/12/3112-3124/4624819. Access in: 31 Dec. 2010.

TANG, S. *et al*. Methane emissions in grazing systems in grassland regions of China: a synthesis. **Science of the Total Environment**, Amsterdam, v. 654, p. 662–670, 2019.

THORNTON, P. K. Livestock production: recent trends, future prospects. **Philosophical Transactions of the Royal Society B: Biological Sciences**, London, v. 365, n. 1554, p. 2853–2867, 2010. Available in: https://royalsocietypublishing.org/doi/10.1098/rstb.2010.0134. Access in: 30 Nov. 2017.

THORNTON, P. K.; HERRERO, M. Potential for reduced methane and carbon dioxide emissions from livestock and pasture management in the tropics. **Proceedings of the National Academy of Sciences**, Washington, DC, v. 107, n. 46, p. 19667–19672, 2010. Available in: http://www.pnas.org/cgi/doi/10.1073/pnas.0912890107. Access in: 21 Jan. 2015.

TORRES, C. M. M. E. *et al*. Greenhouse gas emissions and carbon sequestration by agroforestry systems in southeastern Brazil. **Nature Scientific Reports**, London, v. 7, n. 1, p. 16738, 2017. Available in: http://www.nature.com/articles/s41598-017-16821-4. Access in: 1º Feb. 2018.

TRINDADE, J. K. *et al*. Forage allowance as a target of grazing management: implications on grazing time and forage searching. **Rangeland Ecology & Management**, Denver, v. 65, n. 4, p. 382–393, 2012. Available in: https://linkinghub.elsevier.com/retrieve/pii/S1550742412500631. Access in: 10 July 2015.

UNFCCC – UNITED NATIONS FRAMEWORK CONVENTION ON CLIMATE CHANGE. **Kyoto protocol reference manual on accounting of emissions and assigned amount**. Bonn: UNFCCC, 2008.

VAN GASTELEN, S.; DIJKSTRA, J.; BANNINK, A. Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? **Journal of Dairy Science**, Champaign, v. 102, n. 7, p. 6109–6130, 2019. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0022030219304229. Access in: 1º Feb. 2018.

VAN HOUTERT, M. F. J. The production and metabolism of volatile fatty acids by ruminants fed roughages: a review. **Animal Feed Science and Technology**, Amsterdam, v. 43, n. 3/4, p. 189–225, 1993. Available in: https://linkinghub.elsevier.com/retrieve/pii/037784019390078X. Access in: 1º Apr. 2014.

VAN WYNGAARD, J. D. V.; MEESKE, R.; ERASMUS, L. J. Effect of concentrate feeding level on methane emissions, production performance and rumen fermentation of Jersey cows grazing ryegrass pasture during spring. **Animal Feed Science and Technology**, Amsterdam, v. 241, p. 121–132, 2018. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0377840118301196. Access in: 1º July 2019.

VARGAS, R. S. *et al*. Microbial quality of soil from the Pampa biome in response to different grazing pressures. **Genetics and Molecular Biology**, Ribeirão Preto, v. 38, n. 2, p. 205–212, 2015. Available in: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47572015000200205&lng=en&tlng=en. Access in: 21 Sept. 2016.

VASTA, V. *et al*. Effect of morning vs. afternoon grazing on intramuscular fatty acid composition in lamb. **Meat Science**, Illinois, v. 90, n. 1, p. 93–98, 2012. Available in: https://linkinghub.elsevier.com/retrieve/pii/S0309174011002245. Access in: 6 Feb. 2016.

WAHLQUIST, A. K. Eating beef: cattle, methane and food production. **Asia Pacific Journal of Clinical Nutrition**, Oxford, v. 22, n. 1, p. 16, 2013.

WALL, E.; SIMM, G.; MORAN, D. Developing breeding schemes to assist mitigation of greenhouse gas emissions. **Animal**, Cambridge, v. 4, n. 3, p. 366–376, 2010. Available in: http://www.journals.cambridge.org/abstract_S175173110999070X. Access in: 4 May 2017.

WEIMER, P. J. Manipulating ruminal fermentation: a microbial ecological perspective. **Journal of Animal Science**, Champaign, v. 76, n. 12, p. 3114, 1998. Available in: https://academic.oup.com/jas/article/76/12/3114-3122/4625160. Access in: 1º Aug. 2019.

APPENDIX 1

ANIMAL FEED SCIENCE AND TECHNOLOGY

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

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0377-8401

DESCRIPTION

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

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

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

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

Manuscripts must be prepared in accordance with the journal's Guide for Authors.
Before preparing their manuscript, it is suggested that authors examine the following editorials by the Editors-in-Chief:

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

For an example of a sample manuscript click here.

AUDIENCE

Animal Scientists, Crop Scientists, Feed Manufacturers, Feed Additive Producers.

IMPACT FACTOR

2018: 2.590 © Clarivate Analytics Journal Citation Reports 2019

ABSTRACTING AND INDEXING

Biological Abstracts
Current Contents - Agriculture, Biology & Environmental Sciences
Herbage Abstracts
Index Veterinarius
Nutrition Abstracts
Veterinary Bulletin
Scopus

EDITORIAL BOARD

Co-Editors

V. Bampidis, International Hellenic University, Thessaloniki, Greece
S. Liu, Uni Vets Camden, Camden, New South Wales, Australia
V. Mlambo, University of Mpumalanga, Mbombela, South Africa
A.K. Patra, West Bengal State University, Belgachia, Kolkata, India

Statistical Advisors

J. Craigon, Nottingham, United Kingdom
J.G. Fadel, Davis, CA, United States
T.R. Famula, Davis, CA, United States
M.A. Ibáñez, Madrid, Spain

Book Review Editor

G. Flachowsky, Braunschweig, Germany

Senior Editorial Advisory Board

R. Campbell, Willaston, SA, Australia
J.W. Cone, Wageningen, Netherlands
J.A. Cuarón, Colon, Mexico
K.M. Koenig, Ottawa, AB, Canada
C.J. Lopez-Bote, Madrid, Spain
G.G. Mateos, Madrid, Spain
V.R. Ravindran, Palmerston North, New Zealand
M. Spanghero, Udine, Italy
K-H. Südekum, Bonn, Germany
M.T. Viana, Coronado, Mexico

Editorial Advisory Board

J.F. Aguilera, Granada, Spain
A.K. Bach, Barcelona, Spain
M.A. Bamikole, Benin City, Nigeria
H. Ben Salem, Ariana, Tunisia
A.R.J.B. Cabrita, Porto, Portugal
S. Calsamiglia, Barcelona, Spain
D. Colombatto, Buenos Aires, Argentina
J. De Boever, Merelbeke, Belgium
R. Dixon, Brisbane, QLD, Australia
R.G Elkin, Philadelphia, PA, United States
E.H. Evans, Bowmanville, ON, Canada
L. Fiems, Merelbeke, Belgium
F. Grosjean, Le Plessis Pâté, France
Y. Kotzamanis, Athens, Greece

GUIDE FOR AUTHORS

INTRODUCTION

Types of article

1. Original Research Papers (Regular Papers)
2. Review Articles
3. Short Communications
4. Book Reviews

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Review Articles should cover subjects falling within the scope of the journal which are of active current interest.

A *Short Communication* is a concise but complete description of a limited investigation, which will not be included in a later paper. Short Communications should be as completely documented, both by reference to the literature and description of the experimental procedures employed, as a regular paper. They should not occupy more than six printed pages (about 12 manuscript pages, including figures, tables and references).

Book Reviews will be included in the journal on a range of relevant books which are not more than two years old. Book reviews will be solicited by the Book Review Editor. Unsolicited reviews will not usually be accepted, but suggestions for appropriate books for review may be sent to the Book Review Editor:

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

Manuscripts describing the use of commercial feed products are welcome, but should include the following information: major components, contents of active ingredients (for example enzyme activities). Independent verification, as opposed to a manufacturers guarantee, is always desirable and often avoids difficulties in the review process, especially where there are no, or few, treatment impacts. The Editors reserve the right to reject any manuscript employing such products, wherein this information is not disclosed.

Submissions concerning feedstuff composition are welcome when published and/or accepted analytical procedures have been employed. However, unusual feedstuffs and/or a wide range of data are pre-requisites.
Submissions concerning NIRS may be suitable when more accurate, precise or robust equations are presented. Mathematical, technical and statistical advancement, may constitute the foundation for acceptance. For more details see the editorial in Vol. 118/3-4.

Contact details for submission

For queries concerning the submission process or journal procedures please visit the Elsevier Support Center. Authors can determine the status of their manuscript within the review procedure using Elsevier Editorial System.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.
After acceptance, open access papers will be published under a noncommercial license. For authors requiring a commercial CC BY license, you can apply after your manuscript is accepted for publication.

Open access

This journal offers authors a choice in publishing their research:

Subscription

- Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
- No open access publication fee payable by authors.
- The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peer-reviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

- Articles are freely available to both subscribers and the wider public with permitted reuse.
- A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The gold open access publication fee for this journal is **USD 2750**, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access

Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 12 months.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's Author Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Poorly written and/or presented manuscripts (relative to the journal's guidelines) may be returned to authors for upgrading by the editorial office, prior to a review for scientific merit.
Before preparing their manuscript, it is suggested that authors examine the editorial by the Editors-in-Chief in Vol. 134/3-4, which outlines several practices and strategies of manuscript preparation that the Editors-in-Chief have found to be successful. This editorial also outlines practices that can lead to difficulties with reviewers and/or rejection of the manuscript for publication. There is also an example of an Animal Feed Science and Technology manuscript available on the journal website at https://www.elsevier.com/locate/anifeedsci.

Submit your article
Please submit your article via https://www.evise.com/profile/api/navigate/ANIFEE.

Referees
Please submit the names and institutional e-mail addresses of several potential referees. For more details, visit our Support site. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Use past tense for current findings, and the present tense for "truths" and hypotheses.

Article Structure

Manuscripts should have **numbered lines**, with wide margins and **double spacing** throughout, i.e. also for abstracts, footnotes and references. **Every page of the manuscript, including the title page, references, tables, etc., should be numbered continuously.** However, in the text no reference should be made to page numbers; if necessary, one may refer to sections. Avoid excessive usage of italics to emphasize part of the text.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

If reference is made to AOAC, ISO or similar analytical procedure(s), the specific procedure identification number(s) must be cited. A number of references for neutral and acid detergent fibre (NDF, ADF) assays exist, and an alternative reference to the now out-of-print USDA Agriculture Handbook 379 must be used. There are many options for NDF and ADF assays (e.g. sodium sulfite, alpha amylase, residual ash), which must be specified in the text. For more details see the editorial in Vol. 118/3-4.

The following definitions should be used, as appropriate:

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

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

Reporting 'hemicellulose' values as the difference between NDF and ADF is generally only acceptable if the analyses have been sequential on the same sample. Crude fibre (CF), nitrogen-free extract (NFE) and total digestible nutrients (TDN) are not acceptable terms for describing feeds and should only be referred to in a historical context.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature. Combined 'Results and Discussion' sections are only acceptable for 'Short Communications', except under compelling circumstances.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
- ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

The abstract should be clear, descriptive and not longer than 400 words. It should contain the following specific information: purpose of study; experimental treatments used; results obtained, preferably with quantitative data; significance of findings; conclusions; implications of results if appropriate.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUB: Biochemical Nomenclature and Related Documents for further information.

Authors and Editors are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the *International Code of Botanical Nomenclature*, the *International Code of Nomenclature of Bacteria*, and the *International Code of Zoological Nomenclature*. All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names when the English term is first used, with the exception of common domestic animals. All biocides and other organic compounds must be identified by their Geneva names when first used in the text. Active ingredients of all formulations should be likewise identified.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

All data in figures should have a measure of variation either on the plot (e.g., error bars), in the figure legend itself, or by reference to a table with measures of variation in the figure legend.

Explanations should be given in the figure legend(s). Drawn text in the figures should be kept to a minimum.

If a scale is given, use bar scales (instead of numerical scales) that must be changed with reduction.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article**. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

All publications cited in the text should be presented in a list of references following the text of the manuscript. The manuscript should be carefully checked to ensure that the spelling of authors' names and dates are exactly the same in the text as in the reference list. The accuracy of the references is the responsibility of the author(s).

References published in other than the English language should be avoided, but are acceptable if they include an English language 'Abstract' and the number of non-English language references cited are reasonable (in the view of the handling Editor) relative to the total number of references cited.

In the text refer to the author's name (without initial) and year of publication, followed - if necessary - by a short reference to appropriate pages. Examples: "Since Peterson (1988) has shown that...". "This is in agreement with results obtained later (Kramer, 1989, pp. 12-16)".

If reference is made in the text to a publication written by more than two authors, the name of the first author should be used followed by "et al.". This indication, however, should never be used in the list of references. In this list names of first author and co-authors should be mentioned.

References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on authors' names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 2001a, 2001b, etc.

Reference links
Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/animal-feed-science-and-technology
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data

This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

For more information, visit the Mendeley Data for journals page.

Data statement

To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

Additional Information

Authors should use the 'Track Changes' option when revising their manuscripts, so that any changes made to the original submission are easily visible to the Editors. Those revised manuscripts upon which the changes are not clear may be returned to the author.

Specific comments made in the Author Comments in response to referees' comments must be organised clearly. For example, use the same numbering system as the referee, or use 2 columns of which one states the comment and the other the response.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Author Services. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.

APPENDIX 2

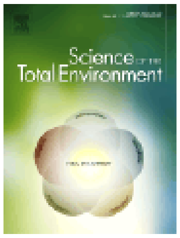
ELSEVIER

SCIENCE OF THE TOTAL ENVIRONMENT

An International Journal for Scientific Research into the Environment and its Relationship with Humankind

AUTHOR INFORMATION PACK

TABLE OF CONTENTS



ISSN: 0048-9697

DESCRIPTION

Science of the Total Environment is an international multi-disciplinary journal for publication of original research on the **total environment**, which includes the **atmosphere**, **hydrosphere**, **biosphere**, **lithosphere**, and **anthroposphere**.

totalenvironment.gif

AUDIENCE

Environmental Scientists, Environmental Toxicologists, Ecologists, Chemical/Environmental Engineers, Environmental Health Scientists and Epidemiologists, Risk Scientists, Environmental Science Managers and Administrators.

IMPACT FACTOR

2017: 4.610 © Clarivate Analytics Journal Citation Reports 2018

ABSTRACTING AND INDEXING

MEDLINE®
CSA Technology Research Database
Current Contents/Agriculture, Biology & Environmental Sciences
Biology & Environmental Sciences
Environmental Periodicals Bibliography
EMBASE
Oceanographic Literature Review
PASCAL/CNRS
Selected Water Resources Abstracts
Sociedad Iberoamericana de Informacion Cientifica (SIIC) Data Bases
Elsevier BIOBASE
Meteorological and Geoastrophysical Abstracts
Scopus

EDITORIAL BOARD

Co-Editors in Chief:

Damià Barceló, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
Environmental analysis; Water and soil quality; Organic mass spectrometry; Emerging organic contaminants; Nanomaterials; Biosensors for: Analysis, Fate and Risk of Emerging Pollutants such as Pharmaceuticals and Nanomaterials in the Environment Water Pollution Control and Protection Bridging analytical chemistry with ecotoxicology- toxicity identification; Evaluation techniques used: GC and LC tandem MS, biosensors, sample preparation, automated on-line techniques for water analysis environmental samples (water, including marine waters, sediments soils, biota samples)
Jay Gan, University of California, Riverside, Riverside, California, USA
Organic Contaminants; Pesticides; Emerging Contaminants; Adsorption; Transformation; Mitigation; Water Quality; Aquatic Toxicology; Remediation; Water Reuse

Special Issues Editor

Elena Paoletti, National Research Council of Italy (CNR), Firenze, Italy
Plant health; Plant ecophysiology; Forests; Climate stressors; Air pollution impacts on terrestrial ecosystems; BVOC; Ground-level ozone

Associate Editors

Julian Blasco, Instituto de Ciencias Marinas de Andalucía (CSIC), Puerto Real (Cádiz), Spain
Metals; Pharmaceuticals; Nanoparticles; Pollution; Ecotoxicology; Risk assessment; Seawater; Sediment
Baoliang Chen, Zhejiang University, Hangzhou, China
Soil pollution control and remediation; Traditional and novel functional materials and environmental applications (biochar, graphene, biosorbent, and organoclay); Sorption and reactions of organic and inorganic contaminants with natural and synthesised media; Novel membrane and pollutant abatement
Jianmin Chen, Fudan University, Shanghai, China
Gaseous and particulate air monitoring and chemistry (particularly urban); Secondary aerosol; Haze formation and fog chemistry; Human toxicity of atmospheric particulates; Aerosols and climate impacts
Frederic Coulon, Cranfield University, Cranfield, Bedfordshire, England, UK
Remediation, hazardous waste, water and wastewater treatment ; Risk assessment and remediation; Bioaerosols; Hydrocarbons; Environmental microbiology; Antarctic science
Adrian Covaci, University of Antwerp, Wilrijk, Belgium
Human exposure; Exposure assessment; Human health effects; Biomarkers; Food safety; Biomonitoring; Indoor pollution; Emerging contaminants; Legacy contaminants; Wastewater epidemiology
Xinbin Feng, Chinese Academy of Sciences (CAS), Guiyang, China
Mercury biogeochemical cycling in the environment and its health impact; Mercury stable isotope geochemistry and remediation of mercury contaminated lands; Cd, Pb, As and Sb biogeochemical cycling in the environment
José Virgílio Matos Figueira Cruz, University of the Azores, Ponta Delgada, Portugal
Groundwater geology; Groundwater geochemistry; Surface water chemistry; Water quality; Water pollution; Water management; Water planning
Pingqing Fu, Tianjin University, Tianjin, China
Organic aerosols; Atmospheric chemistry; Isotopes of atmospheric aerosols; Fog water; Ice-core organics; Dissolved organic matter; Biomarkers
Ashantha Goonetilleke, Queensland University of Technology, Brisbane, Queensland, Australia
Water quality; Water pollution; Water reuse; Water treatment; Stormwater pollutant processes; Integrated Water Resources Management; Water infrastructure resilience; climate change adaptation
Mae Sexauer Gustin, University of Nevada at Reno, Reno, Nevada, USA
Biogeochemical cycling of mercury, metals, and isotopes; Air pollution
Zhen (Jason) He, Virginia Tech, Blacksburg, Virginia, USA
Water pollution and treatment; Environmental biotechnology; Resource recovery from wastes; Bioelectrochemical systems; Bioenergy; Membrane technology; Bioremediation; Desalination
Patricia A. Holden, University of California, Santa Barbara, California, USA
Water quality; Environmental microbiology; Fecal pollution, Biodegradation and bioremediation; Soil pollution and soil processes; Nanomaterials; Wastewater treatment; Biogeochemistry; Emerging contaminants; Hydrocarbons; Metals
Henner Hollert, RWTH Aachen University (RWTH), Aachen, Germany

Bioanalytical environmental toxicology; Aquatic toxicology; Triad (Weight of evidence) approaches; Effect directed analysis; Sediments; In-situ investigations and monitoring; In-vitro bioassays; Waste- and ground water investigations (advanced wastewater treatment); Ecology
Deyi Hou, Tsinghua University, Beijing, China
Sustainability assessment; Life cycle assessment; Environmental footprint analysis; Risk management; Contaminated soil and groundwater remediation; Heavy metal contamination; Biochar production and application; Green synthesis of environmental functional materials; Fate and transport of volatile organic compounds in porous media
Ching-Hua Huang, Georgia Institute of Technology, Atlanta, Georgia, USA
Environmental chemistry; Water quality; Physicochemical treatment processes; Drinking water quality; Wastewater reuse; Contaminants of emerging concern; Reaction kinetics and mechanism
Wei Huang, Peking University, Beijing, China
Exposure assessment; Environmental epidemiology; Health intervention
G. Darrel Jenerette, University of California, Riverside, Riverside, California, USA
Land use/ land cover; Carbon and nitrogen cycling; Ecohydrology; Drylands; Urbanization; Spatial analysis; Remote sensing
Pavlos Kassomenos, University of Ioannina, Ioannina, Greece
Air pollution; Meteorology; Environmental health; Climate change; Particulates; Ozone; Bioaerosols; Dust transportation; Vehicle emissions; Noise
Ewa Korzeniewska, University of Warmia and Mazury, Olsztyn, Poland
Air pollution quality and human health; Contaminant (bio)monitoring and assessment; Ecotoxicology and risk assessment; Environmental management and policy; Human health risk assessment and management; Waste and water treatment; Antibiotic resistance; Biogas production
Ralf Ludwig, Ludwig-Maximilians-Universität München (LMU), Munich, Germany
Hydrology; Water resources management; Climate change; Land use change; Extreme events; Modeling; Remote sensing
Lidia Morawska, Queensland University of Technology, Brisbane, Queensland, Australia
Air pollution; Air quality; Indoor air pollution; Exposure assessment; Contaminated particulates; VOC; anthropogenic; Characterization; Automotive; Apportionment; Pollution transport; Monitoring: Analytical
Huu Hao Ngo, University of Technology Sydney, Sydney, New South Wales, Australia
Water and wastewater treatment and reuse technologies; Alternative water resources; Water management and impact assessment; Solid waste management; Specific green technologies; Water – waste – energy nexus; Greenhouse gas emission control and minimisation
Jose Julio Ortega-Calvo, Consejo Superior de Investigaciones Científicas (CSIC), Sevilla, Spain
Biodegradation and biotransformation of organic pollutants in soils and sediments; Bioremediation; Environmental microbiology; Bioavailability and persistence; Risk assessment
Wei Ouyang, Beijing Normal University, Beijing, China
Water environment and climate risk; Watershed environment management; Non-point source modeling and control; Diffuse pollution assessment
Elena Paoletti, National Research Council of Italy (CNR), Firenze, Italy
Plant health; Plant ecophysiology; Forests; Climate stressors; Air pollution impacts on terrestrial ecosystems; BVOC; Ground-level ozone
Paulo Alexandre da Silva Pereira, Mykolo Romerio universitetas (MRU), Vilnius, Lithuania
Soil degradation, Soil erosion, Soil processes, Forest Fires, Spatial Analysis, Mapping, Geostatistics, Ecosystem Services
Yolanda Picó, Universitat de València, Valencia, Spain
Media / Habitats: drinking water, water quality, water pollution, rivers, lakes, sediments, watersheds, soils, exposure assessment, human health effects, biomarkers, bioindicators, dietary exposure, food contamination, food safety; Human Health Effects: pesticides, endocrine disruptors, pharmaceutical residues, organics, analytical, surveys
Charlotte Poschenrieder, Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
Plant-Environment Interactions; Plant-Soil Relationships; Salinity; Plant- Microbe Interactions; Plant Toxicology; Crop Production; Plant Natural Adaptation
Sergi Sabater, Universitat de Girona, Girona, Spain
River and stream ecology; Biofilm ecology and ecotoxicology; Mediterranean; Water scarcity; Ecosystem functioning; Bioidiversity; Conservation of rivers
Scott C. Sheridan, Kent State University, Kent, Ohio, USA
Human biometeorology, climate change, synoptic climatology, extreme temperature events
Filip M.G. Tack, Universiteit Gent, Gent, Belgium
Heavy metals; Trace element biogeochemistry; Dredged materials; Soil and sediment remediation; Phytoremediation

Kevin Thomas, University of Queensland, Woolloongabba, Queensland, Australia
Contaminants of emerging concern; Non-target analysis; High resolution Mass Spectrometry; Microplastics; Biomonitoring
Daniel Tsang, The Hong Kong Polytechnic University, Hong Kong, China
Green chemistry/engineering; Soil/sediment remediation; Engineered biochar; Waste valorization; Resource recovery; Wastewater/stormwater treatment; Catalytic conversion/degradation; Pollutant transport; Environmental pollution
Paola Verlicchi, Università di Ferrara, Ferrara, Italy
Water treatment; Wastewater treatments; Reuse of reclaimed water; Occurrence and removal of pharmaceuticals from (waste)water; Hospital effluent management and treatment; Petrochemical wastewater treatment; Environmental risk assessment
Daniel A. Wunderlin, Universidad Nacional de Cordoba (Argentina), Córdoba, Argentina
Tracing pollutants from their source to foods; Food Integrity, including the evaluation of bioactive compounds in foods; Studying links between food production and environmental pollution
Shuzhen Zhang, Chinese Academy of Sciences (CAS), Beijing, China
soil contamination; Sorption/desorption of organic contaminants; Bioaccumulation and transformation of organic contaminants in the terrestrial environment; Applications of synchrotron-based spectroscopy techniques in environmental chemistry, NOM analysis and effects on contaminant behaviors

Editorial Board

Jésus R. Aboal Viñas, Universidade de Santiago de Compostela, Santiago de Compostella, Spain
Biomonitoring; Moss biomonitoring; Raptor biomonitoring; Algae biomonitoring; PAHs contamination; Heavy metal contamination; Cellular localization of metals; Hydrological fluxes of forest canopies
Souhail R. Al-Abed, U.S. Environmental Protection Agency (EPA), Cincinnati, Ohio, USA
Environmental implication and applications of nanomaterials; Sediment and water remediation; Contaminant (metals and organics) transformations in the environment; Reuse of materials in environmental applications
Alexandros G. Asimakopoulos, Norwegian University of Science & Technology NTNU, Trondheim, Norway
Takashi Azuma, Osaka University of Pharmaceutical Sciences, Osaka, Japan
Pharmaceuticals and personal care products; Hospital effluent; Water environment; Sewage treatment plant; Occurrence and environmental fate; Water treatment system; Water management; Environmental science; Environmental hygiene; Public health
Roya Bahreini, University of California, Riverside, Riverside, California, USA
Aerosol sources; Formation processes; Composition and microphysical properties; Direct and indirect effects on climate
Carlos Barata, IDAEA-CSIC, Barcelona, Spain
Analytical chemistry; Aquatic toxicology; Environmental risk assessment; Toxicogenomics
Roberto Bargagli, Università degli Studi di Siena, Siena, Italy
environmental biogeochemistry, active and passive biomonitoring of persistent contaminants in terrestrial and aquatic ecosystems
Georgios Bartzas, National Technical University of Athens (NTUA), Athens, Greece
Expertise in Waste management; Environmental monitoring and Risk assessment; Life cycle analysis; Soil and Groundwater decontamination; Geochemical/Thermodynamic modelling; Environmental economics
Ivan Bergier, EMBRAPA Brazil, Corumbá, Brazil
Expertise in sustainable development, particularly in the following areas: environmental services, ecology and biogeochemistry of ecosystems and agroecosystems; Bioenergy; Biofuels; Biochar; Remote sensing; Electron microscopy; Applied to nanotechnology, electronics and automation; Climate change adaptation; Mitigation of greenhouse gases emissions
Harald Biester, Technische Universität Braunschweig, Braunschweig, Germany
Biogeochemical cycling of mercury and trace elements; Biogeochemistry of peatlands
Jayanta Kumar Biswas, University of Kalyani, Kalyani, India
Environmental pollution; Water and soil contamination; Biomonitoring; Toxic metal(loid)s; Ecotoxicology; Bioremediation; Environmental microbiology; Ecological engineering; Ecotechnology; Nanobiotechnology; Wastewater treatment
Paul Bradley, U.S. Geological Survey (USGS), Columbia, South Carolina, USA
Drinking Water Exposure; Water Quality; Environmental and Public Health; Contaminants of emerging concern; Pharmaceuticals; Water Reuse; Remediation; Environmental microbiology; Urban and Aquatic Ecology
Satinder Brar Kaur, Institut National de la Recherche Scientifique (INRS), Québec, Quebec, Canada

Wastewater; Wastewater sludge; Treatment; Emerging contaminants; Antibiotics; Fermentation; Value-added bioproducts, such as enzymes, organic acids, platform chemicals, biocontrol agents, biopesticides, butanol and biohydrogen
Birgit Braune, Environment and Climate Change Canada, Ottawa, Ontario, Canada
Arctic, marine ecosystems, birds, metals, organo-compounds, biomonitoring, biological effects.
Bryan W. Brooks, Baylor University, Waco, Texas, USA
Water Quality, Environmental and Aquatic Eco- Toxicology, Risk and Hazard Assessment, Comparative Pharmacology and Toxicology, Environmental Public Health, Harmful Algal Blooms, Green and Sustainable Chemistry, Urban and Aquatic Ecology, Water Reuse.
Giorgio Buonanno, Università degli Studi di Cassino e del Lazio Meridionale, Cassino (FR), Italy
10.020: Air pollution; 10.030: Air quality; 10.040: Indoor air pollution; 70.040: Clean technologies; 80.050: Incineration
Joanna Burger, Rutgers University, Piscataway, New Jersey, USA
Eco-toxicology; Behaviour; Monitoring and assessment; Birds and reptiles
Glòria Caminal Saperas, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
Biochemical engineering; Environmental engineering (focused on biodegradation of pollutants by microorganisms or enzymes); Bioreactors; Immobilization; Kinetics
Art Chappelka, Auburn University, Auburn, Alabama, USA
Air pollution and global climate effects to terrestrial ecosystems; Native plant community responses (shifts in diversity) to air pollutants and global climate change; Plant-stress-air pollution/global climate change interactions; Urban ecology and ecosystem services
Da Chen, Jinan University, Gangzhou, China
Environmental chemistry; Analytical chemistry; Ecotoxicology; Persistent organic pollutants; Flame retardants; Pesticides; Mass spectrometry; Gas/liquid chormatography.
Wei Chen, Nankai University, Jinnan District, Tianjin, China
Nanoparticles; Nanomaterials; Adsorption; Reactivity; Transport; Remediation; Groundwater; Soil; Organic contaminants
Joaquín Cochero, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
Biofilm; Stream ecology; Biomonitoring; Urban streams; Citizen science
Xinyi (Lizzy) Cui, Nanjing University, Nanjing, China
Organics; Bioavailability
Rui da Silva Coutinho, Universidade Dos Açores, Ponta Delgada, Portugal
Hydrogeology, Volcanology, Natural Hazards, Water Resources Management, Environmental Geology.
Guido Del Moro, National Research Council of Italy (CNR), Bari, Italy
novel processes for wastewater treatment, aerobic granular biomass technologies, integration of chemical oxidation and biological processes for industrial wastewater, advanced oxidation processes, electro-degradation processes, wastewater treatment modelling
José L. Domingo, Universitat Rovira i Virgili, Reus, Catalonia, Spain
Environmental health; Risk assessment; Persistent organic pollutants; Metals; Food contaminants; Toxicology
Zhaozhong Feng, Chinese Academy of Sciences (CAS), Beijing, China
Air pollutant; BVOCs; Crop growth; Forest health; N deposition; N use and allocation; Ozone pollution; Photosynthesis and C cycle; Water use efficiency; Urban environment and forestry
Jose Angel Fernández, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
Air pollution;Air quality; Water pollution; Rivers; Ecological effects; Bioavailability; Bioindicators; Aquatic toxicology; Heavy metals; Biomagnification; Bioaccumulation; Surveys; Moss; Biomonitoring; Western Europe
Bo Gao, China Inst. of Water Resources and Hydropower (IWHR), Beijing, China
Geochemistry of trace metals in environment; Water and sediment transport; Large-scale watershed management
Alejandro García-Gil, Instituto Geológico y Minero de España (IGME), Zaragoza, Spain
Urban hydrogeology; Groundwater quality; Shallow geothermal exploitation impacts on water resources; Groundwater management; hydrogeochemistry; River-groundwater interaction; Groundwater flow and reactive transport numerical modelling; Groundwater microbiology; Enmerging organic contaminants
Jorge Gardea-Torresdey, University of Texas at El Paso, El Paso, Texas, USA
Applications of spectroscopy techniques in environmental chemistry; Phytoremediation; Novel methods for the bioproduction of nanoparticles; Development of analytical methods to detect nanomaterials; Study of the fate of nanoparticles in the environment; Applications of nanotechnology to clean water
Leobardo Manuel Gómez Oliván, Universidad Autónoma del Estado de México, Toluca, Mexico

Aquatic toxicology; Fish toxicity; Emerging contaminants; Metals; Genotoxicity; Citotoxicity; Embryotoxicity; Teratogenesis; Oxidative stress; Biomarkers
Daren Gooddy, British Geological Survey, Oxfordshire, England, UK
Groundwater; Biogeochemical cycles; Residence time indicators
Andrew Gray, University of California, Riverside, Riverside, California, USA
Sediment transport; Hydrology; Water quality; Plastic pollution; Watershed sediment dynamics; Sedimentology; Paleoenvironmental analysis
John Gulliver, University of Leicester, Leicester, England, UK
Noise and air pollution exposure assessment; Air pollution monitoring; Dispersion modelling; Land use regression modelling; Geographical information systems; Geo-statistical techniques (Kriging etc.); Spatial analysis of environmental and health data; Geographical studies of environment and health; Health risk assessments
Ying Guo, New York State Department of Health (NYSDOH), Albany, New York, USA
My research interests: (1) biomonitoring organic chemicals in human body, such as phthalates, PAHs, organophosphate pesticide and environmental phenols; (2) monitoring organic pollutants in environment, e.g., persistent organic pollutants; (3) Analytical method development for novel organic contaminants in various environmental matrix. Recently, I am working on Exposome to women with fertility problems.
Gary Hardiman, Queens University of Belfast, Belfast, UK
Computational biology; Epigenetics; Endocrine disruption; Systems biology; Biomarkers of exposure and human health risk assessment; Diagnostic tool development
Neil S. Harris, University of Alberta, Edmonton, Alberta, Canada
Expertise: cadmium, micronutrients, membrane transporters, trace metal uptake and translocation in plants
Gerard Hoek, Universiteit Utrecht, Utrecht, Netherlands
Exposure assessment; Air pollution modelling; Environmental epidemiology
Peter Hooda, Kingston University, Kingston upon Thames, England, UK
Biogeochemical Cycling of Nutrients and Environmental Contaminants; Catchment Water Quality; Land Degradation; Climate Change Impacts on Soil Processes; Emerging Contaminants
Kiril Hristovski, Arizona State University, Mesa, Arizona, USA
Nanomaterials; Water/Wastewater Quality and Treatment; Solid and Hazardous Waste; Developing Countries
Hafiz M. N. Iqbal, Instituto Tecnológico y de Estudios Superiores de Monterrey, Monterrey NL Mexico, Mexico
Environmental Engineering; Bioengineering; Biomedical Engineering; Bioremediation; Emerging contaminants; Wastewater treatment; Biomaterials; Bio-catalysis; Enzymes; Enzyme-based pollutant degradation; Immobilization; Toxic heavy elements; Liquid and solid waste management; Valorization of agro-industrial wastes and by-products
Rong Ji, Nanjing University, Nanjing, China
Organics; Terrestrial; Biodegradation; Environmental process; Radiotracer
Sunny Jiang, University of California, Irvine, California, USA
Pathogens; Water treatment; Membrane fouling; Microbial water quality; Risk assessments; Water reuse, Virus, bacteria
Weiying Jiang, California Environmental Protection Agency, Sacramento, California, USA
Organics; Pesticides; Dust; Analytics
Wei Jiang, Shandong University, Qingdao, China
Environmental risk of nanomaterials; Nano-bio interaction; Cell membrane damage; Cytotoxicity; Nanoparticle transport
Begoña Jiménez, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain
Persistent Organic Pollutants (POPs); Dioxins; PCBs; Fate of POPs; Contaminants of emerging concern; Organic pollutants in aquatic and terrestrial ecosystems; Bioindicators; Marine mammals; Air Pollution; Environmental chemistry; Monitoring
Sarah Jovan, Pacific Northwest Forest Inventory and Analysis (PNW-FIA), Portland, Oregon, USA
My greatest expertise is in using lichen community composition for monitoring and quantifying nitrogen pollutants. But I also work with lichen/moss tissue assays (for N, S, metals, PAHs), landscape-scale community-based gradient modeling more generally, and biomass modeling for ground-dwelling non-vascular communities in boreal and tundra systems.
Anna Jurado, Technische Universität Dresden, Dresden, Germany
Aquifer recharge quantification; Emerging organic contaminants; Greenhouse gases; Groundwater quality; Groundwater management; Urban groundwater; River-groundwater interaction; Managed aquifer recharge; Numerical modelling; Quantitative hydrogeology
Athanasios Katsogiannis, European Commission, Ispra (VA), Italy

Development and optimisation of analytical chemistry techniques and sampling methodologies to the source understanding; Occurrence and fate of organic contaminants in all environmental compartments, including indoor air, atmospheric air, soil, water and/or wastewater
Nerantzis Kazakis, Aristotle University of Thessaloniki, Thessaloniki, Greece
Groundwater modelling; Groundwater vulnerability; Hydrogeochemistry; Hydrogeophysics; Isotope hydrology; Water resources management; Floods; Climate change impacts on water resources; Managed Aquifer Recharge
M.B. Kirkham, Kansas State University, Manhattan, Kansas, USA
Soil-plant-water relations; Drought stress; Elevated carbon dioxide; Uptake of heavy metals by plants
Charles Knapp, University of Strathclyde, Glasgow, Scotland, UK
Microbial ecology; Bacteria; Microorganisms; Wastewater; Surface water; Nutrients; Eutrophication; Antibiotic resistance; Antimicrobial resistance; Molecular ecology
Dana Kolpin, U.S. Geological Survey (USGS), Iowa City, Iowa, USA
Endocrine disruptors; Pharmaceutical residues; Non-point; Pollution transport; Chemical transport
Prashant Kumar, University of Surrey, Surrey, England, UK
Air quality and health; Airborne ultrafine and nanoparticles; Exposure assessment; Low-cost pollution sensing; Exhaust and non-exhaust emissions; Air pollution control; Grey-grey infrastructure interactions; Indoor air quality; Dispersion modelling; Urban nexus; Future cities/megacities
Keisuke Kuroda, National Institute for Environmental Studies, Fukushima, Japan
Subsurface geochemistry and mitigation technologies of contaminants of emerging concern (CECs)
James Lam, The Education University of Hong Kong, Tai Po, New Territories, Hong Kong
POPs; Emerging contaminants; Risk assessment
Dimitra Lambropoulou, Aristotle University of Thessaloniki, Thessaloniki, Greece
Emerging Contaminants, Organic Pollutants, Transformation Products, Environmental fate, Sample preparation and analysis, Advanced mass spectrometry techniques, Environmental monitoring and risk assessment, water quality, Treatment processes for water and wastewaters
Juying Li, Shenzhen University, Shenzhen, Guangdong, China
Organics; Bioavailability; Isotopes; Analysis; Degradation; Soil-plant system; Transformation; Toxicity
Shibin Li, Syngenta Crop Protection, Greensboro, North Carolina, USA
Environmental toxicology; Regulatory toxicology; Ecotoxicology; Exposure science; Risk assessment; Product safety
Daohui Lin, Zhejiang University, Hangzhou, China
Nanomaterials; Ecotoxicity; Nanotoxicity; Bioavailability; Colloidal behavior; Sorption
Kunde Lin, Xiamen University, Xiamen City, Fujian 361102, China
Organic contaminants; Active sampler
Xiaobo Liu, The University of Hong Kong, Hong Kong SAR, China
Microbial biofilms; Biocatalysis for biosynthesis; Biodegradation of cultural heritages; Microbial electrochemistry; Extracellular electron transfer; Bacterial syntrophy; Environmental microbiology; Fermentation engineering; Biofuel & biomass; Food microbiology and processing; Microbial ecology
Rasha Maal-Bared, EPCOR Water Services, Edmonton, Alberta, Canada
Applied and environmental microbiology; Freshwater microbiology; Drinking water and wastewater; Microorganisms; Pathogens; Biofilms; Antibiotic resistance; Water quality; Water pollution; Food safety; Monitoring
Sheila Macfie, Western University, London, Ontario, Canada
Metal toxicity in plants; Metal localization in plants; Rhizosphere chemistry
Sonia Manzo, ENEA, Portici, Italy
Ecotoxicology; Nanomaterials; Aquatic environment; Seawater; Microalgae; Seaurchin; Risk assessment
Adriaan Albert Markus, Deltares, Delft, Netherlands
Water quality modelling; Numerical modelling and programming in various languages (notably Fortran, in relation to numerical modelling); Transport and fate of nanoparticles and microplastics in the aquatic environment
Ioannis Matiatos, International Atomic Energy Agency (IAEA), Vienna, Austria
Isotope hydrology; Water resources management; Hydrogeochemistry; Groundwater modeling; Applied statistical modeling; Climate change impact; Environmental monitoring; Water quality
Janine McCartney, HHC Services Inc., Lester, Pennsylvania, USA
Chemical Exposures: Toxic tort, Biomarkers, Industrial Hygiene, Employee chemical exposures and community chemical exposures, Safety Engineering; Arc Flash Analyses and Accidents; Electrical Safety; Falls; Equipment & Machinery; Human Factors; Accident Investigation/ Reconstruction; OSHA; Guarding; Construction; Industrial & Premises Accidents; Oil & Gas Extraction; Pipeline Safety and Refinery Safety; Lead and Electrocution

Thomas Meinelt, Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
Alternative treatments in aquaculture; Impact (and interaction) of humic substances on environment and animals.
Derek Muir, Environment and Climate Change Canada, Burlington, Ontario, Canada
Environmental chemistry; Biogeochemistry; Bioaccumulation; Persistent organic pollutants; Chemicals of emerging concern; Chemical inventories; Mercury; Polycyclic aromatic compounds; Arctic; Marine mammals; Fish
Jacek Namieśnik, Technical University of Gdansk, Gdansk, Poland
Environmental analytics and monitoring; Food analysis; QA/QC systems; Green analytical chemistry; Envirometrics
Howard S. Neufeld, Appalachian State University, Boone, North Carolina, USA
The effects of ozone on plants; The role of anthocyanins in vegetative tissues in plants; Climate change impacts on plants in the southern Appalachian mountains; Measuring plant gas exchange and plant water relations, using the Li-Cor 6400 and 6800 gas exchange systems, a Sperry hydraulic conductivity apparatus and Scholander pressure chamber, as well as a variety of other instrumentation (including leaf fluorescence meter) to monitor plant responses to environmental stresses
Hong-Gang Ni, Peking University, Shenzhen, China
Organic pollutants (persistent organic pollutants and environmental molecular markers); Environmental model (process and impact); Human exposure and health risk.
Avelino Núñez-Delgado, Universidade de Santiago de Compostela, Lugo, Spain
Diffuse pollution; Emerging pollutants; Sorption and desorption; Waste recycling; Water treatment systems
Fernando Pacheco-Torgal, University of Minho, Guimarães, Portugal
Eco-efficient construction and building materials; Construction and demolition wastes; Geopolymers; Waste recycling; Durability; Mechanical properties; Alkali-activated cement-based binders; Concrete nanotechnology
Anastasia K. Paschalidou, Democritus University of Thrace, Orestiada, Greece
Air pollution meteorology; Urban meteorology ; Dust transportation; Climate change; Environmental health / Environmental epidemiology; Biometeorology; Synoptic climatology; Dispersion Modeling; Air Quality Indices
Momir Paunovic, University of Belgrade, Beograd, Serbia
Hydrobiology; Aquatic macroinvertebrates; Freshwater mollusks; Invasive aquatic species; Feeding of benthivorous fish; Functional analyses of aquatic ecosystems; Relation of aquatic biota and environmental variables; Bio-monitoring in freshwater; Genotoxicological investigations on aquatic organisms; Microbiology of freshwaters
Alexandra Pavlidou, Hellenic Centre for Marine Research, Mavro Lithari, Anavyssos, Greece
Eutrophication and eutrophication indexes according to WFD and MSFD; Biogeochemical cycles and nutrient dynamics in marine environments (coastal and open sea)
Alexandre R. Péry, AgroParisTech, Paris, France
Toxicokinetic modelling; Toxicodynamic modelling; Ecotoxicology; Mixtures; Integrated risk assessment
Clemens Reimann, Norges geologiske undersokelse - NGU, Trondheim, Norway
Geochemistry; Environmental Geochemistry; Biogeochemistry; Hydrogeochemistry; Regional Geochemistry; Geochemical mapping; Critical Zone Research; Soil chemistry
Tiina Reponen, University of Cincinnati, Cincinnati, Ohio, USA
Indoor air pollution; Exposure assessment; Bacteria; Fungi; Microorganisms; Microbiome; Biohazards; Monitoring
Robert Risebrough
Anacleto Rizzo, IRIDRA, Florence, Italy
Constructed Wetland; Nature-Based Solution for Wastewater Treatment; Sustainable Water Management; Sustainable Sanitation Modelling; Sustainable Urban Drainage Systems; Water Sensitive Urban Design; Low Impact Development; Green Infrastructure; Ecosystem Service
Teresa Rocha-Santos, Universidade de Aveiro, Aveiro, Portugal
Micro(nano)plastic; Plastic; Microfibres; Organic contaminants; Marine monitoring; Environmental monitoring; Wastewater treatment; Biodegradation of microplastics; Sensors; Biosensors
David Roser, UNSW Australia, Sydney, New South Wales, Australia
Mª Jesús Sánchez-Martín, IRNASA, CSIC, Salamanca, Spain
Pesticides, soil, water, organic amendments; Adsorption, desorption, degradation, mobility; Soil and water contamination by pesticides and emerging pollutants; Behaviour of pesticides in soils; Influence of organic amendments
Nan Sang, Shanxi University, Taiyuan, Shanxi, China

Environmental exposure and health risk of chemicals; Biological effect and toxic mechanism of environmental chemicals
Ralf Bernhard Schäfer, Universität Koblenz-Landau, Landau, Germany
Water quality; Rivers; Ecological effects; Chemicals; Aquatic toxicology; Invertebrates; Microorganisms; Modelling; Statistics
Jianwen She, California Department of Public Health, Richmond, California, USA
Environmental analysis; Persistent organic chemical analysis; Biomonitoring; Source apportionment; Non target analysis; Endocrine disruptors; Mass spectrometry
Wei Shi, Nanjing University, Nanjing, China
Environmental fate of emerging organic pollutants; Effect directed analysis based on instrumental analysis and bioassays
Andreas Skouloudis
Athanasios S. Stasinakis, University of the Aegean, Mytilene, Greece
wastewater treatment and reuse; Sludge management; Emerging contaminants; Aquatic pollution; Biodegradation; Ecotoxicity; Risk assessment
Marianne Stuart, British Geological Survey, Wallingford, England, UK
Groundwater pollution; Agrochemicals; Emerging contaminants in groundwater; Industrial contaminants in groundwater; Shale gas exploitation
Qian Sui, East China University of Science and Technology, Shanghai, China
Pharmaceuticals and personal care products; Micro-plastics; Emerging contaminants; Analytical methods; Environmental behaviors; Source apportionment; Advanced oxidation processes; Treatment processes
Piotr Szefer, Medical University of Gdańsk, Gdańsk, Poland
Biomagnification of major and minor elements along the sequential trophic levels of the marine biosphere; Bioavailability of metallic pollutants to benthic organisms as potential biomonitors in relation to the adjacent sediments and sea water; Analytical and chemometric assessment of food quality
Phong Thai, University of Queensland, Woolloongabba, Queensland, Australia
Wastewater analysis; Sewer-based epidemiology; Air quality monitoring; Air pollution epidemiology; Environmental monitoring
Maria Concetta Tomei, Consiglio Nazionale delle Ricerche (CNR), Rome, Italy
Processes and Technologies for Urban and Industrial Wastewater Treatment; Modelling and Control of Biological Processes, Removal of Xenobiotic Compounds, Two-Phase Partitioning Bioreactors (TPPBs); Sludge Treatment; Soil Bioremediation
Ashley Townsend, University of Tasmania, Hobart, Tasmania, Australia
Environmental analysis; Geochemistry; Oceanography; Marine and Antarctic science; Materials science; Human health areas
Richard Van Curen, University of California, Davis, Davis, California, USA
Aerosol Science, atmospheric pollution, climate science, atmospheric modeling
Fang Wang, Chinese Academy of Sciences (CAS), Nanjing, China
Soil pollution and remediation; Persistent organic pollutants; Polycyclic aromatic compounds; Antibiotics; Antibiotic resistance; Phthalate ester; Emerging Contaminants; Biochar; Bioavailability; Biodegradation and biotransformation of organic pollutants; Biofilms; Signaling molecules; Analytical method; Environmental monitoring
Wei (Vivienne) Wang, Zhejiang University, Hangzhou, China
Radio-isotopic tracing and photographing; Pesticides; Organic pollutants; Bioavailability; Degradation; Metabolism: chemical analysis
Xiaoping Wang, Chinese Academy of Sciences (CAS), Beijing, China
Global cycling of POPs; Mechanism of long range atmospheric transport; POPs accumulation in polar region; Risk assessment of POPs, Brown carbon; Emerging contaminants;Tibet Plateau
Shaun Watmough, Trent University, Peterborough, Ontario, Canada
Ecosystem biogeochemistry; ecological impact of trace metals; ecosystem acidification; air pollution impacts on ecosystems
Jianming Xue, New Zealand Forest Research Institute Ltd., Christchurch, New Zealand
Biowaste and wastewater reuse; Emerging contaminants in biowaste and soil; Fate and transport of contaminants in terrestrial ecosystems; Antibiotic pollution and remediation; Biochar for environmental management; Plant uptake and translocation of contaminants; Plant-soil-microbe interactions; Phytoremediation of contaminated soils and water; Biowaste management and climate change
Ishwar Chandra Yadav, Tokyo University of Agriculture and Technology, Tokyo, Japan
Persistent organic pollutants; Brominated and phosphate flame retardants; Heavy metal pollution; Aerosols; South Asia; PM2.5; Solid waste; E-waste; Himalayas

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Aims and Scope

Science of the Total Environment is an international journal for publication of original research on the **total environment**, which includes the **atmosphere**, **hydrosphere**, **biosphere**, **lithosphere**, and **anthroposphere**.

totalenvironment.gif-Total Environment

The total environment is characterized where these five spheres overlap. Studies that focus on at least two or three of these will be given primary consideration. Papers reporting results from only one sphere will not be considered. Field studies are given priority over laboratory studies. The total environment is studied when data are collected and described from these five spheres. By definition total environment studies must be multidisciplinary.

Examples of data from the five spheres are given below:

stoten-banners.jpg-The five spheres of the total environment

Subject areas may include, but are not limited to:

- Agriculture, forestry, land use and management
- Air pollution quality and human health
- Contaminant (bio)monitoring and assessment
- Ecosystem services and life cycle assessments
- Ecotoxicology and risk assessment
- Emerging fields including global change and contaminants
- Environmental management and policy
- Environmental remediation
- Environmental sources, processes and global cycling
- Groundwater hydrogeochemistry and modeling
- Human health risk assessment and management
- Nanomaterials in the environment
- Noise in the environment
- Persistent organic pollutants
- Plant science and toxicology
- Remote sensing
- Stress ecology in marine, freshwater and terrestrial ecosystems
- Trace metals and organics in biogeochemical cycles
- Waste and water treatment

The editors discourage submission of papers which describe results from routine surveys or monitoring programs, studies which are local in scope, laboratory experiments, hydroponic or pot studies measuring biochemical/physiological endpoints, food science studies, screening of new plant species for phytoremediation, testing known chemicals in another setting, and experimental studies lacking a testable hypothesis.

The abstract, highlights and conclusions of papers in this journal must contain clear and concise statements as to why the study was done and how readers will benefit from the results. Articles submitted for publication in *Science of the Total Environment* should establish connections among research findings with implications for environmental quality, ecological health, and/or human health.

Types of paper

Full papers reporting original and previously unpublished work.

Short Communications. A brief communication of urgent matter or the reporting of preliminary findings to be given expedited publication.

Letters to the Editor. A written discussion of papers published in the journal. Letters are accepted on the basis of new insights on the particular topic, relevance to the published paper and timeliness.

Reviews. Critical evaluation of existing data, defined topics or emerging fields of investigation, critical issues of public concern.

Discussion. Opinionated exposition on an important scientific issue or event designed to stimulate further discussion in a broader scientific forum.

Special Issues. Proceedings of symposia, workshops and/or conferences will be considered for publication as a special issue. An Editor or Associate Editor should be contacted early in the conference planning process to get approval and for guidelines on special issues of the journal.

Book Reviews will be included in the Journal on a range of relevant books which are not more than two years old. Book reviews are handled by the Journal Editors. Unsolicited reviews will not usually be accepted, but suggestions for appropriate books for review may be sent to one of the Editors.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Policy and ethics

It is understood that with submission of this article the authors have complied with the institutional policies governing the humane and ethical treatment of the experimental subjects, and that they are willing to share the original data and materials if so requested.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also https://www.elsevier.com/conflictsofinterest.

Editors likewise require reviewers to disclose current or recent association with authors and any other special interest in this work.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies
Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Subscription

- Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
- No open access publication fee payable by authors.
- The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peer-reviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

- Articles are freely available to both subscribers and the wider public with permitted reuse.
- A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The gold open access publication fee for this journal is **USD 3400**, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access
Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 24 months.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Submission

Authors may submit their articles electronically to this journal. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to a PDF file at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail, removing the need for a paper trail.
Note that contributions may be either submitted online or sent by mail. Please do NOT submit via both routes. This will cause confusion and may lead to your article being reviewed and published twice!

For any technical queries please visit our Support Center.

Cover Letter
The corresponding author must state explicitly in a paragraph how the paper fits the Aims and Scope of the journal. Failure to include the paragraph will result in returning the paper to the author.

Referees

All authors must suggest five potential reviewers for their paper upon submission (please include **institutional email addresses ONLY** for all reviewers).
The suggested referees should: (i) not be close collaborators of the authors(s)(ii) not be located in the same institution as the author(s) and(iii) not all be from the home country.Avoid suggesting colleagues you have published with previously as this creates a potential conflict of interest. Do not suggest any Editor of this journal.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Please ensure that your manuscript file contains line numbering. Line numbers should be included manually before uploading your files.

Figures and tables

Please submit Figures and Tables in separate files in an approved format (TIFF, EPS or MS Office files) with the correct resolution.

Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Manuscript Page Limit

There is no restriction on the number of pages but brevity of papers is greatly encouraged. The length of a paper should be commensurate with the scientific information being reported. In particular, the introductory material should be limited to a few paragraphs and results presented in figures should not be repeated in tables.

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- ***Title.*** Be concise and informative. Titles are often used in information-retrieval systems. Acronyms and brand names of products should not appear in the title of a paper. Instead they may be listed in the key words, and spelled out the first time they appear in the body of the paper.
- ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **The inclusion of multiple corresponding authors is strongly discouraged.Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
- ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images also in accordance with all technical requirements.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

The mandatory highlights are important because they appear online in the Table of Contents of the journal. Highlights that list bullet points about the results are therefore not very informative for readers scanning the contents. Here is an outline of what the highlights should contain: What is the overall scientific problem and why did you study it? How did you address the problem, and which spheres are included? What was the major method used? Major finding(s) Take home message Do not repeat the highlights in bullet form for the conclusions. The conclusions should be a narrative about what you found and what it means in the broader scheme.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

The key words of the paper should not contain any words already in the title, but can include abbreviated terms or location information not suitable for the title.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.

- Indicate per figure if it is a single, 1.5 or 2-column fitting image.
- For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
- Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
- Supply files that are too low in resolution.
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Number tables consecutively with Arabic numerals in accordance with their appearance in the text. Type each table double-spaced on a separate page with a short descriptive title typed directly above and place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Tables should never be included within the text, because file(s) containing tables are attached separately in the electronic submission system.

Please submit Figures and Tables in separate files in an approved format (TIFF, EPS or MS Office files) with the correct resolution.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript. More information on how to remove field codes from different reference management software.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/science-of-the-total-environment
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/ book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown ...'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:

Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Mendeley Data
This journal supports Mendeley Data, enabling you to deposit any research data (including raw and processed data, video, code, software, algorithms, protocols, and methods) associated with your manuscript in a free-to-use, open access repository. During the submission process, after uploading your manuscript, you will have the opportunity to upload your relevant datasets directly to *Mendeley Data*. The datasets will be listed and directly accessible to readers next to your published article online.

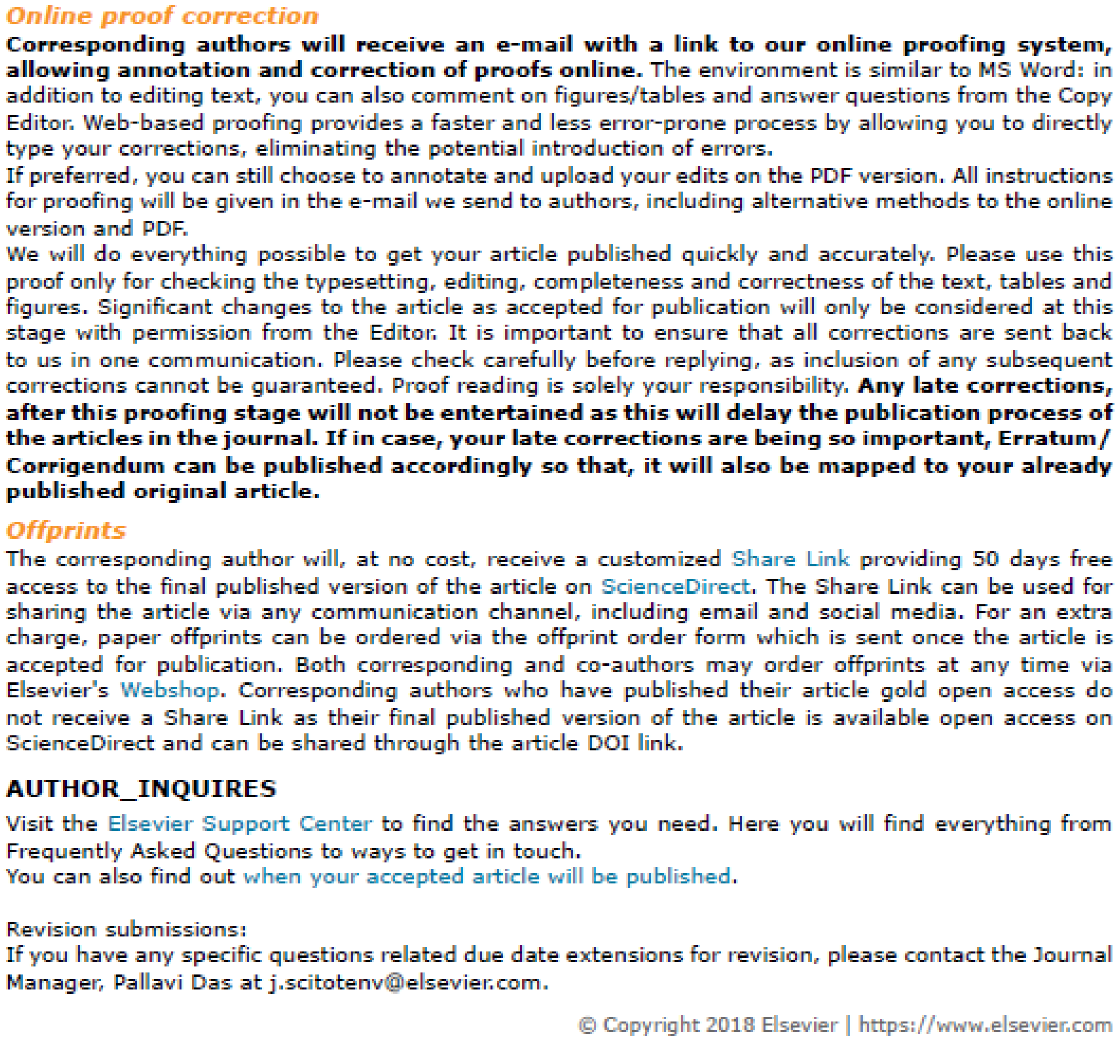
For more information, visit the Mendeley Data for journals page.

Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee of 500 USD is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

MethodsX
You have the option of converting relevant protocols and methods into one or multiple MethodsX articles, a new kind of article that describes the details of customized research methods. Many researchers spend a significant amount of time on developing methods to fit their specific needs or setting, but often without getting credit for this part of their work. MethodsX, an open access journal, now publishes this information in order to make it searchable, peer reviewed, citable and reproducible. Authors are encouraged to submit their MethodsX article as an additional item directly alongside the revised version of their manuscript. If your research article is accepted, your methods article will automatically be transferred over to MethodsX where it will be editorially reviewed. Please note an open access fee is payable for publication in MethodsX. Full details can be found on the MethodsX website. Please use this template to prepare your MethodsX article.

Data statement
To foster transparency, we encourage you to state the availability of your data in your submission. This may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you will have the opportunity to indicate why during the submission process, for example by stating that the research data is confidential. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page.

AFTER ACCEPTANCE



Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proof reading is solely your responsibility. **Any late corrections, after this proofing stage will not be entertained as this will delay the publication process of the articles in the journal. If in case, your late corrections are being so important, Erratum/ Corrigendum can be published accordingly so that, it will also be mapped to your already published original article.**

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR_INQUIRES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also find out when your accepted article will be published.

Revision submissions:

If you have any specific questions related due date extensions for revision, please contact the Journal Manager, Pallavi Das at j.scitotenv@elsevier.com.

AUTHOR's BIOGRAPHY

General Information

Name: Angel Sánchez Zubieta
Birth place: Tenosique, Tabasco, Mexico
Parents: Maria Dolores Zubieta Valenzuela and Ignacio Sánchez Velázquez

Academics

Period	Grade	Institution
February 2020	PhD in Animal Science	Federal University of Río Grande do Sul, Brazil.
June 2013	Specialization on Tropical Livestock Management	Instituto de Ciencia Animal, La Habana, Cuba.
August 2008	Master in Animal Science	Instituto Tecnológico de Conkal, Yucatán, Mexico.
January 2004	Bachelors in Agronomy Engineering	Instituto Tecnológico Agropecuario # 2, Yucatán, Mexico.

Professional experience:

- Researcher at the National Institute of Forestry and Agricultural Research (INIFAP), Yucatán México, assigned to the "Ruminant meat research program". Period: October 2008 to December 2012.

Recent Publications:

- **Paper.** Faniyi, T.O., Prates, Ê.R., Adegbeye, M.J., Adewumi, M.K., Elghandour, M.M., Salem, A.Z., Ritt, L.A., **Zubieta, A.S**., Stella, L., Ticiani, E. and Jack, A.A., 2019. Prediction of biogas and pressure from rumen fermentation using plant extracts to enhance biodigestibility and mitigate biogases. *Environmental Science and Pollution Research*, *26*(26), pp.27043-27051.
- **Book Chapter.** Carvalho, P.C.F; Wallau, M.O; Albuquerque, P.A; Szymczak, L.S; **Zubieta, A.S;** Savian, J.V; Moraes, A. 2019. Forrageiras de clima temperado. In: Plantas forrageiras. Universidade Federal de Vicosa
- **Conference paper.** Carvalho, P. C. F; Bremm, C; Savian, J. V; **Zubieta, A. S**; Szymczak, L. S; Marin, A; Neto, J. F. S; Schons, R. M. T; Moraes, A; dos Santos, D. T. and Bindelle, J. 2017. Como otimizar a ingestão de forragem por vacas leiteiras em pastejo? Proccedings of VIII Simpósio Mineiro de Gado de Leite – III Simpósio Nacional de Produção e Nutrição de Gado de Leite, 10 a 11/11/ 2017 – Uberlândia – MG.

Conferences:

- How can grazing management mitigate enteric CH_4 emissions by grazing ruminants? VIII International Seminar, UNIJUÍ, Ijuí, Brazil, September 2018.

Courses and workshops:

- The SF_6 methodology to assess enteric CH_4 emissions by grazing ruminants. Santiago de Chile, June 2018.

International experience (research group/place):

- Grazing Ecology Research Group, Federal University of Rio Grande do Sul, Brazil.
- Agro-Bio Tech, Liège University, Belgium.
- Laboratory of Animal Nutrition and Ecology, Ghent University, Belgium.
- Instituto de Ciencia Animal (ICA), Cuba.