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

# ASSESSING THE PUT & TAKE, FENCING AND TOPPING TECHNIQUES TO CONTROL SWARD STRUCTURE UNDER CONTINUOUS STOCKING

Thainá Silva de Freitas

Porto Alegre Junho de 2021 Thainá Silva de Freitas

# ASSESSING THE PUT & TAKE, FENCING AND TOPPING TECHNIQUES TO CONTROL SWARD STRUCTURE UNDER CONTINUOUS STOCKING

Dissertação apresentada como requisito para obtenção do Grau de Mestre em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul. Orientador: Paulo César de Faccio Carvalho Coorientador: Jean Víctor Savian

**Porto Alegre** 

2021

CIP - Catalogação na Publicação

de Freitas, Thainá Silva ASSESSING THE PUT & TAKE, FENCING AND TOPPING TECHNIQUES TO CONTROL SWARD STRUCTURE UNDER CONTINUOUS STOCKING / Thainá Silva de Freitas. -- 2021. 71 f. Orientador: Paulo César de Faccio Carvalho. Coorientador: Jean Víctor Savian. Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2021. 1. manejo de pastagens. 2. altura do pasto. 3. desempenho animal. 4. método de lotação. 5. ovinos. I. Carvalho, Paulo César de Faccio, orient. II. Savian, Jean Víctor, coorient. III. Título.

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

Thainá Silva de Freitas Zootecnista

# DISSERTAÇÃO

Submetida como parte dos requisitos para obtenção do Grau de

## MESTRE EM ZOOTECNIA

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

Aprovada em: 08.06.21 Pela Banca Examinadora

PAULO CÉSAR DE FACCIO CARVALHO PPG Zootecnia/UFRGS Orientador

Homologado em: 23/07/2021 Por

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

Jérôme José Bindelle University of Liège

André Fischer Sbrissia Universidade do Estado de Santa Catarina

Julio Ricardo Galli Universidad Nacional de Rosario

CARLOS ALBERTO BISSANI

Diretor da Faculdade de Agronomia

### ACKNOWLEDGEMENTS

First of all, I thank God for his grace and mercy in my life.

I thank my parents, Débora and Pedro Augusto, I will never have enough words to show my gratitude. I will honor your teachings throughout my life. Thanks to João and Isadora. Their lives motivate and inspire me to see life through different eyes daily.

Thanks to my family for understanding my absence and mood swings during this period.

Special thanks to Pedro Henrique, for his love, care e friendship. More than loving you, I admire the virtues and values that you have. I also thank the Sessegolo Ferzola family for their support.

My thanks to Paulo, my advisor, for his time, patience, trust in my work, opportunities and for building and leading this great team. I am very proud to be part of its history.

Thanks to my co-advisor, Jean, for his teachings, friendship and support throughout this period.

Special thanks to Lívia for her dedication and friendship in conducting the experiment. I am honored to share this very important part of our professional lives with you. Thanks to Laís and Arthur for their friendship. You can always count on me!

Thanks to all the members of the GPEP who helped us with the experiment, whether in the field, tabulating data, or by making budgets.

Thanks to the interns from outside UFRGS who contributed to the realization of this experiment and made our days in the field happy: Franco, Édena, Raquel, Carol, Beatriz, Leo, Eduarda, Junior, Mirela, Ravine, Hugo and Renan.

Thanks to Jusi and Aleja for their willingness to help and for the essential comments on the statistical analysis and writing of this dissertation.

I would also like to thank UFRGS, the Agronomic Experimental Station and its professors and staffs.

Thanks to CAPES for granting the scholarship.

To each one who donated their time to this project, thank you very much!

# ASSESSING THE PUT & TAKE, FENCING AND TOPPING TECHNIQUES TO CONTROL SWARD STRUCTURE UNDER CONTINUOUS STOCKING

Author: Thainá Silva de Freitas Advisor: Paulo César de Faccio Carvalho Co-advisor: Jean Victor Savian

# ABSTRACT

'Rotatinuous' is a grazing management concept based on animal behavioral responses to sward structure, to minimize time and consequently maximize herbage intake per unit of grazing time. The application of this strategy under rotational stocking is already known and is eased by the proposal of the control of the pre- and post- grazing sward heights. On the other hand, the application of the concept under continuous stocking can be a challenge. The objective of this dissertation was to evaluate if management interventions on Italian ryegrass, to offer an ideal sward structure, affect sheep and herbage production under continuous stocking. The experiment was carried out in 2019, in a randomized complete block design with three grazing intervention strategies and three replicates, in southern Brazil. All treatments were managed targeting sward height at 15 cm and also had their stocking rate adjustments by put-and-take technique. On T1 treatment just put-and-take; T2 treatment use of fences to concentrate or exclude animals in under- and overgrazed areas, respectively; T3 treatment use of fences in overgrazed areas and the under-grazed areas were mechanically topped. Twenty-seven castrated lambs with an average live weight of 32  $\pm$  2 kg were used. The actual average sward heights were 14.5, 14.7 and 14.9 cm (P = 0.286) for T1, T2 e T3, respectively. Despite the average sward heights that did not differ statistically, the sward height distribution of T1 presented greater heterogeneity (Gini coefficient = 0.273) than T2 (0.227) and T3 (0.248). There were no differences (P > 0.05) for any of the other pasture variables neither animal. In conclusion, our results demonstrated that in the attempt to offer ideal sward structures — using deferment in under-grazed areas and topping or concentration of animals in overgrazed areas — the evaluated techniques just homogeneized sward height distribution, with no detectable benefits to pasture nor animal production. Considering the limits of the experimental spatio and temporal scales, in addition of managing swards at the optimal average sward height, the stocking rate adjustment is enough to maximize animal intake and performance.

**Keywords:** grazing management; sward height; animal performance; stocking method; heterogeneity; sheep

<sup>1</sup>Master dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul,

Porto Alegre, RS, Brazil. (71 p.) June, 2021.

# AVALIAÇÃO DAS TÉCNICAS DE *PUT & TAKE*, CERCA E ROÇADA PARA CONTROLE DA ESTRUTURA DO PASTO SOB PASTOREIO CONTÍNUO

Autora: Thainá Silva de Freitas Orientador: Paulo César de Faccio Carvalho Co-orientador: Jean Victor Savian

## RESUMO

O "Rotatínuo" é um conceito de manejo do pasto baseado nas respostas comportamentais dos animais à estrutura do pasto, para minimizar o tempo e consequentemente maximizar o consumo de pastagem por unidade de tempo pastejado. A aplicação desta estratégia sob o pastoreio rotativo já é conhecida e é facilitada pela proposta de controle de alturas de pré e pós-pastejo. Por outro lado, a aplicação deste conceito sob o pastoreio contínuo pode ser um desafio. O objetivo desta dissertação foi avaliar se intervenções, para oferecer estruturas de pasto ideais, afetam a produção vegetal, o consumo e o desempenho de ovinos sob pastagem de azevém em pastoreio contínuo. O experimento foi conduzido em 2019, em um design experimental em blocos completamente casualizados com três estratégias de manipulação do pasto e três repetições, no sul do Brasil. Todos os tratamentos foram manejados com o objetivo de manter o pasto na média de 15 cm de altura e tiveram o seu ajuste de carga pela técnica do *put-and-take*. No tratamento T1 foi utilizado somente *put-and-take*; no tratamento T2 teve uso de cercas para concentrar ou excluir animais de áreas sub ou super pastejadas, respectivamente; no tratamento T3 teve uso de cercas para isolar áreas super pastejadas e de uma rocadeira costal para cortar o pasto nas áreas sub-pastejadas. Vinte e sete cordeiros castrados com peso vivo médio de 32 ± 2 kg foram utilizados. As alturas médias reais do pasto foram 14,5; 14,7 e 14,9 cm (P = 0,286) para T1, T2 e T3, respectivamente. Apesar de não haver diferença estatística significativa para as alturas de pastagem, a distribuição das alturas do T1 apresentou um maior grau de heterogeneidade (coeficiente de Gini = 0,273) do que T2 (0,227) e T3 (0,248). Não houve diferença estatística (P > 0,05) para nenhuma outra variável do pasto. Além disso, o desempenho animal e o consumo de forragem pelos ovinos não diferiram entre os tratamentos (P > 0,05). Em conclusão, nossos resultados demonstram que a tentativa de oferecer estruturas de pasto ideais - utilizando diferimento em áreas sub-pastejadas e rocada ou concentração de animais em áreas super pastejadas — as técnicas avaliadas apenas homogeneizaram as ditribuições de altura do pasto, sem benefícios encontrados para a produção vegetal nem animal. Considerando os limites das escalas espacial e temporal do experimento, juntamente com o manejo do pasto em uma média de altura ótima, o ajuste de lotação é suficiente para maximizar o consumo e a performance animal.

**Palavras-chave:** manejo de pastagens; altura do pasto; desempenho animal; método de lotação; heterogeneidade; ovinos

# LIST OF FIGURES

# CHAPTER I

**Figure 1** — Spatial and temporal scale of grazing (adapted from Bailey et al. 1996; Bailey e Provenza 2008)

# CHAPTER II

**Figure 1.** Illustration of grazing intervention strategies treatments. All treatments had stocking rate adjustments. (T1) Put-and-take treatment uses just animals for regulating average sward height; (T2) Put-and-take plus fence treatment had deferment of overgrazed areas (<12cm) illustrated by the red X and concentration of animals in areas undergrazed (>18cm) illustrated by the sheep; (T3) Put-and-take plus fence plus mechanical topping treatment had areas deferred when overgrazed (<12 cm) illustrated by the red X and undergrazed areas were topped with a string trimmer (>18 cm) identified by the grey color.

**Figure 2.** Gini coefficient density of Italian ryegrass sward height distribution managed under continuous stocking and grazed by sheep under different grazing intervention strategies. (T1) Put-and-take treatment uses just animals for regulating average sward height; (T2) Put-and-take plus fence treatment had deferment of over-grazed areas (<12cm) and concentration of animals in areas under-grazed (>18cm); (T3) Put-and-take plus fence plus mechanical topping treatment areas were also isolated when over-grazed (<12 cm) and were topped with a string trimmer when under-grazed (>18 cm). Means sward height are plotted to the right of each treatment curve and do not differ (P = 0.2867). Different letters differ significantly at 0.05.

**Figure 3.** Frequency of sward height classes of Italian ryegrass swards managed under continuous stocking and grazed by sheep under different grazing intervention strategies. (T1) Put-and-take treatment uses just animals for regulating average sward height; (T2) Put-and-take plus fence treatment had deferment of over-grazed areas (<12cm) and concentration of animals in areas under-grazed (>18cm); (T3) Put-and-take plus fence plus mechanical topping treatment areas were also isolated when over-grazed (<12 cm) and were topped with a string trimmer when under-grazed (>18 cm). Lowercase letters show the difference of the sward height classes offered within each treatment. Capital letters show the difference between the treatments within each sward height class offered. Different letters differ significantly at 0.05.

# LIST OF TABLES

 Table 1 - Variables of Italian ryegrass pastures grazed by sheep under different grazing manipulation strategies.

**Table 2** - Animal production, intake and feed conversion by sheep grazing Italian ryegrass managed under different grazing manipulation strategies.

# CONTENT

CHAPTER I	9
1. INTRODUCTION	10
2. LITERATURE REVIEW	11
3. HYPOTHESIS	
4. OBJECTIVES	16
CHAPTER 2	17
Abstract	
Implications	19
Introduction	
Material and methods	21
Experimental site	
Experimental design and treatments	
Pasture measurements	24
Sward structure manipulations	
Animal management and measurements	
Estimation of sheep herbage intake	
Statistical analysis	
Results	
Pasture	
Animal performance	
Discussion	
Ethics approval	
References	
CHAPTER 3	42
FINAL CONSIDERATIONS	43
REFERENCES	44
APPENDICES	

CHAPTER I

### **1. INTRODUCTION**

Animal production in pastoral ecosystems is quite complex since the plant and the animal share the same environment, with animals needing leaves to feed and plants needing these same structures to intercept light and perform photosynthesis. Therefore, severe grazing results in the consumption of a large part of the available forage (Benvenutti et al., 2016; Savian et al., 2021), negatively affecting the regrowth of the pasture and, in addition, the animal is also penalized when it is forced to access the bottom of the sward canopy (Schons et al., 2021)

Thus, one of the goals of grazing management is to achieve sward structural characteristics that result in optimal levels of animal performance (Hodgson, 1990), because commercial grazing systems are remunerated based on animal performance. In this way, the "Rotatinuous" concept has been proposed, where the animal is considered as the main agent to define grazing targets (Carvalho, 2013). It is also an efficient alternative to reduce environmental impacts (Savian et al., 2021) and improve animal and pasture production (Savian et al., 2019, 2018; Schons et al., 2021).

However, the "take the best and leave the rest" principle can be challenging to apply in continuous stocking because there is no direct control of the defoliation interval (Carvalho, 2013), which might lead to repeated impacts on preferred areas while others are less or not even used (Teague et al., 2004). Whereas in rotational stocking we can leave an unfavorable sward structure behind and move to the next strip (Schons et al., 2021).

Despite being used in southern Brazil (Carvalho et al., 2021; Carvalho, 2013), we still lack scientific evidence on how to offer ideal sward structures in continuous stocking under the "Rotatinuous" concept. Which makes us question if just the stocking rate adjustment is enough or if other anthropic interventions, like fencing (Anderson et al., 2014; Odintsov Vaintrub et al., 2020; Turner et al., 1997) and topping (Lambert et al., 2000; Mc Donald, 1986) are needed to facilitate the grazing process.

This dissertation shows the results of this concept applied under continuous stocking by analyzing plant production and animal performance. The results of this study are presented in the second chapter of this document in a scientific article format.

### 2. LITERATURE REVIEW

Forage produced on well-managed pasture is one of the most sustainable feed sources that can be produced and utilized in the grazing livestock system (Rao et al., 2015; Savian et al., 2021). Yet, grazing is a complex process that varies in time and space and involves animals that need to search, gather, and process herbage (Ungar & Noy-Meir, 1988; Bailey, 1996). Grazing management, the art of guiding the grazing process aiming to feed animals (Allen et al., 2011), emerged only after animal domestication, during the Neolithic period. Since then, men, as a shepherd, determine the choices trajectory, displacement speed, and total trajectory duration, as well as the animal's grazing opportunities.

The barbed wire arising, which was recent (created in 1876), led to distance men from the necessities of deep comprehension of the preferences and behavior dynamics of grazing animals (Carvalho et al., 2019). This distance is not an isolated phenomenon because the disconnection between agriculture and nature is a global process (Gordon et al., 2017). In commercial grazing systems, the same pattern is observed, through grazing managers' practices which promote homogenous sward, multi-paddock grazing, and the overuse of resources to control the grazing process.

So, pastoralism has evolved basically in terms of two grazing methods: rotational and continuous (di Virgilio et al., 2019). Essentially, both of them converge to a variation in the animal distribution in time (e.g., occupation time and rest) and space (e.g., the multi-paddock area in rotational stocking method controlled by the fence) (di Virgilio et al., 2019). The traditional pasture management is static and based on the balance between productivity and dynamics of plants growth and their utilization by the animals by manipulating different variables such as stocking rate, herbage allowance and frequency of defoliation (Carvalho et al., 2009; Pontes-Prates et al., 2020) leading to a management focused mainly on the plant (Schons et al, 2021).

Despite the different propositions of stocking methods, it is defined or justified by controlling the frequency and intensity of the defoliation (Carnevalli et al., 2006; Barbosa et al., 2007), a combination that directly affects the sward structure. As a rule, rotational stocking is capable of defining the space-time allocation of bites through the definition of occupation periods and rest. Yet, continuous stocking has a lower capacity of controlling where and when the defoliation will happen (Carvalho et al., 2009).

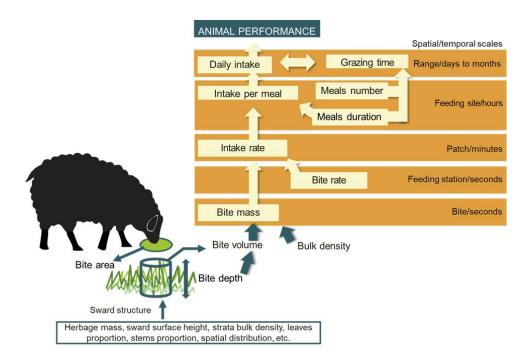
In order to try to control sward structures in paddocks continuously stocked, many interventions are used to reach an intensification of commercial grazing systems, which are usually translated into the better usage of forage resources. For example, paddocks are highly subdivided to homogenize the area, which is believed to guarantee uniform grazing (Turner et al., 1997). Also, topping (defoliation by mowing the top stratum of the sward) has been largely used in order to reduce dead matter material and early inflorescences (between the emergence and flowering) to improve pasture quality (McDonald, 1986). Nevertheless, these interventions try to sort out problems created by the management itself, disregarding animal evolutionary knowledge (Carvalho et al., 2019)

Briske et al. (2008) indicate that animal and plant productivity do not differ between continuous and rotational stocking methods in most of the published grazing experiments when under similar stocking rate, but concepts guiding matter the most since bites are a detached event and thus, in any grazing management system it is "rotational" at the plant level. (Pontes-Prates, 2020).

Some studies (e.g., Bergman et al., 2001) suggest that ruminants adopt a minimum grazing time strategy. Harvesting a higher quantity even though this strategy offers them the harvest of a diet with lower nutritional quality (Gordon & Lascano, 1993; Bergman et al., 2001). When it comes to time restriction, some studies suggest that both cattle and sheep prefer forages that can be consumed faster, or a high ingestion per unit of time (Kenny & Black, 1984; Black & Kenny, 1984; Illius & Gordon, 1990; Laca & Demment, 1991; Illius et al., 1992; Demment et al., 1993; Laca et al., 1993; Utsumi et al., 2009).

Allden and Whittaker (1970) state that when the structure is not favorable for bite mass, sheep are able to compensate for the difficulty of prehension by an increase in grazing time (from 6 to 13 hours). From the same point of view, Hodgson (1981) claims that bite rate and grazing time are often considered as the primary compensatory responses of animals to ingestive limitations. However, when the sward conditions diverge from optimal increases, compensation becomes progressively more inefficient as animals have other social activities to perform during the day and their physical conditions are also limiting.

Grazing is a complex combination of various movements and activities performed at different temporal and spatial scales. In Figure 1 it is shown why daily dry matter intake and consequently animal performance are built through bites (Laca & Ortega, 1995; Bailey, 1996). Hence, the major concern in grazing management is to offer an adequate sward structure in order to optimize bite mass, and consequently, intake rate (Carvalho, 2013).



**Figure 1** – Spatial and temporal scale of grazing (adapted from Bailey et al. 1996; Bailey e Provenza 2008)

Laca and Lemaire (2000) defined sward structure as the spatial arrangement of morphological components of a plant, in other words, the arrangement of the aerial part of the plants in a plant community. Two types of dry matter distribution can characterize sward structure: vertical and horizontal. In the vertical dimension, the pasture has height, leaf/stem ratio, and distribution of pasture components (blade, stem, and pseudostem) or species in different horizons of the pasture, with the horizontal dimension, there is variation in tillers density, plant component, and species composition, thus varying in density and mass (Carvalho et al., 2008; Gordon & Benvenutti, 2006).

The variable of sward structure most related to bite mass is sward height (Laca, 1992). Mezzalira et al. (2014) and Gonçalves et al. (2009) observed that the short-term intake rate of cattle and sheep is optimized in sward heights that also optimize bite mass. Bite mass has a quadratic relationship with increasing sward height (Mezzalira et al 2017). It is explained because bite depth has a linear and positive relationship with sward height, but the density increases to a certain point and then decreases by the distribution of more dispersed leaves (Cangiano et al., 2002; Laca et al., 1992).

On the other hand, the lower the sward surface height and the denser the pasture, the less effective is the ability of animals to increase the amount of forage brought to the mouth. Laca et al. (1992) argue that animals obtain heavier bite mass on higher heights and scattered leaves than on dense pastures with lower heights.

Therefore, sward height affects the ingestive behavior of animals (Fonseca et al., 2013).

Carvalho (2013) proposed a pasture management strategy designed to offer to the animals an optimal sward structure to maximize forage intake per unit of grazing time based on the concepts of grazing behavior. This grazing management strategy, which was named "Rotatinuous", is characterized by low intensity and high grazing frequency, which means that animals eat predominantly the top grazing stratum of the sward, almost exclusively composed of leaves. For that, Carvalho (2013) integrated the principles of sward structure (optimal sward height) and animal behavior (shortterm intake rate), to create a grazing management strategy that maximizes and maintains the highest forage intake rate.

This grazing management concept with animal-based pasture targets has been already discussed under rotational stocking method (Savian et al., 2018; Savian et al, 2019; Schons, et al., 2021). The ideal pre-grazing height that maximizes the short-term intake rate by animals was investigated for different forage species, such as native grassland (Gonçalves et al., 2009), *Sorghum bicolor* (Fonseca et al., 2012), *Pennisetum glaucun* (Mezzalira et al., 2013), *Lolium multiflorum* (Amaral et al., 2013), *Cynodon* sp. and *Avena strigosa* (Mezzalira et al., 2014) and *Festuca arundinacea* Schreb. (Szymczak et al., 2020).

The same theoretical basis of grazing behavior was used to determine post grazing sward height. In order to remain at a constant intake rate, the average post-grazing height should not be reduced by more than 40% of the pre-grazing height (Fonseca et al., 2013). When animals are forced to graze the bottom canopy strata, significant reductions in forage intake occur (Mezzalira et al., 2014; Fonseca et al., 2013), so, the decline in intake rate is associated with changes in sward structure.

The "Rotatinuous" concept is already applied at the farm level (Carvalho, 2013) under rotational stocking method. Mezzalira (2012) noticed that, at bite level, there is no difference between stocking methods in the definition of optimal structure. Sward height target in continuous stocking is the average between the optimal sward height. However, grazing trials in continuous stocking have not been done yet (Carvalho, 2013). One of its challenges is the spatial control of grazing distribution because animals naturally create patches, preferring and avoiding areas in the paddock (Anderson et al., 2014).

Animals face different spatial heterogeneity of sward height under "Rotatinuous" when compared to traditional rotational method and also to the most common continuous stocking in Brazil. Opportunities for grazing animals to select preferred parts of the plant, such as green leaves, results in greater conversion of forage consumed in kg of animal product (Savian et al, 2021; Schons et al., 2021). Also, when different patches are available, animals can select feeding stations that maximize their nutrients intake.

However, besides adjusting stocking rates, and in order to create a more favorable sward structure for animals, is it necessary to use different tools to offer them? If so, how to offer the structure that maximizes the intake rate under continuous stocking, using stocking rate adjustment, fences and topping to modify sward height distribution, is the main focus of this Dissertation.

### 3. HYPOTHESIS

The grazing intervention strategies with fencing or topping to offer plants that optimize intake rate do not improve pasture production, herbage intake and performance of sheep managed under continuous stocking because the optimum sward height is already maintained by stocking rate adjustement.

# 4. OBJECTIVES

To test if interventions to control the sward structure affect/improve pasture production, herbage intake and performance of sheep grazing Italian ryegrass under continuous stocking method oriented by the Rotatinuous concept.

**CHAPTER 2** 

# Assessing the put & take, fencing and topping techniques to control sward structure under continuous stocking

T. S. Freitas <sup>a,1</sup>, L.C. Lima <sup>a,1</sup>, J. V. Savian <sup>b,2</sup>, P.C. F. Carvalho <sup>a,1</sup>

<sup>a</sup> Grazing Ecology Research Group, Federal University of Rio Grande do Sul, Av. Bento Gonçalves, 7712, Porto Alegre, 91540-000, Brazil

<sup>b</sup> Instituto Nacional de Investigación Agropecuaria (INIA), Programa Pasturas y Forrajes. Estación Experimental INIA Treinta y Tres. Ruta 8 km 281, Treinta y Tres, 33000, Uruguay

Corresponding author: Thainá Silva de Freitas. Email: thain.freitas@gmail.com

### Abstract

Livestock use 2.5 billion ha of land and consume 6 billion tonnes of feed, of which 86% is not eaten by humans. Despite the diversity of production systems, grasslands are the main source of ruminant feeding. Thus, grazing is the pivotal feeding process and grazing management should seek to provide opportunities to obtain better animal performance. These targets are met with the "Rotatinuous" concept, a grazing management strategy based on the responses of animal behavior to sward structures, which means minimizing time costs and consequently maximizing herbage intake per unit of grazing time. However, its application under continuous stocking can be a challenge due to the lower control of animals in the paddock. This work was conducted in 2019, in a randomized complete block design, analyzing the effects of sheep grazing Italian ryegrass (*Lolium multiflorum* Lam.) in continuous stocking with three different

grazing interventions strategies targeting sward height at 15 cm. Which were: stocking rate adjustment (Put & Take); use of fences to concentrate animals and mechanical topping to reduce sward heights in undergrazed areas or the use of fences to exclude animals from overgrazed areas. Twenty-seven castrated lambs with an average live weight of  $32 \pm 2$  kg were used. Results indicated that the evaluated techniques just homogeneized sward height distribution and did not improve pasture production neither increased sheep performance, when Italian ryegrass is managed in a range of 12 to 18 cm. Considering experimental limits of spatio and temporal scales, stocking rate adjustment was enough to offer ideal Italian ryegrass sward structures for sheep grazing under continuous stocking.

**Keywords**: grazing systems, sheep performance, stocking rate adjust, sward structure, Italian ryegrass, resource heterogeneity

### Implications

Our results showed that in continuous stocking method, pasture production and sheep performance on Italian ryegrass swards do not improve with manipulations in the sward structure with fencing or mechanical topping. This techniques just homogeneize sward height distribuition on the spatial and temporal scales studied. Also, we confirmed that to achieve high sheep intake and performance, and production per area, Italian ryegrass swards under continuous stocking should be managed at 15 cm, on average.

### Introduction

Grazing is a singular process composed of animal and plant, which has been developed and evolved throughout time on grassland ecosystems. Managers try to

control and improve this action through the use of interventions over space and time (with fences and rest and occupation periods). Nonetheless, their main goal is to achieve a more efficient use of forage resources, which might have negative results, depending on the limits imposed.

However, our ability to control environmental effects is inefficient due to traditional paradigms and the set of factors selected to manage grazing systems (Laca, 2009). The aim of grazing management should be to provide grazing opportunities with a high rate of herbage intake and at appropriate grazing intensities, in order to obtain better individual performance of the animal.

Thus, the "Rotatinuous" grazing management concept suggests a new approach, using pre- and post-grazing sward heights aiming to allow animals to maximize and sustain their intake rate (Carvalho, 2013). Studies applying this approach have observed an improvement in animal performance and pasture production (Savian et al., 2018; Schons et al., 2021), associated with a reduction in methane emissions (Savian et al., 2019, 2021).

The application of this concept in rotational stocking is eased by the nature of control of entry and exit moment of the animals from the strips. Yet, under continuous stocking, the use of the same concept reaches a new level of challenge. Even though animal control is lesser, the concept does not change and animals may find the ideal structures on their own. Studies suggest the need of manipulating the temporal foraging pattern considering that grazing creates sward heterogeneity due to the selection or avoidance of plants (Rook and Tallowin, 2003). As managers, we may have a stocking control in order to provide patches with an optimum average of sward heights in the paddock for animals to maximize their intake rate.

20

Despite facilitating grazing management and animal control, just controlling paddock size does not eliminate uneven sward heights because animals seek preferred areas regardless of paddock size (Anderson et al., 2014). This leads us to our main question: Would anthropic intervention ease the grazing process in a continuously stocked paddock? If yes, would this ease provide improvements to herbage intake, animal performance and pasture production?

Thus, our objective was to analyze if the use of interventions to provide grazing opportunities improves herbage intake, animal performance and pasture production. To offer these opportunities, we controlled sward height distribution with stocking adjustment, fencing and topping using sheep grazing Italian ryegrass under continuous stocking method oriented by the "Rotatinuous" concept.

### Material and methods

### Experimental site

The experiment was conducted at the Experimental Station of the Faculty of Agronomy (EEA) of the Federal University of Rio Grande do Sul (UFRGS), in southern Brazil (30°05"22'S, 51°39"09'W, and 46 m a.s.l.), with a subtropical humid "Cfa" climate according to the Köppen classification. In 2019, the annual mean air temperature was 19.8°C and annual rainfall was 964.8 mm (EEA-UFRGS).

An experimental area of 2.25 ha was divided into nine square-shaped paddocks (experimental units) of 0.25 ha each. The soil of the experimental site was classified as a Typic Paleudult (USDA, 1999), with 17.5% clay, 20% silt and 62.5% sand. Yet, in the 0–20 cm layer, the soil presented a pH of 4.05, SMP index of 6.05, 23.2 mg/dm<sup>3</sup> of

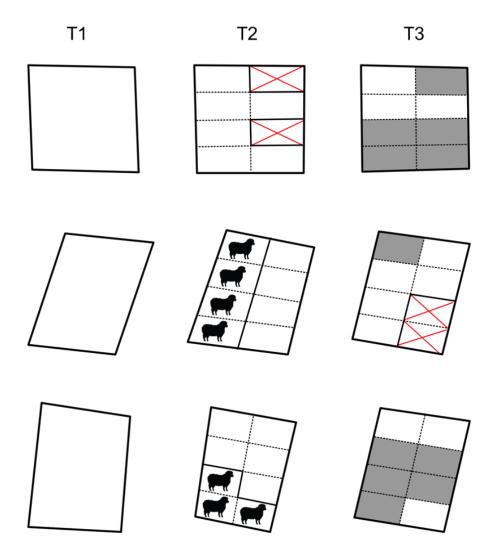
available P by Bray method, 105.5 mg/dm<sup>3</sup> of available K by Mehlich 1 extraction method, 22.7 g/kg of OM, 0.60 cmol<sub>c</sub>/dm<sup>3</sup> of exchangeable Al3<sup>+</sup>, cation exchange capacity of 3.77 cmolc/dm<sup>3</sup> and 39.5% of base saturation (Schons et al., 2021). Italian ryegrass (*Lolium multiflorum* Lam.) seeds (36 kg/ha) were broadcasted on May 20th and June 7th, 2019, after conventional soil preparation (plowing and disking). A total of 70 kg of nitrogen in the form of urea were broadcasted equally in two moments, on June 25th and August 30th, 2019.

The experimental stocking season was 95 days, starting on July 25th and finishing on October 28th, 2019.

### Experimental design and treatments

A randomized complete block design with three replicates (paddocks) was used to test three grazing intervention strategies, and the slope of the area was used as a blocking criterion. The pasture manipulation treatments were applied under continuous stocking, and the sheep grazed Italian ryegrass swards in a target sward height of 15 cm because it is the average between optimal sward heights for rotational stocking (18 and 12 cm for pre- and post-grazing) as proposed by Amaral et al. (2013) and Carvalho (2013). T1) only put-and-take (Mott and Lucas, 1952), which means that the stocking rate was adjusted weekly to maintain the sward height target of 15 cm; T2) put-and-take plus fence, which means that the stocking rate was adjusted weekly and animals were concentrated in under-grazed areas of the paddock (where the average sward height was above 18 cm) and a grazing deferment with the fence was promoted in over-grazed areas of the paddock (where the average sward height was below 12 cm), both of them for short periods until Italian ryegrass reach 15 cm, on average; and T3)

put-and-take plus fence plus topping, which means that the stocking rate was adjusted weekly and pasture of under-grazed area of the paddock was mechanical topping with a string trimmer at 15 cm when it exceeded 18 cm, and over-grazed areas also were fenced for grazing deferment when sward height was below 12 cm. In T2 and T3 treatments, the paddocks were divided into eight virtual quadrants of 312 m<sup>2</sup> (Figure 1) in order to apply the pasture manipulation treatments (fence or mechanical topping).



**Fig. 1.** Illustration of grazing intervention strategies treatments. All treatments had stocking rate adjustments. (T1) Put-and-take treatment uses just animals for regulating average sward height; (T2) Put-and-take plus fence treatment had deferment of overgrazed areas (<12cm) illustrated by the red X and concentration of animals in areas undergrazed (>18cm) illustrated by the sheep; (T3) Put-and-take plus fence plus mechanical topping treatment had areas

deferred when overgrazed (<12 cm) illustrated by the red X and undergrazed areas were topped with a string trimmer (>18 cm) identified by the grey color.

#### Pasture measurements

The sward height was measured through 280 points per paddock every 15 days using a sward stick (Barthram, 1985) coupled to an RTK-GPS (EMLID REACH RS GNSS RTK) to record spatial distribution of sward height, and to identify over-grazed or subgrazed areas in the paddock in order to apply the interventions in each paddock. Between the georeferenced measurements, the sward stick without RTK-GPS was used to make 150 undisturbed readings of sward heights per paddock, which means that every week the sward heights in all paddocks were measured.

Herbage mass was determined by clipping five random quadrats (0.25 m<sup>2</sup> each) per paddock at ground level every 21 days. At the end of the stocking season, five random quadrats were clipped in each paddock to measure residual herbage mass.

Five grazing exclusion cages randomly distributed in each paddock were used to estimate daily herbage accumulation rate every 21 days (Klingman et al., 1943). Herbage accumulation rate was obtained by dividing the accumulated herbage by the number of days between cuts. Total herbage production (THP) was calculated as the mean daily herbage accumulation rate along the stocking period, multiplied by the number of days of the stocking season and summed to the herbage mass at the beginning of the experiment.

Italian ryegrass sub-samples were taken to morphological separation. Each subsample was manually separated into leaf blades, stem plus sheath, dead material and inflorescences. To know the partial dry matter (DM) of herbage, all herbage samples

24

were dried in a forced-air oven at 55°C for 72 hours until constant weight. The herbage samples were not corrected to DM at 105°C.

Average herbage allowance was calculated according to Sollenberger et al. (2005) [herbage allowance (kg DM/kg LW) = herbage mass (kg DM/ha) / animal LW (kg/ha)]. We used the Gini coefficient as an indicator of the sward height heterogeneity, which is a mathematical measure of inequality that is applied in several fields (Gini, 1921). Gini values quantify the relative inequality height among points of sward heights in a paddock when applied to sward structures.

### Sward structure manipulations

The pasture interventions were applied right after the accomplishment of sward height measurements with the RTK-GPS. During the stocking season, georeferenced sward heights were measured on 7 occasions (period).

In the first two weeks of the stocking season, the target sward height was maintained using only the put-and-take method for all treatments. After that, in addition to animal adjustment by put-and-take method, it was necessary to fence 21% and 46% of the overgrazed areas in T2 and T3 treatments, respectively. These areas were evaluated daily with 30 points of sward height to determine the duration of sward manipulations, which varied from 3 to 16 days. From September 9th until the end of the stocking season, we increased the grazing pressure in 46% of the area of T2 treatment. The duration of these manipulations varied from 2 to 7 days. In the T3 treatment, 45% of the surface was topped to 15 cm once on September 20th.

### Animal management and measurements

25

The experimental animals were Corriedale and Texel castrated male lambs with 32 ± 2 kg of LW and an average of 10-month-old. The sheep were separated by breed and LW and allocated randomly in blocks at the beginning of the stocking season. Each paddock had three test-sheep (permanent animals over the whole stocking season). Sheep had free access to freshwater. At the beginning and end of the stocking season, to quantify LW, sheep were fasted for 12 hours.

The stocking rate (kg LW/ha) was obtained by the relationship between the number of animals and the total area of the paddock. Average daily gain (ADG; kg/animal/day) was obtained by the difference of the final and initial LW of the test-sheep divided by the number of days of the stocking season. The LW gain per hectare (LWG, kg/ha) was calculated by the number of animals per hectare multiplied by ADG of test-animals and its result was divided by stocking season duration in days to obtain the daily LW gain per hectare. Feed conversion (kg OM intake/kg LW gain) was calculated by dividing the organic matter intake per sheep per day divided by the ADG.

### Estimation of sheep herbage intake

To estimate daily organic matter intake, we used the faecal crude protein technique (Penning, 2004). For that, we used an Italian ryegrass equation proposed by Azevedo et al. (2014) [OM intake (g/sheep per day) =  $111.33 + 18.33 \times$  faecal crude protein (g/sheep per day)].

Two daily OM intake measurement periods were performed during the stocking season, each period consisted of total faeces collection during five consecutive days. For that, three test-sheep per paddock were used (n = 54; 3 sheep per paddock, 9 paddocks, and 2 measurement periods). The sheep were equipped with faeces collecting bags (Penning, 2004), which were emptied every morning. The faeces were

weighed and homogenized and a sub-sample of 20% of the total was taken. Then, these faecal samples were dried at 55°C for 72 h, grouped per animal, ground with a knife mill (1-mm screen), and analysed for DM, OM and crude protein (N  $\times$  6.25) (AOAC, 1975).

### Statistical analysis

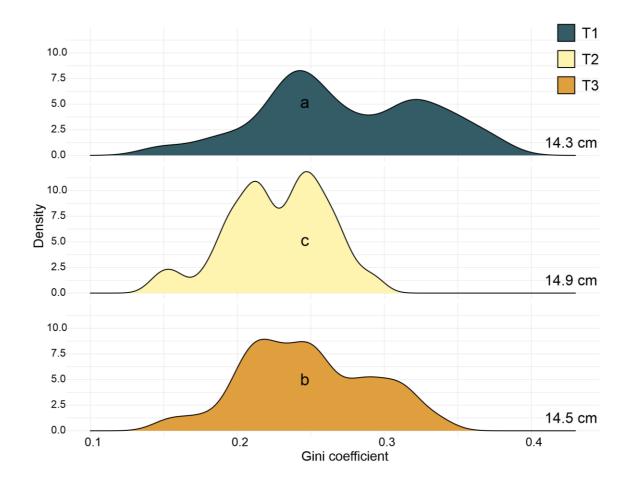
All response variables were checked for normality by the Shapiro test (P>0.05), homogeneity of variance by Bartlett's test (P>0.05) and visual residual analysis. When the assumptions of the analysis of variance (ANOVA) were not achieved, the data were log<sub>10</sub> transformed.

Data were subjected to ANOVA at a 5% level of significance, using linear mixed-effects models with the LMER function from the *Ime4* package in R Studio software version 3.6.1 (R Core Team, 2020). The grazing intervention effect was considered as a fixed effect in the statistical models, and we tested the inclusion of different random effects. Then, to define the best fit model the Akaike's Information Criterion was used. Also, sward height classes were considered fixed effects for sward height frequency as well. The model for sward height frequency, herbage mass and herbage intake that was evaluated in different periods over time includes the effect of paddock nested in each period as random effects to account for a potential lack of independence among repeated observations on the same paddocks over the periods. For average sward height, Gini coefficient, herbage allowance, morphological composition of herbage, random effects were period and block. For the herbage accumulation rate, total herbage production, stocking rate, gain per area, daily gain per area and average daily gain variables the final model included block as a random effect. For the variable forage mass residual, the final model included only paddock as a random effect.

### Results

### Pasture

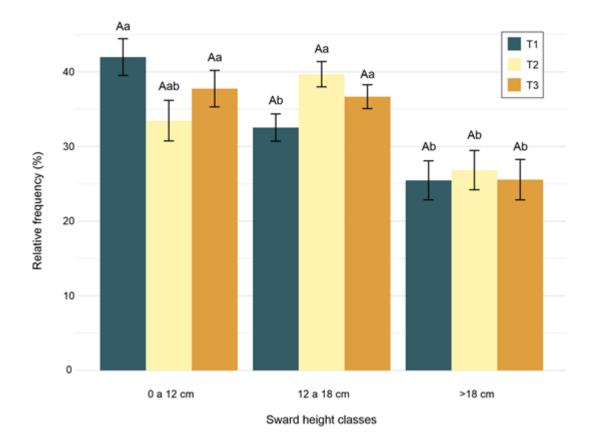
Italian ryegrass sward heights did not differ (P = 0.286) between the grazing intervention treatments (Figure 2), with an average of  $14.7 \pm 0.3$  cm during the stocking season, which was in line with the sward height target proposed. Despite the average sward heights that did not differ statistically, when the Gini coefficient was performed the average sward height heterogeneity was different between treatments (P < 0.001). That is, the sward heights distribution of T1 treatment presented greater heterogeneity (0.273) than T2 (0.227) and T3 (0.248) treatments (Figure 2).

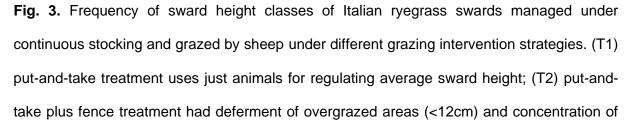


**Fig. 2.** Gini coefficient density of Italian ryegrass sward height distribution managed under continuous stocking and grazed by sheep under different grazing intervention strategies. (T1) put-and-take treatment uses just animals for regulating average sward height; (T2) put-and-take plus fence treatment had deferment of overgrazed areas (<12cm) and concentration of animals in areas undergrazed (>18cm); (T3) put-and-take plus fence plus mechanical topping treatment areas were also isolated when overgrazed (<12 cm) and were topped at 15 cm with a string trimmer when undergrazed (>18 cm). Mean sward height is plotted to the right of each treatment curve and do not differ (P = 0.286). Different letters differ significantly at 0.05.

The frequency of sward height classes that were below (from 0 to 12 cm), above (> 18 cm), and in the ideal animal intake height range (between 12 and 18 cm) over the entire grazing season are presented in Figure 3. We observed a difference in the sward

height classes offered (P < 0.001). On T1 treatment, sward heights in the range 0 to 12 cm represented 42%, which are 9.5% and 16.5% higher than ideal (32.5%) and above (25.5%) classes, respectively. On T2 treatment, the ideal class was 39.7%, which was numerically higher than the below class (33.5%) and significantly higher than the above class (26.8%). Moreover, for T3 treatment, both below and ideal classes were significantly higher than the above class, being 37.8, 36.7 and 25.6%, respectively. However, no differences among treatments were observed for each sward height class offered (P > 0.05).





animals in areas undergrazed (>18cm); (T3) put-and-take plus fence plus mechanical topping treatment areas were also isolated when overgrazed (<12 cm) and were topped at 15 cm with a string trimmer when undergrazed (>18 cm). Lowercase letters show the difference of the sward height classes offered within each treatment. Capital letters show the difference between the treatments within each sward height class offered. Different letters differ significantly at 0.05.

**Table 1** shows the average value for variables of Italian ryegrass pastures for each treatment. There were no statistical differences (P > 0.05) for any of the following variables: herbage accumulation rate (mean 56.1 ± 6.9 kg DM/ha), total herbage production (mean 6765 ± 807 kg DM/ha), herbage allowance (mean 2.8 ± 0.22 kg DM/kg LW), herbage mass (mean 1625 ± 46 kg DM/ha), and residual herbage mass (mean 1503 ± 81 kg DM/ha).

 Table 1 - Variables of Italian ryegrass pastures grazed by sheep under different grazing manipulation strategies.

Variables	T1	T2	Т3	SEM	P-value
Herbage mass (kg DM/ha)	1716	1618	1542	46	0.221
Morphological composition					
Leaf blade (%)	61.8	63.5	63.8	2.85	0.808
Stem+sheath (%)	24.7	21.7	21.0	1.78	0.092
Dead material (%)	11.2	11.1	11.7	1.29	0.981
Inflorescence (%)	2.9	2.3	1.9	0.379	0.398
Leaf blade:Stem+sheath ratio	0.93	1.17	1.22	0.698	0.166
Herbage allowance (kg DM/kg LW)	3.28	2.63	2.55	0.22	0.235
Daily Herbage accumulation rate (kg DM/ha)	62.5	47.6	58.2	6.87	0.527
Total herbage production (kg DM/ha)	7666	6042	6587	807	0.569

Residual herbage mass (kg DM/ha)	1503	1440	1581	81	0.751
----------------------------------	------	------	------	----	-------

T1 = only put-and-take treatment uses just animals for regulating average sward height; T2 = put-and-take plus fence treatment had deferment of over-grazed areas (<12cm) and concentration of animals in areas under-grazed (>18cm); T3 = put-and-take plus fence plus mechanical topping treatment areas were also isolated when over-grazed (<12 cm) and were topped at 15 cm with a string trimmer when under-grazed (>18 cm).

DM = dry matter; LW = live weight; SEM = standard error of the mean.

### Animal performance

Table 2 shows the effect of grazing manipulation strategies under continuous stocking on animal performance in each treatment. No differences (P > 0.05) between treatments were observed for all variables. The ADG was 0.117 ± kg/sheep, on average. The total LW and daily LW gain per area were, on average, 240 ± 30 kg LW/ha and 2.6 ± 0.3 kg LW/ha/day, respectively. The stocking rate was 728 ± 63 kg LW/ha, on average.

**Table 2 -** Animal production, intake and feed conversion by sheep grazing Italian ryegrass managed under different grazing manipulation strategies.

Variables	T1	T2	Т3	SEM	P-value
Average daily gain (kg/sheep)	0.123	0.120	0.108	0.008	0.189
LW gain (kg LW/ha)	255	260	206	30.0	0.4956
Daily LW gain (kg LW/ha)	2.8	2.9	2.3	0.34	0.505
Stocking rate (kg LW/ha)	702	806	676	63.0	0.845
OM intake (g/sheep/day)	901	787	770	26	0.308
OM intake (% LW)	2.25	2.04	1.92	0.1	0.185
OM intake per area (kg OM/ha/day)	16.9	16.2	13.6	1.2	0.460
Feed conversion (kg OM/kg LW gain)	7.6	6.7	7.6	0.4	0.506

T1 = only put-and-take treatment uses just animals for regulating average sward height; T2 = put-and-take plus fence treatment had deferment of over-grazed areas (<12cm) and concentration of animals in areas under-grazed (>18cm); T3 = put-and-take plus fence plus mechanical topping treatment areas were also isolated when over-grazed (<12 cm) and were topped at 15 cm with a string trimmer when under-grazed (>18 cm).

LW = live weight; OM = organic matter; SEM = standard error of the mean.

No difference was observed between the treatments for herbage intake (P = 0.308; mean 819 ± 26 g OM/sheep/day), OM intake expressed as a percentage of LW (P = 0.185; mean 2.07 ± 0.1 %), OM intake per area (P = 0.459; mean 7.3 ± 0.4 kg OM/ha/day), and feed conversion (P = 0.506; mean 7.3 ± 0.4 kg OM/kg LW gain).

### Discussion

Our study showed that managing under continuous stocking at moderate defoliation intensity (e.g., range of 12 and 18 cm throughout the stocking cycle) offers a high proportion of green leaf to the grazing animal, and intervention with fences or mechanical topping (in order to offer ideal sward structures) does not improve their herbage intake and performance, neither affects herbage production. Hence, in Italian ryegrass swards managed under continuous stocking, it is conceivable to maintain optimum average sward heights only with stocking rate adjustment, promoting a nonlimiting sward heterogeneity for the animals to find out ideal sward heights that support a high herbage intake per unit of time and consequently per day.

The sward height frequency between 12 and 18 cm, which means 1/3 of sward heights offered among treatments (Figure 3), endorse the animals were managed under sward structures that favor high herbage intake per grazing time. Therefore, our main question "How to offer optimal sward structures for grazing lambs in continuous

stocking?" has been answered. Overall, all grazing manipulation strategies were efficient in offering ideal sward heights.

Despite the expectation to observe less heterogeneity under the put-and-take plus fence plus topping strategy (T3 treatment), this study showed that, in fact, this grazing manipulation strategy had a higher Gini coefficient than the put-and-take plus fence (T2 treatment) (Figure 2). It might be explained due to the intervention duration difference between T2 and T3 treatments. While on T3 — with mechanical topping — the sward height target (15 cm) was achieved almost instantaneously, on T2 treatment — by grazing down with fence concentration — it was required 2 to 7 days to reach the same goal. It is important to remember that put-and-take intervention was applied to all treatments and that the Gini coefficient difference is a result of fencing and topping.

In addition, a higher Gini coefficient was observed when only stocking rate adjustment (T1) was used. This result makes sense because grazing is the main creator of spatial heterogeneity (Adler et al., 2001), especially when associated with the lower control on the sward height distribution of this treatment. We hypothesized that no differences would be observed on animal and plant production because all treatments aimed to maintain the offer of ideal structures throughout the stocking season. The lack of differences between sward structure variables between treatments (Table 1) confirms our hypothesis and can be explained by the moderate grazing defoliation regime.

Recent studies confirmed that set grazing management goals with moderate defoliation levels promote a canopy with more leaf area and growing points to intercept and use light for herbage accumulation (Lemaire and Chapman, 1996; Silveira et al., 2013; Martins et al. 2019). Our findings suggest that continuous stocking at moderate defoliation intensity produces a high proportion of green leaves. Consequently, a

higher proportion of green leaves in the grazed horizon may explain the high performance of the animals. According to da Silva et al. (2013), grazing the higher quality leaves frequently results in a better balance among the rate of leaf growth, reduced leaf senescence, and amount of leaf removal by the animals.

In the same way, the sward variables were consistent with previous findings by other authors (Savian et al., 2018; Schons et al., 2021) that found similar herbage mass and morphological components managing sward heights of Italian ryegrass under rotational stocking under "Rotatinuous" grazing concept. Herbage allowance ranged between 3.2 and 2.5 among treatments which are in agreement with the findings of Schons et al. (2021).

Similarly, animal results (Table 2) are in agreement with those reported on the literature, studies using Italian ryegrass swards managed under continuous stocking at an average of 15 cm, which means moderate grazing intensity, offered the opportunity to improve herbage production while favoring animal ADG and LWG/ha (Farias et al., 2020; Planisich et al., 2021). Also, Schons et al. (2021) found similar ADG values (0.119 kg sheep/day) to sheep grazing Italian ryegrass under rotational stocking and under the "Rotatinuous" concept.

Although there was no difference for OM herbage intake, T1 treatment was 14,54% and 12,65% higher than T3 and T2 treatments, respectively. It might have happened due to the higher heterogeneity of sward height distributions on T1 treatment. In paddocks continuously grazed with no interventions other than stocking adjustment, animals modify sward spatial distributions and maintain patches throughout the grazing season (Cid and Brizuela, 1998). It happens as a result of animal behavior (e.g., resting areas) and because grazing is a discrete process in space and time (Laca, 2009). Also,

35

spatial heterogeneity of sward height benefits instantaneous intake rate, allowing animals to select a better diet than the average available (Pontes-Prates et al., 2020). Anthropic interventions (fence and topping) are usually used to seek homogeneity of sward structures in a paddock (Turner et al., 1997) and to induce growth and nutritional quality (Kolver et al., 1999; Mc Donald, 1986). Those practices are very labor-intensive and probably more expensive. Then, appropriate grazing management strategies by themselves result in a more practical and probably cheaper alternative for producers. In this study, the main objective was to provide grazing opportunities through different forms of controlling sward height distributions by the "Rotatinuous" concept guidelines. Our results reinforce the importance of keeping proper management. Once a nonlimiting situation for grazing behavior is set, the selection of the stocking method becomes secondary (Briske et al., 2008). Ergo, we have different pathways to achieve the same result. From this point on we should consider management parameters (e.g., costs, familiarity, goals and farm strategies) not animal performance, herbage intake nor pasture production.

Considering the experimental spatio-temporal scales, grazing intervention strategies used in this experiment (fence and topping) homogeneized the sward height distribution but did not improve any of the pasture and animal variables analyzed. Maybe in an longer stocking season and in a larger area, these interventions could impact positively since they were managed in an optimal sward height. When it comes to grazing management, humans control only spatio-temporal aspects at paddock level and we often do it poorly because our focus is to intensify the management with supplies. This tells us that we should learn from animals, not teach them (Carvalho, 2005). This study is important to give a step forward to comprehend animal and sward

36

heterogeneity interface and how to offer an ideal sward height in continuous stocking under the "Rotatinuous" concept.

# Ethics approval

All animal handling and care attended the guidelines of the law of procedure for the scientific use of animals and were approved by the Ethics Committee for the Use of Animals of the UFRGS (protocol 3571).

# **Declaration of interest**

None.

# References

- Adler, P., Raff, D., Lauenroth, W., 2001. The effect of grazing on the spatial heterogeneity of vegetation. Oecologia 128, 465–479. https://doi.org/10.1007/s004420100737.
- 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.
- Anderson, D.M., Estell, R.E., Holechek, J.L., Ivey, S., Smith, G.B., 2014. Virtual herding for flexible livestock management – a review. Rangel. J. 36, 205. https://doi.org/10.1071/RJ13092.
- AOAC, 1975. Official Methods of Analysis, twelfth ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Azevedo, E.B., Poli, C.H.E.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, 42e50.

- Barthram G.T., 1985. Experimental techniques: the HFRO sward stick. HFRO Biennial Report 1984–85, pp. 29–30.
- 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.
- 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.
- Carvalho, P. C. de F., 2005. O manejo da pastagem como gerador de ambientes pastoris adequados à produção animal. In: Pedreira, C. G. S.; Moura, J. C. de; Silva, S. C.; Faria, V. P. (Org.). Teoria e Prática da Produção Animal em Pastagens. Piracicaba, p. 7-32.
- Carvalho, P.C. de F., Trindade, J.K., Mezzalira, J.C., Poli, C.H.E.C., Nabinger, C., Genro, T.C.M., Gonda, H.L., 2009. Revista Brasileira de Zootecnia Do bocado ao pastoreio de precisão: compreendendo a interface planta- animal para explorar a multi-funcionalidade das pastagens From the bite to precision grazing: understanding the plant-animal interface to exploit the m. Rev. Bras. Zootec. 3598, 109–122.
- 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.C. de F., Savian, J.V., Chiesa, T. Della, Souza Filho, W. de Terra, J.A., Pinto, P., Martins, A.P., Villarino, S., Trindade, J.K., Nunes, P.A. de A., Piñeiro, G., 2021. Landuse intensification trends in the Rio De La Plata region of South America: toward specialization or recoupling crop and livestock production. Front. Agric. Sci. Eng. 8, 97. https://doi.org/10.15302/J-FASE-2020380
- Cid, M.S., Brizuela, M.A., 1998. Heterogeneity in tall fescue pastures created and sustained by cattle grazing. J. Range Manag. 51, 644–649. https://doi.org/10.2307/4003606

- Da Silva, S.C., Gimenes, F.M.A., Sarmento, D.O.L. et al. (2013). Grazing behaviour, herbage intake and animal performance of beef cattle heifers on marandu palisade grass subjected to intensities of continuous stocking management. J. Agric. Sci. 151: 727–739.
- Di Virgilio, A., Lambertucci, S.A., Morales, J.M., 2019. Sustainable grazing management in rangelands: Over a century searching for a silver bullet. Agric. Ecosyst. Environ. 283, 106561. https://doi.org/10.1016/j.agee.2019.05.020
- Farias, G. D., Dubeux, J. C. B., Savian, J. V., Duarte, L. P., Martins, A. P., Tiecher, T., ... & Bremm, C., 2020. Integrated crop-livestock system with system fertilization approach improves food production and resource-use efficiency in agricultural lands. Agronomy for Sustainable Development, 40, 1-9.
- Gini, C., 1921. Measurement of inequality of incomes. The economic journal, 31, 124-126.
- Klingman, D. L., Miles, S. R., & Mott, G. O. 1943. The cage method for determining consumption and yield of pasture herbage. Journal of the American Society of Agronomy, 35, 739-746.
- Kolver, E.S., Penno, J.W., Macdonald, K.A., Mcgrath, J.M., Carter, W.A., 1999. Mowing pasture to improve milk production. Proc. New Zeal. Grassl. Assoc. 145, 139–145. https://doi.org/10.33584/jnzg.1999.61.2326
- Laca, E.A., 2009. New approaches and tools for grazing management. Rangel. Ecol. Manag. 62, 407–417. https://doi.org/10.2111/08-104.1
- Lambert, M.G., Paine, M.S., Sheath, G.W., Webby, R.W., Litherlandr, A.J., Fraser, T.J., Stevens, D.R., 2000. How do sheep and beef farmers manage pasture quality? Proc. New Zeal. Grassl. Assoc. 121, 117–121. https://doi.org/10.33584/jnzg.2000.62.2378.
- Lemaire, G., & Chapman, D.,1996. Tissue flows in grazed plant communities. The ecology and management of grazing systems, CAB International. https://hal.inrae.fr/hal-02836461.
- Martins, C. D. M., Schmitt, D., Duchini, P. G., Miqueloto, T., & Sbrissia, A. F., 2021. Defoliation intensity and leaf area index recovery in defoliated swards: implications for forage accumulation. Scientia Agricola, 78.

- Mc Donald, R.C., 1986. Effect of topping pastures 1. Pasture accumulation and quality. New Zeal. J. Exp. Agric. 14, 279–289.
- Mott, G. O., & Lucas, H. L. 1952. The design, conduct and interpretation of grazing trials on cultivated and improved pastures. In International grassland congress. Vol. 6, No. 1952, pp. 1380-1395.
- Penning, P.D., 2004. Animal-based techniques for estimating herbage intake. In: Penning,P.D. (Ed.), Herbage Intake Handbook. British Grassland Society, pp. 53e93.
- Planisich, A., Utsumi, S. A., Larripa, M., & Galli, J. R., 2021. Grazing of cover crops in integrated crop-livestock systems. Animal, 15(1), 100054.
- Pontes-Prates, A., Carvalho, P.C. de F., Laca, E.A., 2020. Mechanisms of grazing management in heterogeneous swards. Sustain. 12, 1–17. https://doi.org/10.3390/su12208676
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
- 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., de Souza Filho, W., Zubieta, A.S., Kindlein, L., Bindelle, J., Bayer, C., Bremm, C., Carvalho, P.C. de F., 2021. 'Rotatinuous' stocking as a climatesmart grazing management strategy for sheep production. Sci. Total Environ. 753. https://doi.org/10.1016/j.scitotenv.2020.141790
- 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

- Silveira, M.C.T., Da Silva, S.C., De Souza, S.J., Barbero, L.M., Rodrigues, C.S., Limao, V. A., Pena, K.D., Do Nascimento, D., 2013. Herbage accumulation and grazing losses on Mulato grass subjected to strategies of rotational stocking management. Sci. Agric., 70, pp. 242-249.
- Schons, R.M.T., Laca, E.A., Savian, J.V., Mezzalira, J.C., Schneider, E.A.N., Caetano, L.A.M., Zubieta, A.S., Benvenutti, M.A., Carvalho, P.C.D.F., 2021. 'Rotatinuous' stocking: an innovation in grazing management to foster both herbage and animal production. Livest. Sci. 245, 104406.
- Sollenberger, L. E., Moore, J. E., Allen, V. G., & Pedreira, C. G. 2005. Reporting forage allowance in grazing experiments. Crop Science, 45, 896-900.
- Turner, L.W., Absher, C.W., Evans, J.K., 1997. Planning Fencing Systems for Intensive Grazing Management.

**CHAPTER 3** 

# FINAL CONSIDERATIONS

Stocking adjustment, fencing and mechanical topping are common interventions used by managers aiming to have a better herbage nutritive value and homogenize sward structure distributions. These actions might ease the grazing management and can help offer good sward structure to be grazed by animals. However, depending on the limits imposed they can reduce the selective behavior of animals, reducing the productive potential of the field.

In this dissertation, we hypothesized that by offering an ideal sward structure for sheep grazing Italian ryegrass in continuous stocking under the "Rotatinuous" concept, no differences would be observed for pasture production, herbage intake and sheep performance because animals are able to graze in an ideal sward height distribution (range of 12 and 18 cm), regardless the use of intervention such as fencing and mechanical topping.

Finally, in order to achieve high sheep herbage intake and performance, and production per area, we recommend that Italian ryegrass swards under continuous stoking should be managed at 15 cm, on average.

# REFERENCES

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**, East Melbourne, v. 21, n. 5, p. 755-766, 1970.

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.

ANDERSON, Dean M. *et al.* Virtual herding for flexible livestock management–a review. **The Rangeland Journal**, Cotlesloe, v. 36, n. 3, p. 205-221, 2014.

BAILEY, Derek W. *et al.* Mechanisms that result in large herbivore grazing distribution patterns. **Journal of Range Management**, Denver, v. 49, n. 5, p. 386-400, 1996.

BARBOSA, Cristina Maria Pacheco *et al.* Terminação de cordeiros em pastagens de azevém anual manejadas em diferentes intensidades e métodos de pastejo. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 36, p. 1953-1960, 2007.

BERGMAN, C. M. *et al.* Ungulate foraging strategies: energy maximizing or time minimizing? **Journal of Animal Ecology**, London, v. 70, n. 2, p. 289-300, 2001.

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

BRISKE, D. *et al.* Rotational grazing on rangelands: reconciliation of perception and experimental evidence. **Rangeland Ecology & Management**, Lakewood, v. 61, v. 1, p. 3-18, 2008.

CANGIANO, C. A. *et al.* Effect of live weight and pasture height on cattle bite dimensions during progressive defoliation. **Australian Journal of Agricultural Research**, East Melbourne, v. 53, n. 5, p. 541-549, 2002.

CARVALHO, P. C. F. *et al.* Características estruturais do pasto eo consumo de forragem: o quê pastar, quanto pastar e como se mover para encontrar o pasto. *In*: SIMPÓSIO SOBRE MANEJO ESTRATÉGICO DA PASTAGEM, 4.; SIMPÓSIO INTERNACIONAL SOBRE PRODUÇÃO ANIMAL EM PASTEJO, 2., 2008, Viçosa. **Anais** [...]. Viçosa, MG: UFV, 2008. p. 101-130.

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

CARVALHO, P. C. F. *et al.* Métodos de pastoreio: uma perspectiva alternativa a décadas de debate e pouco avanço conceitual. *In*: SIMPÓSIO DE PRODUÇÃO ANIMAL A PASTO, 5., 2019, Maringá. **Anais** [...]. Maringá: Universidade Estadual de Maringá, 2019. p. 283-318.

CARNEVALLI, R. A. *et al.* Herbage production and grazing losses in *Panicum maximum* cv. Mombaça under four grazing managements. **Tropical Grasslands**, Brisbane, v. 40, n. 3, p. 165-176, 2006.

DEMMENT, M. W. *et al.* Selective behaviour of cattle grazing ryegrass swards with horizontal heterogeneity in patch height and bulk density. *In:* INTERNATIONAL GRASSLAND CONGRESS, 17., 1993, Queensland. **Proceedings of the** [...]. Palmerston North: New Zealand Grassland Association, 1993 p. 712-714.

FONSECA, L. *et al.* Effect of sward surface height and level of herbage depletion on bite features of cattle grazing sorghum bicolor swards. **Journal of Animal Science**, Champaign, v. 91, n. 9, p. 4357-4365, 2013.

GONÇALVES, Edna Nunes *et al.* Relações planta-animal em ambiente pastoril heterogêneo: processo de ingestão de forragem. **Revista Brasileira de Zootecnia**, Viçosa, MG, v. 38, p. 1655-1662, 2009.

GORDON, I. J.; LASCANO, C. Foraging strategies of ruminant livestock on intensively managed grasslands: potential and constraints. *In:* INTERNATIONAL GRASSLAND CONGRESS, 17., 1993, Queensland. **Proceedings of the** [...]. Palmerston North: New Zealand Grassland Association, 1993. p. 208-216.

GORDON, Iain J. *et al.* Food in 3D: how ruminant livestock interact with sown sward architecture at the bite scale. *In*: BELS, V. (ed.). **Feeding in domestic vertebrates:** from structure to behaviour. Wallingford: CABI, 2006. cap. 15, p. 263-277.

GORDON, Iain J.; PRINS, Herbert H. T.; SQUIRE, Geoff R. (ed.). Food production and nature conservation: conflicts and solutions. Florence: Taylor & Francis, 2016.

HODGSON, J. Variations in the surface characteristics of the sward and the shortterm rate of herbage intake by calves and lambs. **Grass and Forage Science**, Oxford, v. 36, n. 1, p. 49-57, 1981.

HODGSON, John. **Grazing management:** science into practice. Harlow: Longmans Scientific & Technical, 1990.

ILLIUS, A. W.; GORDON, I. J. Prediction of intake and digestion in ruminants by a model of rumen kinetics integrating animal size and plant characteristics. **The Journal of Agricultural Science**, London, v. 116, n. 1, p. 145-157, 1991.

ILLIUS, A. W.; CLARK, D. A.; HODGSON, J. Discrimination and patch choice by sheep grazing grass-clover swards. **Journal of Animal Ecology**, London, v. 61, n. 1, p. 183-194, 1992.

KENNY, P. A.; BLACK, J. L. Factors affecting diet selection by sheep. I. Potential intake and acceptability of feed. **Australian Journal of Agricultural Research**, East Melbourne, v. 35, n. 4, p. 551-563, 1984.

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.

LACA, E. A. Field test of optimal foraging with cattle: the marginal value theorem successfully predicts patch selection and utilisation. *In*: INTERNATIONAL GRASSLAND CONGRESS, 17., 1993, Queensland. **Proceedings of the** [...]. Palmerston North: New Zealand Grassland Association, 1993. p. 709-710.

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, 1995. p. 129-132.

MCDONALD, R. C. Effect of topping pastures 1. Pasture accumulation and quality. **New Zealand Journal of Experimental Agriculture**, Wellington, v. 14, n. 3, p. 279-289, 1986.

MEZZALIRA, J. C. *et al.* Manejo do milheto em pastoreio rotativo para maximizar a taxa de ingestão por vacas leiteiras. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 65, n. 3, p. 833-840, 2013.

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.

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.

PONTES-PRATES, Arthur; CARVALHO, Paulo César de Faccio; LACA, Emilio Andrés. Mechanisms of grazing management in heterogeneous swards. **Sustainability**, Basel, v. 12, n. 20, [art.] 8676, 2020.

RAO, Idupulapati M. *et al.* **LivestockPlus:** the sustainable intensification of foragebased agricultural systems to improve livelihoods and ecosystem services in the tropics. Cali: CIAT, 2015. (CIAT Publication, no. 407).

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.

SAVIAN, J. V. *et al.* A comparison of two rotational stocking strategies on the foraging behaviour and herbage intake by grazing sheep. **Animal**, Cambridge, v. 14, n. 12, p. 2503-2510, 2020.

SAVIAN, J. V. *et al.* 'Rotatinuous' stocking as a climate-smart grazing management strategy for sheep production. **Science of the Total Environment**, Amsterdam, v. 753, [art.] 141790, 2021.

SCHONS, R. M. T. *et al.* 'Rotatinuous' stocking: an innovation in grazing management to foster both herbage and animal production. **Livestock Science**, Amsterdam, v. 245, [art.] 104406, 2021.

SZYMCZAK, Leonardo Silvestri *et al.* Tall fescue sward structure affects the grazing process of sheep. **Scientific Reports**, London, v. 10, [art.] 11786, [p. 1-10], 2020.

TEAGUE, W. R.; DOWHOWER, S. L.; WAGGONER, J. A. Drought and grazing patch dynamics under different grazing management. **Journal of Arid Environments**, London, v. 58, n. 1, p. 97-117, 2004.

TURNER, L. W.; ABSHER, C. W.; EVANS, J. K. **Planning fencing systems for intensive grazing management**. Lexington: University of Kentucky, College of Agriculture, Cooperative Extension Service, 1986.

UTSUMI, Santiago A. *et al.* Resource heterogeneity and foraging behaviour of cattle across spatial scales. **BMC Ecology**, London, v. 9, [art.] 9, [p. 1-10], 2009.

VAINTRUB, M. Odintsov *et al.* Precision livestock farming, automats and new technologies: possible applications in extensive dairy sheep farming. **Animal**, Cambridge, v. 15, n. 3, [art.] 100143, 2020.

# **APPENDICES**

Single appendix - Rules to elaborate and submit a manuscript for Animal Journal

# The International Journal of Animal Biosciences

# **INSTRUCTIONS FOR AUTHORS**

Last updated 24 July 2020

animal – The International Journal of Animal Bioscience is a Gold Open Access peer-reviewed journal, published monthly in English, online formats (12 issues making a volume). Special issues or supplements may also be produced upon agreement with the Editorial Board.

Note: As **animal** moves to a gold open access model from 1st January 2021, all articles submitted after 3<sup>rd</sup> July 2020 and subsequently accepted, will be subject to an Article Publishing Charge (APC). Such articles will be published as open access content and be covered by a <u>Creative Commons Licence</u>.

# Contents

Before you start	2
Scope	2
Authorship and Ethics	2
Length recommendations for different types of article	2
Style sheet and pre-submission checklist	
Initial screening	
Writing your paper - About Contents	4
Your paper is understandable	4
Your paper is complete	
Each section of your main text provides the required information	5
Complying with Image Integrity and Standards	
Supplementary material	
Presenting your paper - About Format.	
General presentation	
Presentation of statistical results	
Numbers and units	
Style	
References	
Illustrations	
Supplementary materials	
Submitting your manuscript	
Submission system	
Files	
File/item types in Editorial Manager	
Required information	
Licences to publish & article processing charge	

Visit https://www.journals.elsevier.com/animal for additional resources.

# **BEFORE YOU START**

## Scope

The scope of the journal, the expected standards of published articles, the article types published by *animal*, ethics, the authorship policy, the pre-print policy, plagiarism falsification fabrication and the handling of misconducts, the licences to publish and the article processing charge, the evaluation procedures and peer-review criteria, procedures for complaints and appeals, proofs and information on publication of the manuscript are presented in the Publication policies available at https://animal-journal.eu/instructions-and-policies/.

Submitted manuscripts should not have been published previously, except in a limited form (e.g. abstract or short communication to a symposium or part of MSc or PhD theses) and should not be under consideration for publication by another journal. Book reviews are not accepted.

# **Authorship and Ethics**

- Authorship complies with the authorship and other ethics policies described in the Publication policies.
- Research described in the manuscript complies with ethical guidelines available on the website https://animal-journal.eu/instructions-and-policies/
- Authors have obtained permission to use copyrighted material in the manuscript prior to submission.

## Length recommendations for different types of article

Recommendations on article length and number of illustrations are described below. Articles may deviate from those guidelines as justified by the research.

# Table 1 Specifications for the articles published in animal

Article type	Maximum length (all text except figures)	Maximum number of tables plus figures	Maximum number of references	Additional information
Research article	7 500 words (= 9 journal pages)	8	35	
Short communication	3 000 words	3	10	
Review article	10 000 words (= 12 journal pages)	10	50	
Opinion paper	1700 words (= 2 journal pages) or 1 200 if a figure is submitted	1	5	
All article types			5 references per 1000 words	Supplementary material can be proposed and will be made available online

# Style sheet and pre-submission checklist

When preparing their manuscript authors are advised to use the journal's style sheet (https://animaljournal.eu/instructions-and-policies/), and to self-evaluate their manuscript before submission using the Pre-submission Checklist (https://animal-journal.eu/instructions-and-policies/). The checklist is provided as a service to authors to aid them getting their manuscript ready for peer-review.

## Initial screening

At submission, manuscripts are viewed by the Editorial Office, the Editor-in-Chief and/or a member of the Editorial Board for compliance with scope, standards and presentation of the manuscript. If the manuscript is incomplete or the quality of the manuscript is insufficient for peer-review, it will be returned to authors for improvements before being assigned to an Editor. If the submission is out of scope or not up to standards, it will be removed by the Editor-in-Chief and no longer considered. Any deviations from instructions will be at the discretion of the Editor-in-Chief Note that the quality of the manuscript is under the responsibility of authors. A good quality is expected to facilitate peer-review and ensure that manuscripts are peer reviewed exclusively on academic merit.

# WRITING YOUR PAPER – ABOUT CONTENTS

# Your paper is understandable

## Scientific writing

A good quality of scientific writing is required. The research must be understandable by the general scientific readership of animal and by specialists.

The structure of the whole text is efficient. The research problem is identified, the context and existing knowledge relevant to the problem is analysed, the hypothesis is clear. The reporting is complete. The central message is identified. Arguments and evidence are presented in a well organised, clear, logical and balanced way from the most general to the specific point. Discussion connects all results obtained in an organised and proper way with a clear interpretation. Sentences are simple, short and direct, the style is concise yet informative and precise. The take-home messages are clear.

## English

i A good quality of written English (syntax, spelling, grammar) is required to facilitate the peer-review process.

Spelling may be in British or American English, but must be consistent throughout the paper. Care should be exercised in the use of agricultural terminology that is ill-defined or of local familiarity. If the English is not good enough, the manuscript will be sent back to the authors with a recommendation that authors have their manuscripts checked by an English language native speaker before re-submission. Elsevier lists third-party services specialising in language editing and / or translation at http://webshop.elsevier.com/languageediting/ and suggests that authors contact them as appropriate. Use of any of these services is at the author's own expense.

## Presentation

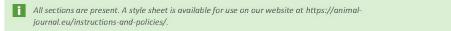
Poor presentation may hamper the understandability of the paper.

The responsibility for the preparation of a paper in a form suitable for a good understanding of the research lies with the author. Authors should follow the Instructions for Authors (see <u>Presenting your</u> paper) and consult recent articles of *animal*, available at https://www.journals.elsevier.com/animal, to make themselves familiar with the layout and style of *animal*.

- The format of (sub-) headings is in accordance with recommendations in order to clarify the structure of the text, especially in the Material and Methods section
- Tables are clearly presented. Treatments are in columns and variables are in lines, as relevant.

# Your paper is complete

Main text - Required sections and order



Full title, Authors, Authors' affiliations including department and post/zip codes, Corresponding author, Abstract, Keywords, Implications, Introduction, Material and methods, Results, Discussion, Ethics

approval, Data and model availability statement, Author ORCIDs, Author contributions, Declaration of interest, Acknowledgements, Financial support statement, References, Tables, List of figure captions

## Figures

Figures are submitted as relevant.

## Supplementary Materials

Supplementary Materials are submitted as relevant.

## Each section of your main text provides the required information

## Full title

The title provides sufficient information to allow the reader to judge the relevance of a paper to his/her interests.

- Concise and informative; no more than 170 characters including spaces
- Include the animal species on which the study has been carried out
- Exclude the name of the country or of the region where the study took place
- Exclude Latin names, if there is a common name
- Exclude non-standard abbreviations. Follow the link to find the standard abbreviations.
- The title of companion manuscripts should reflect it with a Part 1 and a Part 2.
- Title of a review article should start with "Review:", "Invited review:" or "Animal board invited review:" as relevant
- Title of an invited opinion paper should start with "Opinion paper:"
- Title of a short communication should start with "Short communication:"

# Authors and affiliations

Information, such as author names and affiliations should be presented as below.

## Example

J. Smith<sup>a,1</sup>, P.E. Jones<sup>b</sup>, J.M. Garcia<sup>a,c</sup>, P.K. Martin Jr<sup>d</sup> [initials only for first names]

<sup>a</sup>Department of Animal Nutrition, Scottish Agricultural College, West Main Road, Edinburgh EH9 3JG, UK

<sup>b</sup>Animal Science Department, North Carolina State University, Raleigh, NC 27695-7621, USA

<sup>c</sup>Laboratorio de Producción Animal, Facultad de Veterinaria, Universidad de Zaragoza, C. Miguel Servet, 177, 50013, Zaragoza, Spain

<sup>d</sup>Dairy Science Department, North Carolina State University, Raleigh, NC 27695-7621, USA

<sup>1</sup>Present address: Dairy Science Laboratory, AgResearch, Private Bag 11008, Palmerston North, New Zealand (for any author of the list whose present address differs from that at which the work was done)

Corresponding author: John Smith. E-mail: John.Smith@univ.co.uk.

- Only one corresponding author is indicated in the manuscript.
- The corresponding author who submits and manages the manuscript during the submission/review process must be registered on Editorial Manager. He or she can be different from the corresponding author indicated in the manuscript who will be the correspondent for the published paper.

## Abstract (max 400 words, single paragraph)

The abstract should be complete and understandable, without reference to the paper. It follows the same structure as the text.

## WRITING YOUR PAPER - ABOUT CONTENTS

- The context and the rationale of the study are presented succinctly to support the objectives. Experimental methods and main results are summarised but should not be overburdened by numerical values or probability values. The abstract ends with a short and clear conclusion.
- Citations and references to tables and figures are not acceptable.
- Abbreviations used in the abstract must be defined in the abstract.
- The whole abstract is written as a single paragraph.

# Keywords (5 keywords)

Keywords are essential in information retrieval and should not repeat words in the title with respect to indicating the subject of the paper.

- Five keywords (no more, no less)
- Keywords should be different from words in the title
- Keywords should be short and specific
- The animal species or type can be among the keywords but differently from the title
- The use of non-standard abbreviations in the list of keywords is not allowed. Follow the link to find <u>the standard abbreviations</u>.

## Implications (max 100 words)

Implications must explain the expected impact that the results may have on practice, when they will be applied. Impact may be economic, environmental or social.

- After a brief description of the context and the scientific question, highlight your main findings, and describe the potential applications of your own results and their field of application for the livestock industry.
- Be careful not to oversell your results.
- The Implications section should stand alone, be clear to non-specialists while being precise enough for specialists
- Write in simple English suitable for non-specialists or even non-science readers.
- Use of non-standard abbreviations is discouraged.

#### Introduction

The introduction briefly outlines the context of the work, presents the rationale of the scientific issue, and clearly defines the objectives.

- The context is only briefly described. The scientific question is developed and supported by the critical analysis of published work
- For hypothesis-driven research (Sections 1 to 3), the hypothesis under test should be clearly stated.
- Scarcity of studies is not sufficient to justify research. Increasing the knowledge on a subject is not an objective per se.
- If the implementation of the research results in practice could contravene health, animal wellbeing and environmental standards in countries other than where the work was undertaken, then authors should consider how this will impact on the international relevance of the research.
- If the manuscript is companion to another submission, the introduction presents the links between manuscripts.
- If preliminary results have been published in an abstract form, it is indicated at the end of the introduction.

## Material and methods

Material and methods should be described in sufficient detail so that others can reproduce the experiment. References to previously published work may be used to give details of methods, provided that references are readily accessible and in English.

- *Reporting.* Material and methods are reported according to "The ARRIVE Guidelines for Reporting Animal Research" detailed in Kilkenny *et al.* (2010)<sup>1</sup> and summarised at <u>www.nc3rs.org.uk</u>.
- *Experimental design.* When relevant, the experimental design should be presented in a separate sub-section at the beginning of the "Material and methods" section. It explains and justifies the structure of the experimental units (e.g. individual animal, group/pen of animals) and how the controlled experimental factors were organised in treatments to test the hypothesis or answer the specific questions under study. The known and expected sources of variability in experimental units are identified to address replication, blocking or randomization. A power analysis of the experimental design is recommended. Distinction between quantitative and qualitative factors, use of control treatments are presented.
- **Description of critical methods.** For the sake of reproducibility, the methods that are most important for the conclusions of the study, including mathematical equations, must be described in detail. They can be presented in the Supplementary Materials as necessary.
- Validation and Quality Assurance. Validation is defined as a comparison of the research predictions with the real world to determine whether the results of the research are suitable for their intended purpose. Validation highlights the strengths and the limits of the results obtained, and their applicability. A wide range of validation techniques can be applied, including: comparison with reference measurements (e.g. recovery rates for markers or gaz exchange measurements), robustness of measurements (e.g. intra- and inter-observer reliabilities for observational measurements), statistical tests (e.g. regression analysis of observed vs. predicted data), deviance measures (e.g. Mean Absolute Error, Root Mean Squared Error), visual techniques (e.g. plot of observed vs. predicted data), subjective assessment (e.g. evaluation by experts). For laboratory methods, results of Quality Assurance tests or method validation procedures refer to performance of assays (e.g. intra/inter-assay CV, reportable range, specificity, normalisation...). Validation and/or Assurance quality procedure and output<sub>7</sub> must be reported for the methods that are most important for the conclusions of the study in the Material and methods or as Supplementary materials. Alternatively they must be addressed in the Discussion section.
- Statistical analysis of results. The statistical analysis of results should be presented in a separate sub-section at the end of the "Material and methods" section. The software used and the models of statistical analysis must be described, as well as each of the statistical methods used. Sufficient statistical details must be given to allow replication of the statistical analysis. Generally, and when there are more than 2 treatments, an analysis of variance with F-tests is preferred to multiple *t*-tests. A statistical guide for authors is available on the website at <a href="https://animal-journal.eu/instructions-and-policies/">https://animal-journal.eu/instructions-and-policies/</a>. The publication of Lang and Altman (2013)<sup>2</sup> can also be used as a reference. We also recommend you include the code for the statistical model, as programmed in the relevant software, in the Supplementary materials.
- **Proprietary product.** If a proprietary product is used as a source of material in experimental comparisons, it should be described using the appropriate chemical name. If the trade name is helpful to the readers, provide it in parentheses after the first mention.

<sup>&</sup>lt;sup>1</sup> Kilkenny C, Browne WJ, Cuthill IC, Emerson M and Altman DG 2010. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biology 8, e1000412, doi: 10.1371/journal.pbio.1000412.

<sup>&</sup>lt;sup>2</sup> Lang T and Altman D 2013. Basic statistical reporting for articles published in clinical medical journals: the SAMPL guidelines. In Science editors' handbook (ed. Smart P, Maisonneuve H and Polderman A), pp. 175-182. European Association of Science Editors, Exeter, UK. This document may be reprinted without charge but must include the original citation.

Results - Discussion

Separation between Results and Discussion is preferred to highlight the interpretation of results.

- Presentation of Results and Discussion in a single section is possible but discouraged.
- If the implementation of the research results in practice could contravene health, animal wellbeing and environmental standards in countries other than where the work was undertaken, then authors should consider how this will impact on the international relevance of the research. A short conclusion appears at the end of the text and is merged in a single paragraph.

## Ethics approval

Where research involves animal experimentation, authors should assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on animal experimentation.

- When relevant, the full reference/number of the committee approval should be provided.
- Due consideration of the 3Rs (Refinement, Reduction, Replacement) is expected.
- When the study did not require approval by an institutional committee, include the following statement: 'Not applicable'.

## Data and model availability statement

Authors must indicate whether their data, the model or the software developed as an outcome of the study are deposited in an official repository. Access rights should also be specified.

- If data, models or software are deposited in an official repository, provide the full reference.
- If not applicable, you can use one of the following statements as appropriate: 'None of the data were deposited in an official repository.' or 'The model was not deposited in an official repository.'
- Whether deposited or not, always indicate the access rights to data, software or model (available to reviewers, available upon request, public, confidential...).
- Do not indicate the software that were used in the study, they belong to the Material and methods section.

## Author ORCIDs

The corresponding author should provide his/her ORCID number.

• All co-authors are encouraged to indicate their ORCID number.

## Author contributions

The contribution of each co-author should be explained: conception or design of the work, acquisition, analysis and interpretation of data, drafting and critically revising the manuscript.

- Author contributions should be described according to the CRediT taxonomy
- They have to be formatted by the author's name followed by the relevant credit role(s). More details and a sample CRediT author statement is available at <u>https://www.elsevier.com/authors/journal-authors/policies-and-ethics/credit-author-statement</u>.

#### Declaration of interest

Please provide details of all known financial, professional and personal relationships with the potential to bias the work.

• Where no known conflicts of interest exist, include the following statement: "None."

#### Acknowledgements

In this section, the authors may briefly acknowledge individuals or organisations that provided advice, their credits to companies, preliminary publications of the research, etc.

- Individuals who contributed to the article but do not meet the full criteria for authorship should be acknowledged here.
- If the research was conducted as part of a thesis, it should be acknowledged here, and the full reference should be provided.
- If the article was deposited in a pre-print repository, it should be acknowledged here, and the full reference should be provided.

## Financial support statement

Please provide details of the sources of financial support for all authors, including grant numbers.

- Example of statement: 'This work was supported by the European Commission (grant number XXXXXX)'
- Grants held by different co-authors should be identified according to individual authors by the author's initials.
- When no specific funding has be provided, you may use the following statement: 'This research received no specific grant from any funding agency, commercial or not-for-profit section'.

#### References

References from international refereed journals or from national refereed journals with at least an English abstract are preferred.

- References from non peer-reviewed articles or from national abstracts/conference proceedings, MSc or PhD thesis, institutional/technical reports, documents that cannot be obtained easily by the reader should be minimized.
- If a submitted manuscript has previously been published in a limited form (e.g. abstract or short communication to a symposium or part of MSc or PhD theses), the previous publication form should be cited and the full reference should be provided.
- In general, no more than 3 references can be given for the same statement (except for reviews and meta-analyses).
- The list of references used in a meta-analysis should be presented in Supplementary materials.

## Tables

Tables should be explicit while concise and should not include details on materials and methods in the captions or footnotes. The reporting of statistical results complies with simple basic rules.

• Tables are recommended when exact numerical values are important or may be re-used later in meta-analysis. The same material should not be presented in tabular or graphical form.

- The animal species and the experimental treatments (or the issue) under study are indicated in each caption.
- When data are analysed by analysis of variance *animal* requests that a residual error term such as the pooled standard error, the residual standard deviation (RSD), or the root mean square error (RMSE) is reported in tables and not SE/SD for each treatment. Indeed, anovas are based on the hypothesis of homogeneous variance among treatment groups.
- Probabilities are given as numerical values and not as "NS" when considered not significant.
- The number of decimals of P values for means and/or the error term should be homogenized or should follow a systematic rule.
- The number of "decimal places" is different from the number of "significant figures". This is especially important when reporting coefficients in equations. In the equation  $Y=a+bX+cX^2$ , the number of meaningful decimal places depends on the value of X.

## Figures

Figures should be explicit while concise and should not include details on materials and methods in the captions or footnotes.

- Figures are recommended to illustrate trends. The same material should not be presented in tabular or graphical form.
- The animal species and the experimental treatments (or the issue) under study are indicated in each caption.

# **Complying with Image Integrity and Standards**

## Image Integrity and Standards

Any image produced by an instrument (e.g. scanner, microscopy) with the objective of being used to derive quantitative results is considered as original data. Manuscripts that report images without any quantitative findings are not acceptable. Digitalisation of an image converts the image into numerical values that can be analysed like any other numerical values. The full information may prove important beyond what the author would like to show. Hence images submitted with a manuscript should be minimally processed; some image processing is acceptable (and may be unavoidable), but the final image must accurately represent the original data are used just to illustrate a point, this should be accompanied by a clear statement in the manuscript telling the reader this and explaining what is being demonstrated. Please refer to the <u>Office of Research Integrity</u> <u>guidelines</u> on image processing in scientific publication.

- Image acquisition: Equipment and conditions of image acquisition and processing must be detailed in the Material and methods section. This includes the make and model of equipment, the acquisition and the image processing software, and the image treatment if any. If you export files from an acquisition device, make sure to use a format with no loss of information and do not file them into a higher resolution than that of acquisition. Authors have the responsibility to archive original images, with their metadata, in their original format without any compression or compressed without loss of information.
- Preparation of images for a manuscript: For guidance, we refer to the Journal of Cell Biology's
  instructions to authors (<u>http://jcb.rupress.org/site/misc/ifora.xhtml#image\_aquisition</u>) which
  states:
  - No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
  - 2) The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (i.e., using dividing lines) and in the text of the figure legend.
  - 3) Adjustments of brightness, contrast, or colour balance are acceptable if they are applied to every pixel in the image and as long as they do not obscure, eliminate, or misrepresent any information present in the original, including backgrounds. Non-

linear adjustments (e.g., changes to gamma settings) must be disclosed in the figure legend.

For further information, image examples, and more detailed guidance, we advise reading <u>What's in a picture? The temptation of image manipulation</u> (reprinted in the *Journal of Cell Biology* (2004) 166, 11-15).

- If a cropped image is included in the main text of a paper (e.g. a few lanes of a gel), display the full original image, including the appropriate controls, the molecular size ladder and/or the scale as relevant, as a single figure in a Supplementary Material file to facilitate peer-review and for subsequent on-line publication.
- The statistical analysis applied to the quantitative data associated with images must clearly define the statistical unit considered (e.g. the animal, the sample).
- Image screening prior to acceptance: Digital images from submissions will be screened for any evidence of improper manipulation or quality. If the original images cannot be supplied by authors on request, the journal reserves the right to reject the submission or to withdraw the published paper.

# Supplementary material

**1** Authors can include supplementary material in any type of article. It will be peer-reviewed along with the rest of the manuscript.

- Detailed description of critical methodologies and procedures and results of validation and Quality Assurance should be reported in Supplementary materials if not included in the Material and methods section.
- The main text of the article must stand alone without the supplementary material.
- A link to this on-line supplementary material will be included by the Copy Editor at the proof stage.
- The title of the article, the list of authors, their affiliations and the journal name are included at the top of the Supplementary materials.

# PRESENTING YOUR PAPER – ABOUT FORMAT

Authors should consult recent articles of *animal*, available at https://www.journals.elsevier.com/animal, to make themselves familiar with the layout and style of *animal*.

A style sheet summarising the below indications is available on our website at https://animaljournal.eu/instructions-and-policies/. We recommend that you use it to insert your text.

## **General presentation**

## Manuscript layout

Manuscripts should be prepared using a standard word processing programme such as Microsoft Word, and presented in a clear, readable format with easily identified sections and headings.

- Typed with double-line spacing with wide margins (2.5 cm)
- Lines must be continuously numbered; the pages must also be numbered
- Arial 12 should be used for the text, and Arial 11 for tables and references
- Use of small paragraphs with less than 6 to 8 lines must be avoided
- Footnotes in the main text are to be avoided

## Title and headings

The format of title and headings is in accordance with instructions in order to clarify the structure of the text.

- Title use bold, with an initial capital for the first word only and for words that ordinarily take capitals
- Authors' names use lower case with initials in capitals (e.g. J. Doe)
- Authors' addresses use italics
- Headings are left aligned with an initial capital for the first word only, and are not numbered.
- Limit sections to three heading levels Heading 1, Heading 2, Heading 3.
  - Examples: Material and Methods Experimental design The experiment was designed as... Analytical methods Feed analyses Feeds were analysed... Milk fatty acid composition The composition of...

#### Abbreviations

# Standard abbreviations (Table 2) are not defined.

- Define non-standard abbreviations at first appearance in the abstract and in the main text
- Authors should avoid excessive use of non-standard abbreviations. A maximum of 10 is advised
- No non-standard abbreviation in the (short) titles, in (sub)headings or in keywords
- Non-standard abbreviations used in tables and figures must be defined either as footnotes or in the caption
- Do not start a sentence with an abbreviation

Item	Definition		
Standard abbreviation			
ACTH	Adrenocorticotropic hormone		
ADF	Acid detergent fibre		
ADL	Acid detergent lignin		
ADP	Adenosine diphosphate		
ANOVA	Analysis of variance		
ATP	Adenosine triphosphate		
BLUP	Best linear unbiased prediction		
BW	Body weight		
CoA	Coenzyme A		
CP	Crude protein		
DM	Dry matter		
DNA	Deoxyribonucleic acid		
ELISA	Enzyme-linked immunosorbent assay		
FSH	Follicle-stimulating hormone		
GLC	Gas-liquid chromatography		
GLM	General Linear Model		
HPLC	High performance (pressure) liquid chromatography		
IGF	Insulin-like growth factor		
IR	Infrared		
LH	Luteinising hormone		
MS	Mass spectrometry		
n	Number of samples		
NAD	Nicotinamide adenine dinucleotide		
NADP	Nicotinamide adenine dinucleotide phosphate		
NADPH <sub>2</sub>	Reduced nicotinamide adenine dinucleotide phosphate		
NDF	Neutral detergent fibre		
NIRS	Near infrared stectrophotometry		
PAGE	Polyacrylamide gel electrophoresis		
PCR	Polymerase chain reaction		
PMSG	Pregnant mare serum gonadotropin		
RNA	Ribonucleic acid		
SDS	Sodium dodecyl sulfate		
UV	Ultraviolet		
Standard statistical abbreviat			
CV	coefficient of variation		
df	degrees of freedom		
EMS	expectation of mean square		
F	variance ratio		
LSD	least significant difference		
MS	mean square		
Р	probability		
use ns	$P \leq 0.05$ , in tables		
use *	$P \le 0.05$ , in tables		
use **	$P \leq 0.01$ , in tables		
use ***	$P \leq 0.001$ , in tables		
r	simple correlation coefficient		
R	multiple correlation coefficient		
$R^2$	coefficient of determination		
RSD	residual standard deviation		
RMSE	root mean square error		
SD	standard deviation		
SE	standard error		
SED	standard error of difference		
SEM	standard error of mean		
Sy.x	standard error of estimate		
$\chi^2$	chi square		

Table 2 Standard abbreviations that do not require definition

The names of the chemicals do not need to be written in full; chemical symbols are sufficient. Fatty acids are abbreviated using the rule: cis-18:1 for the sum of cis octadecenoic acids. When isomers are

described, the double bond positions are identified by numbering from the carboxylic acid end: c9,t11-18:2; iso-15:0. The terms "omega 3" and "omega 6" are discouraged and replaced by "n-3" and "n-6", e.g. 18:3n-3. Trivial names can be used for most known fatty acids (myristic, palmitic, oleic, linoleic, linolenic) and abbreviations in some cases: CLA for conjugated linoleic acids, EPA for eicosapentaenoic acid, DHA for docosahexaenoic acid. Chemical names and trivial names cannot be mixed in a same table.

# Presentation of statistical results

In the text and tables, presentation of statistical results follows simple rules.

- Treatment means are reported with meaningful decimals. For guidance, the last digit of a treatment mean corresponds to 1x10 of standard error (e.g., for a standard error of 1.2, the mean values should be reported as 15)
- In the text, the probability of significance is indicated either by the exact level of probability (e.g. P = 0.07) or by the following conventional standard abbreviations (which need not be defined): P < 0.05, P < 0.01 and P < 0.001 for significance at these levels.</li>
- In tables, when data are analysed by analysis of variance, a residual error term, is given for each criteria/item/variable/trait in a separate column
- In tables, probabilities are indicated in a separate column. The numerical P values (e.g. P = 0.07) are reported. In figures differences can be indicated by \*, \*\* and \*\*\* for P < 0.05, P < 0.01 and P < 0.001, respectively
- In tables, differences between treatments (or comparison of mean values) are indicated using superscript letters with the following conventional standard: a, b for P < 0.05; A, B for P < 0.01.</li>

# Numbers and units

The format of numbers and units should be consistent.

#### Numerals

- In the text, use words for numbers zero to nine and numerals for higher numbers. In a series of two or more numbers, use numerals throughout irrespective of their magnitude
- Do not begin sentences with numerals
- For values less than unity, 0 is inserted before the decimal point
- For large numbers in the text, substitute 10<sup>n</sup> for part of a number (e.g. 1.610<sup>6</sup> for 1 600 000)
- Do not use a comma separator for numbers greater than 999 (e.g. 100 864)
- The multiplication sign between numbers should be a cross (x)
- Division of one number by another should be indicated as follows: 136/273.
- Use numerals if a number is followed by a standard unit of measurement (e.g. 100 g, 6 days, 4th week).
- Use numerals for dates, page numbers, class designations, fractions, expressions of time, e.g. 1 January 2007; type 2
- Dates are given with the month written in full and the day in numerals (i.e. 12 January *not* 12th January).
- For time use 24-h clock, e.g. 0905 h, 1320 h

## Units of measurement

The International System of Units (SI) should be used. A list of units is found at <u>http://physics.nist.gov/cuu/Units/units.html</u>. Recommendations for conversions and nomenclature appeared in *Proceedings of the Nutrition Society* (1972) 31, 239-247. Some frequently used units that are not in the SI system are accepted: e.g. I for litre, ha for hectare, eV for electron-volt, Ci for curie. Day, week, month and year are not abbreviated. The international unit for energy (energy value of feeds, etc.) is Joule (or kJ or MJ).

 A product of two units should be represented as N·m and a quotient as N/m (e.g. g/kg and not g.kg<sup>-1</sup>).

• When there are two quotients, represent as: g/kg per day (not g/kg/day).

## Concentration or composition

Composition is expressed as mass per unit mass or mass per unit volume. The term *content* should not be used for concentration or proportion.

# Style

The style should be consistent.

## Capitals

- Initial capitals are used for proper nouns, for adjectives formed from proper names, for generic names and for names of classes, orders and families
- Names of diseases are not normally capitalised

# Italics

Use italics for:

- Authors' addresses (see above)
- Subheadings (see above)
- Most foreign words, especially Latin words, e.g. ad hoc, ad libitum, in situ, inter alia, inter se, in vitro, per se, post mortem, post partum, m. biceps femoris but no italics for c.f., corpus luteum, e.g., etc., i.e., NB, via
- Mathematical unknowns and constants
- Letters used as symbols for genes or alleles e.g. *HbA*, *TfD* (but not chromosomes or phenotypes of blood groups, transferrins or haemoglobins, e.g. HbAA, TfDD)

# References

It is the author's responsibility to ensure that all references are cited and accurate.

- All sources must be cited in the text using the author-date system and must have an entry in the reference list.
- Names of organisations used as authors (e.g. Agricultural and Food Research Council) should be written in full in the list of references and on first mention in the text. Subsequent mentions may be abbreviated (e.g. AFRC).

## In-text citation conventions

Cite references by name(s) of author(s) and year of publication by chronological order.

- For single authors, use Doe (2014) or (Doe, 2014)
- For two authors, use Doe and Smith (2014) or (Doe and Smith, 2014)
- For three or more authors, use Doe et al. (2014) or (Doe et al., 2014)
- When multiple references are cited, rank them preferably by chronological order using commas and semicolons: (Doe, 1999; Smith and Doe, 2001; Doe et al., 2014 and 2015; Wright et al., 2018a and 2018b)

## List of references

In the reference list, references should be listed in alphabetical order by authors' names. Their formatting and style should be as detailed below.

Authors' information and publication year

Author, A., Author, B., Author, C.D., Author, E., Year.

- Include a comma after every family name and in-between different authors' names
- Include a period after every initial
- Commas before and full stops after publication years
- Note that all authors must be listed

Publisher/Conference/University location

Publisher, City, State (2-letter abbreviation) for US places, Country

Examples:

- AOCS Press, Champaign, IL, USA
- Cambridge University Press, Cambridge, UK
- International Organization for Standardization, Geneva, Switzerland
- FAO, Rome, Italy
- Louisiana State University, Baton Rouge, LA, USA
- Cambridge University, Cambridge, UK

## Journal article

Author(s), Year. Article title. Full Name of the Journal Volume, first-last page numbers.

- Journal names are given in full, not in abbreviated form.
- Issue numbers are not required.

#### Examples:

- Martin, C., Morgavi, D.P., Doreau, M., 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351-365.
- Berry, D.P., Wall, E., Pryce, J.E., 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 (suppl. 1), 115–121.
- Knowles, T.G., Kestin, S.C., Haslam, S.M., Brown, S.N., Green, L.E., Butterworth, A., Pope, S.J., Pfeiffer, D., Nicol, C.J., 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. PLoS ONE 3, e1545.
- Pérez-Enciso, M., Rincón, J.C., Legarra, A., 2015. Sequence- vs. chip-assisted genomic selection: accurate biological information is advised. Genetics Selection Evolution 47, 43. doi:10.1186/s12711-015-0117-5.
- When the article is online but not yet printed, the right format is:
  - Zamaratskaia, G., Squires, E.J., 2008. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal, doi:10.1017/S1751731108003674, Published online by Cambridge University Press 17 December 2008.

## Book (or official report)

Author(s)/Editor(s)/Institution, Year. Book title, volume number if more than 1, edition if applicable. Publisher's name, City, State (2-letter abbreviation) for US places, Country.

- If a publisher is based in more than one place, use only the first one.
- If multiple publishers are listed, it is acceptable to use only the first one.

## Examples:

- Association of Official Analytical Chemists (AOAC), 2004. Official methods of analysis, volume 2, 18th edition. AOAC, Arlington, VA, USA.
- Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., 1996. SAS system for mixed models. Statistical Analysis Systems Institute Inc., Cary, NC, USA.
- Martin, P., Bateson, P., 2007. Measuring behaviour. Cambridge University Press, Cambridge, UK.

- National Research Council (NRC), 2012. Nutrient requirements of swine, 11th revised edition. National Academy Press, Washington, DC, USA.
- Statistical Analysis Systems Institute, 2002. SAS user's guide, version 9.00. SAS Institute Inc., Cary, NC, USA.

## Book chapter (or part of an official report)

Author(s), Year. Chapter title. In Title of book (ed. A Editor and B Editor). Publisher's name, City, State (2-letter abbreviation) for US places, Country, pp. first-last page numbers.

- If a publisher is based in more than one place, use only the first one.
- If multiple publishers are listed, it is acceptable to use only the first one.

Example:

 Nozière, P., Hoch, T., 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In Nutrient digestion and utilization in farm animals (ed. E Kebreab, J Dijkstra, A Bannink, WJJ Gerrits and J France). CABI Publishing, Wallingford, UK, pp. 40–47.

## Proceedings (or Conference papers)

Author(s), Year. Paper title. Proceedings of the (or Paper presented at the) XXth Conference title, date of the conference, location of the conference, pp. first-last page numbers or poster/article number.

- · Conference dates in the form Day Month Year.
- Note If proceedings are published in a journal, the article should be formatted as for a journal article. If they have been published as chapters in a book, the article should be formatted as for a chapter in a book.

## Examples:

- Bispo, E., Franco, D., Monserrat, L., González, L., Pérez, N., Moreno, T., 2007. Economic considerations
  of cull dairy cows fattened for a special market. In Proceedings of the 53rd International Congress of
  Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.
- Martuzzi, F., Summer, A., Malacarne, M., Mariani, P., 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, 26-29 August 2001, Budapest, Hungary.

#### Website

Author(s)/Institution, Year. Document/Page title. Retrieved on DD Month YYYY (i.e. accessed date) from http://www.web-page address (URL).

#### Example:

 Bryant, P., 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from http://darwin.bio.uci.edu/~sustain/bio65/Titlpage.htm

## Thesis

Author, A.B., Year. Thesis title. Type of thesis, University with English name, City, State (2-letter abbreviation) for US places, Country (i.e. location of the University).

#### Example:

 Vlaeminck, B., 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. PhD thesis, Ghent University, Ghent, Belgium.

# Illustrations

Tables and Figures should be simple. The same material should not be presented in tabular and graphical form.

## Tables

- Each table is on a separate page at the end of the main text (one table per page)
- Tables are numbered consecutively using Arabic numbering. They are referred to as Table 1, Table 2, etc., with capital 'T', no italics
- Each table has its own explanatory caption. The caption is sufficient to permit the table to be understood without reference to the text but remains concise. The animal species and the experimental treatments or the issue under study are indicated in each caption.
- Units are clearly stated either in the caption (only if a limited number of units are used), or for each (sub-)item. Standard abbreviations for units are used
- Tables are created in MS Word using the table function within the programme (without using tabs). Layout can be portrait or landscape
- Single spacing is possible for long tables
- Variables are in rows and treatments in columns
- Separate columns are included to present the basic statistical results: error terms (preferably residual error terms) and exact probabilities
- · No vertical lines between columns and no horizontal lines between rows of data
- Main items are aligned on the left-hand side. Sub-items are indented. For any (sub-)item, only the first letter of the first word is in capitals
- Footnotes are referenced using superscript numbers
- Abbreviations used in a table are defined as footnotes (preferred option) or in the caption
- Treatment means are reported with meaningful decimals. For guidance, the last digit corresponds to 1/10 of standard error
- Number of decimals for the indicators of residual variability (e.g. RSD, SEM, RMSE) are either identical to that chosen for mean values or have one more decimal. The choice is consistent in all the tables
- Follow the link to Presentation of statistical results for the presentation of statistical results in tables

#### Figures

Specific guidelines are provided for images (see Image Integrity and Standards), and detailed information for preparing your artwork is available at https://www.elsevier.com/authors/author-schemas/artwork-and-media-instructions.

- Figure captions are all listed on a separate page at the end of the main text. They are sufficiently detailed to allow the figures to be understood without reference to the text, e.g. 'Fig. 1. Effect of fat source and animal breed on carcass composition in pigs' is preferred to 'Fig. 1. Carcass composition'. The animal species and the experimental treatments or the issue under study are indicated in each caption.
- Abbreviations used in each figure have to be defined in the caption and kept to a minimum
- Figures are numbered consecutively in the text using Arabic numbering. They are referred to as Fig. 1, Fig. 2, etc., with capital 'F', no italics
- In figures statistical differences can be indicated by \*, \*\* and \*\*\* for P < 0.05, P < 0.01 and P < 0.001, respectively
- Figures are not inserted in the text. Each figure (without caption) is uploaded separately with one separate file per figure and no embedded captions in these files
- Figure size should be readable in a width of approximately 175 mm (i.e. the maximum size of printing over two columns). Easy reading of the figure is required
- Ensure that the font size is large enough to be clearly readable at the final print size (should not be less than 8 point, or 2.8 mm, after reduction).
- Symbols and line types should allow different elements to be easily distinguished (generally, solid symbols are used before open symbols, and continuous lines before dotted or dashed lines)
- Figures should be provided as TIFF or EPS files. Other formats, such as MS Word, MS Excel, MS PowerPoint, AI and layered PSD (up to CS5), are permitted, provided that figures have

been originally created in these formats and that the embedded artwork is at a suitable resolution. If your drawing/graphics application does not provide suitable 'export' options, then copy/paste or import the graphic into a Word document

- Resolutions for TIFF figures at the estimated publication size must be:
  - for line figures (e.g. graphs) 1000 dpi (3600 px for 1 column, 7500 px for 2 columns)
    for figures with different shadings (e.g. bar charts) 500 dpi (1800 px for 1 column,
    - 3800 px for 2 columns) 200 dri (1100 px for 1 columns) 2200 px for 1
  - for halftone images (e.g. photographs) 300 dpi (1100 px for 1 column, 2300 px for 2 columns)

# Supplementary materials

Supplementary material should be presented according to the instructions for the main text. It will not be copyedited and authors are entirely responsible for the presentation of the supplementary material according to animal's style.

- In the main text, supplementary material is referred to as: "Supplementary Table S1", "Supplementary Table S2", etc. for tables "Supplementary Figure S1", "Supplementary Figure S2", etc. for figures "Supplementary Material S1", "Supplementary Material S2", etc. for other material For example: "The list of references used for the meta-analysis is given in Supplementary Material S1 and Supplementary Table S1 reports, etc."
- Supplementary material is submitted along with the main manuscript in a separate file and identified at uploading as "Supplementary File for Online Publication Only"
- The title of the article, the list of authors and the journal name are included at the top of the supplementary material
- No line numbering
- Single spacing
- Unlike the figures included in the main text, each supplementary figure has its own title embedded below the figure

# SUBMITTING YOUR MANUSCRIPT

Before submitting your manuscript, you should self-evaluate if your manuscript is in scope and standards of the journal and ready for peer-review using the submission checklist at (https://animal-journal.eu/instructions-and-policies/).

Companion manuscripts should be submitted at the same time.

# Submission system

Manuscript submission is made electronically through Editorial Manager directly via http://www.editorialmanager.com/animal. Authors should ensure that the email address of the corresponding author is correct.

- Any query about a submission to the Editorial Office or the Editor should be addressed through Editorial Manager.
- Authors can check the status of their manuscript using Editorial Manager.
- Before submission, any query to the Editorial Office for clarification of instructions to authors, to ask if paper is within the scope or if a review article is of interest to the journal etc should be addressed through <u>questions@animal-journal.eu</u>.
- Please see <u>https://www.ariessys.com/wp-content/uploads/EM-Author-English.doc</u> for the author tutorial, including the Logging In details.

## Files

Separate files are submitted for the main text, figures, Supplementary materials, Covering letter, Permissions and responses to reviewers.

# You must submit separate files for:

- The main text which includes the full text, tables, figure captions, but excludes figures. The file should be in DOC/DOCX or RTF format (PDF is not accepted)
- Each figure (without captions). At submission in *Editorial Manager*, enter a description of each figure (Figure 1, Figure 2a, etc.) in the appropriate box
- Supplementary online-only materials, if relevant
- The covering letter stating any comment and information that might be helpful to the editors and Editorial Office. If the submission was recommended by PCI Animal Science, the link to the review reports and the PCI recommendation should be provided.
- The response to referees' comments for revised versions
- Written permissions to reproduce copyrighted material.

The authors must obtain a written permission to reproduce material that is owned by a third party (for example in review papers); they must also include the relevant credit in their paper. Forms and guidance are available at

https://www.elsevier.com/about/policies/copyright/permissions.

# File/item types in Editorial Manager

Authors are required to select the appropriate item for each file to be uploaded at the 'Attach Files' step.

- Select 'Manuscript' for the main text
- Select 'Figure' for each figure
- Select 'Supplementary File for Online Publication Only' for the supplementary material
- · Select 'Covering Letter' for the covering letter
- Select 'Copyright and Permissions' for the permissions to reproduce copyrighted material
- Select 'Response to Referee Comments' for the response to referees' comments

# **Required information**

At submission in Editorial Manager, detailed information is required

Authors who submit a manuscript to the online submission system also have to provide:

- The type of article (research article, short communication, review article, special issue paper, invited opinion paper, etc.).
- An ORCID identifier for the corresponding author at least.
- The email address of each co-author
- The section of the scope which is the most appropriate for their manuscript (https://animaljournal.eu/instructions-and-policies/).
- The names and e-mail addresses of at least 3 potential reviewers. Reviewers should have no conflict of interest with the authors or the submission. Authors should nominate *an international spread of reviewers; they* should not nominate reviewers who are their regular collaborators or who work in the same institution or university. The editorial board will use its discretion when selecting reviewers and the suggested reviewers may not be used.
- The names of up to 3 opposed reviewers in case of established conflict of interest
- The confirmation that it is an original contribution approved by all authors, that all permissions to reproduce copyrighted material have been obtained and that any conflict of interest is declared in the 'Declaration of interest' section.

# LICENCES TO PUBLISH & ARTICLE PROCESSING CHARGE

Animal is a Gold Open Access journal, which publishes articles under Gold Open Access. As described in the Publication policies document available at https://animal-journal.eu/instructions-and-policies/, authors will be asked to complete a 'User Licence Agreement' and accepted articles will be subject to a Gold Open Access Publication Fee.

# VITA

Thainá Silva de Freitas, daughter of Pedro Augusto Inácio de Freitas and Débora Santos da Silva, was born on June 27<sup>a</sup> in 1996, in Porto Alegre/RS. She attended elementary school at E.M.E.F. Aurora Pedroso de Camargo and high school at E.E.E.F.M. Presidente Vargas, in Dourados/MS. In 2014, Thainá started her major in Animal Science at the Federal University of Rio Grande do Sul (UFRGS), in Porto Alegre/RS. During this period, she developed research activities in the Grazing Ecology Research Group lab advised by Prof. Dr Paulo César de Faccio Carvalho. Thainá graduated in Animal Science in April/2019, and in the same month, she started her Master's degree in Animal Science at the Postgraduate Program in Animal Science — UFRGS, also advised by Prof. Dr Paulo César de Faccio Carvalho.