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

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# SHEEP INGESTIVE BEHAVIOR AND TANNIN SELECTION IN PAMPA GRASSLAND AND FEEDLOT

Porto Alegre
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Orientador: César H. E. C. Poli
Coorientador: Juan J. Villalba

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# COMPORTAMENTO INGESTIVO DE OVINOS E SELEÇÃO DE TANINOS NO CAMPO NATIVO DO BIOMA PAMPA E EM CONFINAMENTO

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## RESUMO

O campo nativo do bioma Pampa possui grande biodiversidade de plantas, algumas das quais com concentrações significativas de compostos secundários, como taninos. A inclusão de taninos na dieta de ruminantes pode melhorar a qualidade e a produtividade da carne. No entanto, a ingestão voluntária desse composto fenólico ainda é um desafio. Esta tese inclui três estudos dentro deste contexto. O primeiro estudo questionou se os ovinos consomem a tanífera *Desmodium incanum* (DI), a espécie de leguminosa mais comum no Pampa. O segundo estudo avaliou como a seleção da dieta em ovinos é influenciada pela concentração de taninos e proteínas, além de avaliar os efeitos da dieta escolhida em parâmetros de desempenho. O terceiro estudo avaliou o comportamento alimentar de ovinos em pastagem nativa do Pampa, e como ele é influenciado pela estação do ano, características do pasto e infecção parasitária. O pastejo de DI foi observado através do método de plantas marcadas. Após três dias de pastejo pelo menos 70% das plantas foram pastejadas. No segundo estudo, o consumo diário total diminuiu quando o tanino foi adicionado à ração preferida, com alto teor de proteína. No entanto, quando a alimentação isenta de tanino não foi uma opção, a inclusão de 4% extrato de tanino não afetou o consumo diário total, além de diminuir nitrogênio ureico do sangue e aumentar excreção de nitrogênio fecal. Ganho médio diário não foi afetado pela adição de tanino. O tempo de pastejo foi maior no outono, enquanto a primavera e o verão não apresentaram diferença. O tempo de ruminação diminuiu com a duração da luz do dia: o valor da primavera foi maior que o do verão, que foi maior do que o do outono. Houve uma correlação positiva moderada entre a porcentagem de material morto no pasto e o tempo percentual de pastejo ($P<0,000$, $r = 0,462$), e uma correlação negativa moderada entre o material morto e o tempo de ruminação ($P<0,000$, $r = -0,488$). Quando bem manejado, o campo nativo do bioma Pampa oferece condições ótimas para a ovinocultura sustentável, produzindo carne de alta qualidade, resultante da ingestão de taninos no pasto. Mais estudos são encorajados a abordar o efeito da ingestão de taninos de DI nos resultados de desempenho.

**Palavras-chave**: *Desmodium incanum*, comportamento alimentar, campo nativo, ruminante

**SHEEP INGESTIVE BEHAVIOR AND TANNIN SELECTION IN PAMPA GRASSLAND AND FEEDLOT**

**Author:** Marina Terra Braga
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## ABSTRACT

The Pampa grassland biome has high plant biodiversity, some with significant secondary compound concentrations such as tannins. Tannin inclusion in ruminant diets may improve meat quality and productivity. However, voluntary intake of this phenolic compound can be a challenge. This dissertation includes three studies within this context. The first study documented whether sheep consume tannin-rich *Desmodium incanum* (DI), the most common Pampa herbaceous legume species. The second study evaluated how forage tannin and protein concentration influenced sheep feedlot feed selection and the diet affects performance parameters. The third study evaluated sheep feeding behavior in a Pampa native pasture, and how season, sward characteristics and parasite infection influenced it. Tagging individual DI plants allowed identification of those sheep consumed. After 3 days of grazing, >70% were grazed. In the second study, feed intake decreased when tannin was added alongside a high-protein feed. However, when tannin-free feed was not a choice, the 4% tannin extract inclusion did not affect feed intake, aside from decreasing blood urea nitrogen and increasing fecal nitrogen excretion. Average daily gain was not affected by the inclusion of tannins. Fall grazing time was greater, while spring and summer showed no difference. Ruminating time decreased with season progression: spring > summer> fall. There was a moderate positive correlation between dead material percentage in the pasture and grazing time ($P < 0.000$, $r = 0.462$), and a moderate negative correlation between dead material and ruminating time ($P < 0.000$, $r = -0.488$). When managed correctly, Pampa offers optimal conditions for sustainable sheep farming, producing high-quality meat resulting from forage tannin ingestion. Further studies are encouraged to address DI tannin intake influence on sheep performance.

**Keywords**: *Desmodium incanum*, feeding behavior, natural grasslands, ruminant

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## LIST OF ABBREVIATIONS

ADF – acid detergent fiber

ADG – average daily gain

ADL – acid detergent lignin

BUN – blood urea nitrogen

BW – bodyweight

CP – crude protein

CT – condensed tannin

Da – Dalton

DI – *Desmodium incanum*

DM – dry matter

EDTA – ethylenediaminetetraacetic acid

FCR – feed conversion ratio

FEC – fecal egg count

g – gram

Ha – hectare

HM – herbage mass

HP – high protein

HP+T – high protein with tannin

Kg – kilogram

LP – low protein

LP+T – low protein with tannin

mm – millimeters

mg – milligrams

MM – mineral matter

MW – metabolic weight

N – nitrogen

OMD – organic matter digestibility

PBCT – protein-bound condensed tannin

PPP – precipitation of proteins by phenol

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# CHAPTER I

# 1. INTRODUCTION

Tannins are plants' secondary compounds often related but not limited to protection against grazing. For decades, their effects have been studied on ruminant feeding and nutrition particularly because of the consequences brought by their affinity for dietary protein. Depending on the concentration and structure, tannin inclusion on ruminant diets may bring benefits to meat production both in quantity and quality. However, the main challenge is the fact that tannin often restrict feed intake.

The Pampa biome is a diverse and vast grassland historically tied to herbivore farming in South America. Unfortunately, these prairies have been ravaged by mismanage and replacement by row crops. Studies on livestock farming in this environment are essential to unveil solutions for a sustainable and lucrative production. The biodiversity of the Pampa flora offers optimal conditions for producing good quality meat. In this context, Tontini *et al*. (2019) evaluated secondary compounds and nutritional values of seven common tropical forage species. *Desmodium incanum*, a Pampa native, had the highest concentration of tannin, more than 4x the second highest reported concentration, which was found in pinto rhizoma peanut (*Arachis pintoii*). Such findings suggest that *D. incanum* could be an important source of tannin for herbivores in the Pampa, since this species is the main legume of this biome and it is found essentially year-round, except in winter (BOLDRINI, 1993).

This dissertation thesis is composed of five chapters:

- ✓ Chapter I comprises this introduction, a literature review, hypotheses and objectives.
- ✓ Chapter II presents the manuscript "An evaluation of the role of *Desmodium incanum* as a source of condensed tannins for ruminants in the Pampa biome grasslands."
- ✓ Chapter III presents the manuscript "Trade-offs between selection of nutrients and tannin in growing lambs."
- ✓ Chapter IV presents the manuscript "Sheep diurnal ingestive behavior in a Pampa biome pasture."
- ✓ Chapter V comprises final considerations, annexes, and the author's *vita*.

The first study addresses whether sheep actually eat the tanniferous legume *Desmodium incanum*. This research was carried out in the three seasons where this plant is found (spring, summer and fall). The second study focuses on how sheep selection of feed is influenced by tannin and protein levels. The effects of the chosen diet on performance parameters were also observed. Finally, the third study characterizes sheep feeding behavior in a Pampa native pasture, and how it is influenced by season, sward characteristics and parasite infection.

# 2. LITERATURE REVIEW

## 2.1. Concerning tannins

### 2.1.1. Etymology, concept, and location in the plant

Tannins have been known and used for many centuries, being present in feeds, foods and drinks. The name tannin derives from French “tan” meaning the bark of a tree, particularly the holm oak or other trees used in tanning (FRUTOS *et al*., 2004).

Tannins are secondary compounds, meaning they are not involved directly in plant survival and reproduction, but rather in secondary functions. They are broadly distributed all over the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants (FRUTOS *et al*., 2004). In fact, tannins are found in 80% of woody perennial dicotyledons and 15% of annual and herbaceous perennial dicotyledon species approximately (WAGHORN, 2008). They play a role in the plant’s defense against microbial pathogens, insects and larger herbivores (DIXON; XIE; SHARMA, 2005).

Tannins occur in almost every part of the plant. However, they are generally more abundant in the most valuable parts for herbivores, such as new leaves and flowers (FRUTOS *et al*., 2004). Within the cells, they are found inside the vacuoles (MIN *et al*., 2003). For this reason, only when the cell is broken by mastication, tannins are liberated, interfering on animal metabolism. Tannin availability in the plant varies according to the season and physiological state of the plant, being a consequence of the different demand for nutrients. During their growth, plants have few resources available for the synthesis of secondary compounds. Conversely, during flowering, the plant growth is reduced, and more carbon is available for tannin synthesis (IASON; HARTLEYT; DUNCAN, 1993). Tannin availability increases in pathological conditions (JERÓNIMO *et al*., 2016) and is also induced by abiotic stress, such as UV-light (MELLWAY; CONSTABEL, 2009), hydric stress, temperature, ozone and nutrient availability (TREUTTER, 2006).

### 2.1.2. Chemical structure

Most of the effects tannins have on animals upon ingestion are related to their protein binding capacity which is explained by their chemical structure. The high affinity for proteins is due to the phenolic groups present in tannins, providing several points at which the carbonyl groups of peptides bind (FRUTOS *et al*., 2004). Tannins are composed of 12 to 16 phenolic groups and 5 to 7 aromatic rings per 1000 units of relative molecular mass (JERÓNIMO *et al*., 2016).

Traditionally, tannins have been divided into two types: hydrolysable and condensed. Hydrolysable tannins are formed by a carbohydrate core (often glucose) esterified with phenolic acids (mostly gallic and ellagic acid). Condensed tannins are non-branched polymers or oligomers of flavonoids units (flavan-3-ol, flavan-3,4-diol), commonly linked by carbon-carbon bonds in the 4/6 or 4/8 position (JERÓNIMO *et a*l., 2016). Hydrolysable tannins occur only in dicotyledonous, while condensed tannins occur in angiosperms and gymnosperms. However, it’s not impossible to find both tannins in the same plant (SILANIKOVE; PEREVOLOTSKY; PROVENZA, 2001). The chemical structures of both hydrolysable and condensed tannins are illustrated in Figure 1.

Hydrolysable tannins have a molecular weight of 500-3000 Da and are more soluble in water. They are also more prone to enzymatic and non-enzymatic hydrolysis than condensed tannins (JERÓNIMO *et al*., 2016). Hydrolysable tannins can be toxic to ruminants, particularly when eaten in large amounts, leaving insufficient time for rumen microbial adaptation. Nevertheless, animals may adapt to diets containing hydrolysable tannin, enabling acceptable levels of production with appropriate feeding management (WAGHORN, 2008).

Condensed tannins have higher molecular weight compared to hydrolysable tannins: 1000-20000 Da. They are also called proanthocyanidins, since they can be oxidatively degraded and release anthocyanidin pigments in acid conditions (JERÓNIMO *et al*., 2016). They are the most common type of tannin in forage legumes, shrubs, and tree leaves (MIN *et al*., 2003).

Tannins from different species of plants have distinct physicochemical properties, resulting in different bond affinity levels. The affinity with proteins comes from the number of phenolic groups in the tannin. However, the degree of affinity also depends on the proteins (FRUTOS *et al*., 2004). Proteins most likely to show affinity for tannins are large, with open and flexible structure and rich in prolines and other hydrophobic amino acids rather than small proteins with compact globular structures (FRUTOS *et al*., 2004; JERÓNIMO *et al*., 2016). The complex formed by the binding tannin-protein is generally unstable. According to (KUMAR; SINGH, 1984), bonds may be of tour types:

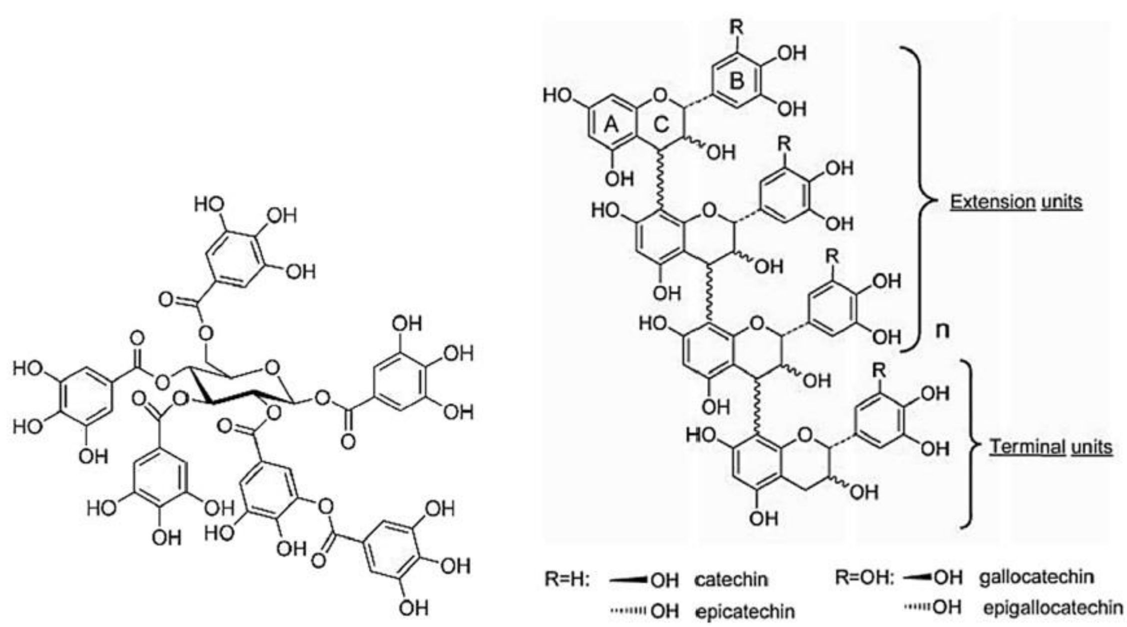
1) Hydrogen bonds (reversible and dependent of pH) between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of protein;

2) Hydrophobic interactions (reversible and dependent of pH) between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein;

3) Ionic bonds (reversible, exclusive of hydrolysable tannins) between the phenolate ion and the cationic site of the protein;

4) Covalent bonding (irreversible) through the oxidation of polyphenols to quinones and their subsequent condensation with nucleophilic groups of the protein.

**Figure 1 -** Chemical structure of hydrolysable tannin (a) and condensed tannin (b**)**




**A** **B**

**From** Huang *et al*., 2018.

### 2.1.3. Effects on ruminant feeding and nutrition

The effect of tannins on ruminant metabolism is mostly related to their affinity for proteins. Whether the consequences of tannin ingestion will be beneficial, neutral or detrimental depend on many factors such as tannin type, structure and weight, species and category of animal and amount ingested (MUELLER-HARVEY *et al*., 2019). According to Frutos *et al*. (2004), three mechanisms can explain the negative effects of high concentrations of tannin on feed intake: decrease in feed palatability, slowing of digestion and development of conditioned aversion.

The decrease in palatability may be caused by binding with protein saliva or by a direct reaction with taste receptors, leading to an astringent sensation. In their review, Frutos *et al*. (2004) explained that proline, a protein present to different degrees in ruminant species' saliva, have a high capacity to bind with tannin. The complex tannin-proline remains stable across the whole pH range of the digestive tract. This might withdraw the negative effect on food intake, negate the benefits to ruminant nutrition and health, as well as promote higher intake of tannin-rich feeds. The authors suggest that proline-rich saliva may be an adaptive mechanism for the consumption of tannin-rich plants, since browsing animals secrete it constantly and sheep produce them when consuming plants rich in tannin.

Slow digestion results in a full rumen during a greater amount of time. Consequently, satiety is signaled to the centers involved in feed intake. Previous studies using tannin infusion directly on the sheep's rumen found a significant decrease on dry matter intake (NARJISSE; ELHONSALI; OLSEN, 1995). This decrease was independent of palatability and could be explained by slower digestion of dry matter in the rumen.

As for conditioned aversion, it may be linked to slow digestion. Aversion to food is nearly always generated by post-prandial experience (FRUTOS *et al*., 2004). Additionally, when animals experience toxicity due to tannin ingestion, they will probably develop food conditioned aversion.

In his review, Waghorn (2008) explains that when diets exceed the amount of protein required by the animal, tannins may reduce degradation without limiting amino acid availability for absorption. Conversely, when protein concentrations are close to or bellow animal needs, tannins are detrimental to animal performance. One of the consequences of dietary tannin is a reduction in proteolysis. Although microbial flow to the intestine is not necessarily reduced, high concentrations of tannins will decrease volatile fatty acid concentration, since the rumen pool size tends to increase due to slower rate of digestion. A reduced rate of digestion, especially fiber, results in more rumination and reduces voluntary feed intake – such process may lead to negative impact in productivity, especially if the tannin limits microbial growth or amino acid absorption in the rumen. Nevertheless, it is important to remember that the effect on protein digestion and amino acid absorption will depend on the tannin type (according to the bonds formed), as well as the quantity ingested (TEDESCHI; RAMÍREZ-RESTREPO; MUIR, 2014).

## 2.2. Implications on ruminant productivity and sustainability

### 2.2.1. Weight Gain

Some studies reported improved weight gain when offering condensed tannin to ruminants. Quebracho tannin offered at 1 and 2% (kg condensed tannin / kg herbage dry matter) to steers resulted in linear increase of final weight and live weight gain (MIN *et al.*, 2006). The 2% treatment resulted in 20.8% of increase in live weight gain when compared to the control treatment, with no tannin. However, apart from tannin type and quantity ingested, other factors also contribute to either a beneficial or a detrimental effect on ruminants. For instance, when dietary crude protein (CP) concentrations surpass animal needs, tannin may enhance dietary protein use, leading to improved animal performance, such as liveweight gain. In contrast, when dietary CP concentrations are low and fiber concentrations are high, condensed tannins are no longer an advantage (WAGHORN, 2008).

### 2.2.2. Anthelmintic effect

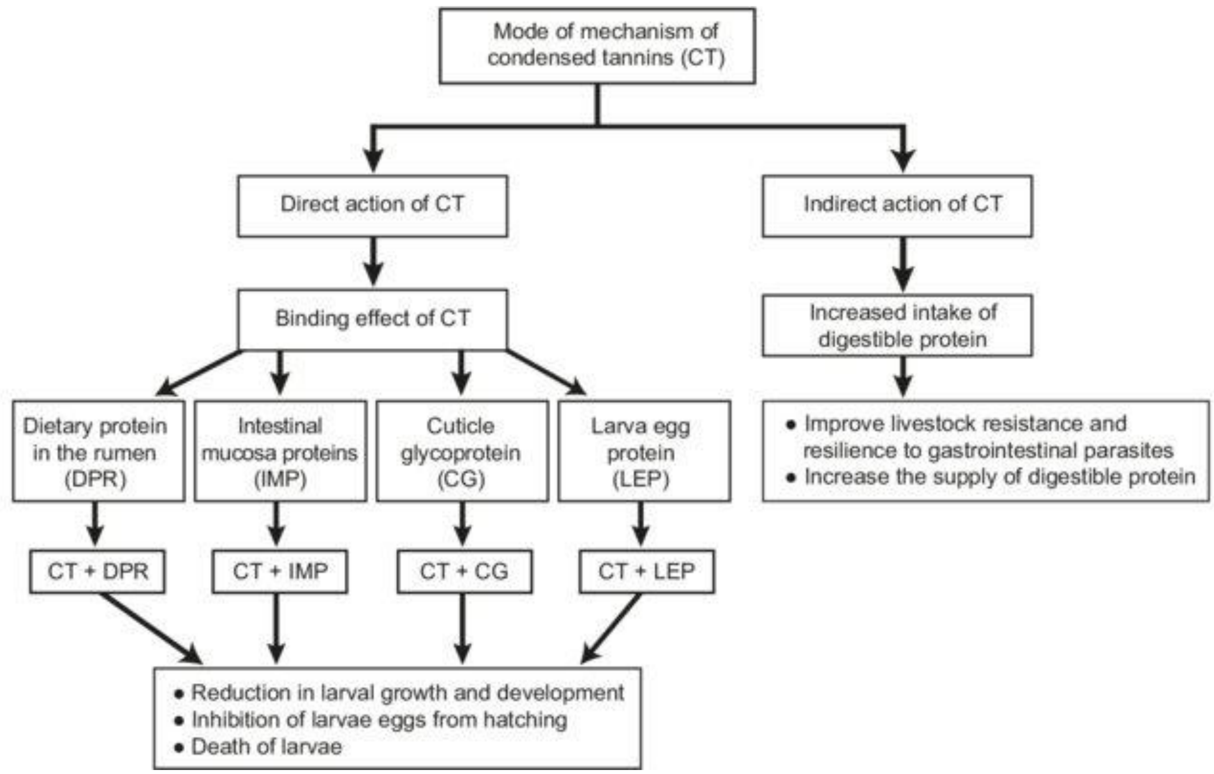
Another indirect effect of tannins on ruminant nutrition and performance is achieved by minimizing the negative effects of blood-consuming gastro-intestinal nematode parasites (TEDESCHI *et al*., 2014). The anthelmintic properties of tannins result of direct and indirect mechanisms (ATHANASIADOU *et al*., 2001). Direct mechanisms are occasioned by tannin binding capacity. Firstly, while binding to dietary proteins, they left less proteins for larvae nutrition, leading to larvae starvation and death. Tannins can also bind to the glycoproteins of the larvae cuticle and kill it directly. Another direct mechanism is achieved by impairing female fertility (number of eggs) as a result of poor nutrition left to parasites. The indirect mechanism against internal parasites results of enhanced immunological response. When the host benefits from improved dietary protein use, as a consequence of a diet including tannin, there is a better chance that its intestinal mucosa can recover and that the animal will endure/tolerate parasitic infection. A scheme with condensed tannin's mechanisms of action in reducing worm burdens is illustrated in Figure 2.

A study using 18% of *Acacia mearnsii* tannin offered to naturally infected sheep found a decrease in fecal egg count (FEC) after 8 weeks of treatment (CENCI *et al*., 2007). However, no difference was found for parasite burden within the period of the experiment.

### 2.2.3. Antioxidant effect and environmental impact

Phenolic compounds such as tannins are also known to have antioxidant properties (JERÓNIMO *et al*., 2016). Studies offering tanniferous to sheep and cattle found positive impacts on antioxidant status of animals (LÓPEZ-ANDRÉS *et al*., 2013; LUCIANO *et al*., 2011). Additionally, diets containing tannins also improve the oxidative stability of meat during storage (LUCIANO *et al*., 2011). Finally, tannins can also reduce environmental impacts by lowering rumen methane emissions (TEDESCHI *et al*., 2014) and by increasing the urea recycling efficiency, which results in lower nitrogen (N) loss via urine into the environment (BARRY; MCNABB, 1999).

**Figure 2 –** Mechanisms of action of condensed tannins in reducing worm burdens in ruminant animals.

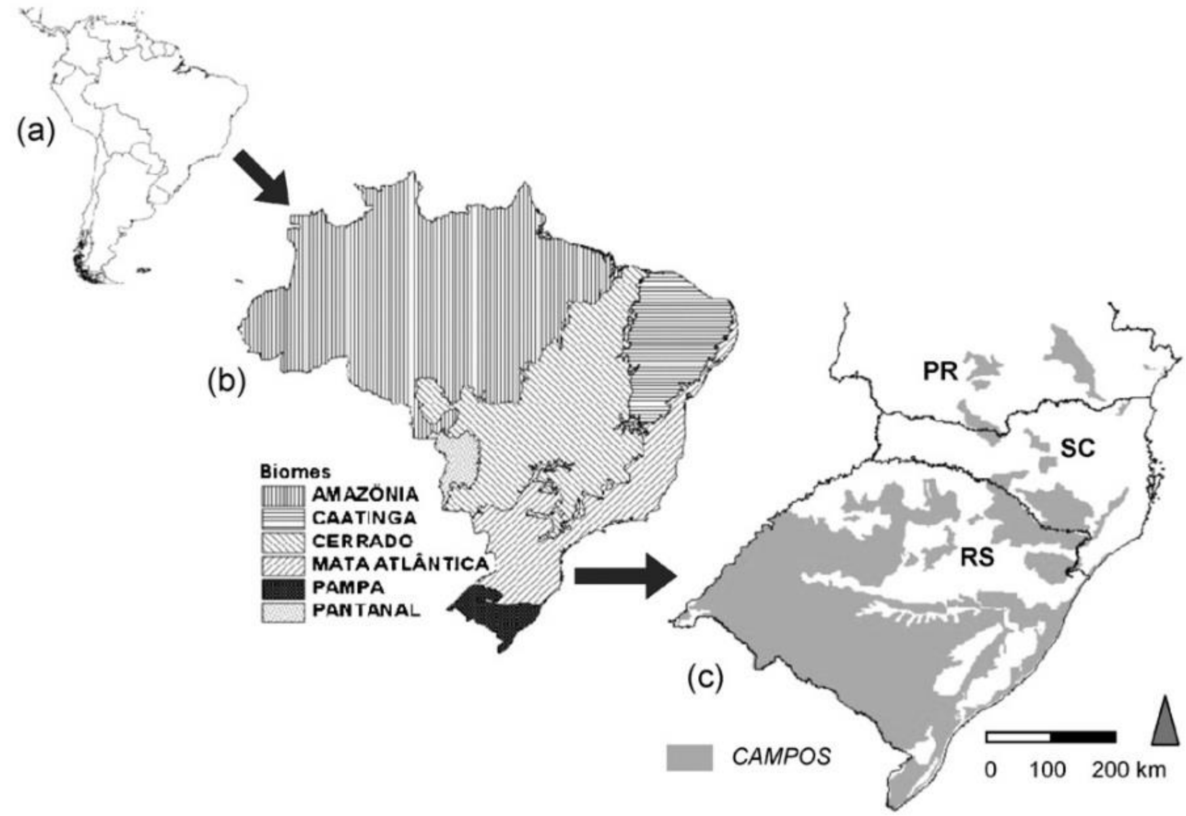



**From** Idamokoro *et a*l. (2016)**.**

## 2.3. The Pampa biome grasslands

The Pampa biome is a vast and rich grassland which covers part of the southernmost state of Brazil: Rio Grande do Sul. It is part of the Río de la Plata natural grasslands, the largest in South America, which also covers parts of Argentina and Uruguay. In Brazil, the pampa is one of the six officially recognized biomes and represents roughly 2% of the country's territory (CARVALHO *et al.*, 2009). The Pampa grasslands are the foundation of domestic herbivorous feeding since early European colonization. Location of Pampa grasslands in Brazil is illustrated in Figure 3.

**Figure 3 -** Location of South Brazilian grasslands: (a) overview, (b) official Brazilian classification of biomes (IBGE, 2004) and (c)distribution of grasslands in Brazil's southern region (abbreviation of states: RS: Rio Grande do Sul; SC: Santa Catarina, PR: Parana).




**From** Overbeck *et al*. (2007).

### *Desmodium incanum*

In the Pampa biome, previous studies reported that the most common legume species is *Desmodium incanum* (CAPORAL; BOLDRINI, 2007; CASANOVA *et al*., 2013; FERREIRA; SETUBAL, 2009; HERINGER; JACQUES, 2002). A study on tropical pastures evaluated concentrations of condensed tannin on several species used in grazing: *Panicum maximum*, *Cynodon spp*., *Cajanus cajan*, *Arachis pintoii* and *D. incanum* (Tontini et al., 2019). After analyzing samples collected during summer and autumn, they found that *A. pintoii* and *D. incanum* were the only species that contained condensed tannin (concentrations of 2.45 and 8.04% of dry matter respectively. In fact, there was an interaction between species and season for extracted condensed tannin, with an increase during autumn. Moreover, condensed tannin biological activity was detected only in *D. incanum*, with a mean of 60.1 g kg-1 DM. The results of this study disclose the potential of *D. incanum* as a source of condensed tannin in south Brazilian native pastures.

## 2.4. Tannin voluntary intake by ruminants

The reduction in palatability of tannin-rich feeds and plants is mainly caused by the astringency sensation as tannin binds to salivary proteins (Jerónimo *et al*., 2016). However, the amount of tannin that an animal intakes voluntarily may vary according to internal and external changes.

One important internal change that may lead to increased tannin intake is parasitic infection. A study with parasitized and non-parasitized lambs found that the first group ingested significantly more of the tannin-containing food during the first 12 days of the experiment (LISONBEE *et al*., 2009). Subsequently, this difference became smaller and disappeared by the end of the study when parasite burdens decreased. The authors suggested that parasitized lambs may have sensed the beneficial effects of consuming a tannin-rich feed (i.e., reduced parasitic burdens), which encouraged further consumption. Another study using artificially parasitized lambs offered dietary choice with or without tannin (JUHNKE *et al*., 2012). They found that infected lambs ingested more of the tannin feed and less of the tannin-free feed compared to non-infected animals. Moreover, increased preference for the tannin-rich diet coincided with the days when infection was at its highest (based on fecal egg count – FEC), which also suggests that animals identify and select a diet with more tannin when coping with gastrointestinal parasites.

Nevertheless, diet selection in practical cannot be solely attributed to tannin presence and quantities. Tannins are not ingested alone, but with other nutrients present in the selected plants. To address this question, a recent study evaluated not only intake of tannin, but preference for diets of high (HEP) or low (LEP) energy to protein ratios before, during and after parasitic infection (COSTES-THIRÉ *et al*., 2019). During the parasitic infection, all groups increased both preference for HEP and intake of digestible energy (DE) in relation to the previous period, and this pattern that remained after infection. As for tannin intake, it only increased during infection when the tannin was added to the high energy feed (HEP), suggesting that energy to protein ratio of the food selected was more consequential than the presence of tannins.

An herbivore's preference for a plant chemically defended by secondary compounds is affected by the context of nutrient availability in the moment of the exposure to that plant (BARAZA; VILLALBA; PROVENZA, 2005). A study with sheep and goats demonstrated that diets with higher concentrations of protein resulted in increased voluntary intake of one-seed juniper (*Juniper monosperma*), a tannin-rich plant (UTSUMI *et al*., 2009). Another study, also using sheep and goats, offered concentrate either high in energy or high in protein, and a third treatment containing both (VILLALBA; PROVENZA; BANNER, 2002). This study also evaluated the effect of polyethylene glycol on tannin intake, since it attenuates the negative effects of tannins. After the supplementation, animals were offered a diet containing 15% of quebracho tannin extract. Animals that had received protein-rich concentrate (both exclusively and associated with energetic concentrate) had higher intakes of the tannin-rich diet compared to the treatment that received energy-rich diet exclusively. Supplementation with polyethylene glycol also increased the intake of tannin extract. These results reveal the conspicuous role of the nutritional context on voluntary tannin intake.

In order to take advantage of tannin's potential benefits, it is important to know the factors that lead the animal to consume this secondary compound. Seasonal variations generally result in differences not only in biomass availability, but also in concentrations of nutrients and secondary compounds. These variations are a reality in several countries. Therefore, it is important to consider nutritional context to understand the animal's interaction with condensed tannins. Furthermore, internal changes, such as parasitical burden may also lead to increased tannin ingestion, but nutritional characteristics appear to play even more important role.

# 3. HYPOTHESES AND OBJECTIVES

## 3.1. Hypotheses

**CHAPTER II:**

*Desmodium incanum*, the most common legume species in the Pampa biome grasslands, is rich in tannins. I hypothesize that this plant is an important source of condensed tannins for sheep, as they willingly eat it in the three seasons when it is found.

**CHAPTER III:**

The inclusion of tannin may alter sheep feed preference and nutrient selection depending on diet protein content. Tannin inclusion may enhance dietary protein use and reflect on better performance and blood parameters.

**CHAPTER IV:**

Seasonal Pampa native grassland herbage characteristics induce differences in sheep ingestive behavior, caused by differences in daylight duration, weather conditions, sward structure and parasite infection.

## 3.2. Objectives

**CHAPTER II:**

1) Elucidate if sheep graze *D. incanum* in the Pampa.
2) Measure condensed tannin concentration across the annual cycle of *D. incanum*.

**CHAPTER III:**

1) Evaluate the trade-offs between selection of nutrients and tannins.
2) Assess the effects of diet on performance and blood parameters of growing lambs.

**CHAPTER IV:**

1) Describe sheep ingestive behavior in spring summer and fall as correlated with sward characteristics and parasite burden.

# CHAPTER II

## An evaluation of the role of *Desmodium incanum* as a source of condensed tannin for ruminants in the Pampa biome grasslands[1]

[1]Elaborado de acordo com as normas do African Journal of Range and Forage Science

**An evaluation of the role of *Desmodium incanum* as a source of condensed tannins for ruminants in the Pampa biome grasslands**

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**Abstract**

*Desmodium incanum* (DI) is the predominant legume species in the Pampa. It contains considerable amounts of condensed tannins (CT), which may be beneficial for ruminants but may also reduce intake. The aims of this study were to evaluate sheep consumption of DI and to assess CT concentration across DI annual cycle. Six paddocks of 0.13 ha were grazed by 5–6 sheep for two weeks per season. Before grazing, each paddock was evaluated for botanical composition and forty-five plants of DI were tagged using colored wires. Concentration of protein-bound CT (PBCT) and crude protein (CP) were analyzed in DI samples. DI cover did not differ among the seasons (9.17 ± 1.47 %). Tagged DI grazing was similar between seasons with an average of 76.46

± 10.64 % ($P$ > 0.05). Values of PBCT and CP found in DI were, respectively, 12.9 and 12.62 % (spring), 13.5 and 13.18 % (summer) and 9.9 and 13.94 % (fall). The tagging method showed that DI was grazed through its entire cycle. In all seasons, animals grazed at least 70% of the tagged plants within the first 3 days in the paddocks regardless the high content of PBCT observed in DI tissues.

Key words: creeping beggarweed, native grasslands, secondary compounds, sheep, tanniferous plants

**Introduction**

Condensed tannins are secondary compounds found in plants, related, but not limited to protection against grazing. Nevertheless, their ingestion may bring beneficial effects to ruminants, particularly due to their protein binding capacity (Waghorn 2008). Because of this affinity for proteins, CT reduce protein degradation in the rumen, increasing in certain conditions the nutritional value of feed (Mueller-Harvey et al. 2019). Hence, when available dietary CP concentrations exceed animal requirements, these effects can improve animal performance (Waghorn 2008). Improvements are reported for weight gain (Min et al. 2006; Montossi et al. 1996), milk production and quality (Jerónimo et al. 2016) and wool production (Montossi et al. 1996). Condensed tannin are also known for their antioxidant properties, bringing positive impacts on antioxidant status of the carcasses (Luciano et al. 2011; López-Andrés et al. 2013) and increasing the oxidative stability of meat during storage (Luciano et al. 2011). Finally, CT has anthelmintic properties (Athanasiadou et al. 2001) and can reduce environmental impacts by lowering rumen methane emissions (Tedeschi, Ramírez-Restrepo and Muir 2014) and by increasing urea recycling efficiency, which results in lower nitrogen loss via urine into the environment (Barry and McNabb 1999).

The Pampa biome is a vast and rich grassland which covers parts of Argentina, Uruguay and southern Brazil. It is one of the largest fertile prairies in the world and has been the main source of feed for domestic herbivores for centuries (Carvalho et al. 2009). However, in the past decades, the use of this native pasture has been inefficient and often inadequate, and the biome is being devastated by activities that in the short term give greater economic return (Nabinger et al. 2009).

Studies concerning the multidisciplinary aspects of these grasslands are essential to improve their use as a feed source for ruminants, aiming at more sustainable and profitable production systems.

A recent study (Tontini et al. 2019) evaluated secondary compounds and nutritional values of seven common tropical forage species: guinea grass (*Panicum maximum*), bermudagrass (*Cynodon* spp.), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), creeping beggarweed (*Desmodium incanum*), macrocephala stylo (*Stylosanthes macrocephala*) and pinto rhizoma peanut (*Arachis pintoii*). The highest concentration of CT was found in the legume *D. incanum* (66.5 ± 13.8 g $kg^{-1}$ dry matter [DM]), more than 4x the second highest reported concentration, which was found in pinto (15.7 ± 5.2 g $kg^{-1}$ DM). *D. incanum* also had the highest percentage of extractable CTs (68%) of the total CT and was the only species for which the CT exhibited biological activity (protein precipitation by phenols = 60.1 g $kg^{-1}$ DM). Such findings suggest that *D. incanum*, commonly known as creeping beggarweed, is potentially an important source of CT for herbivores in the Pampa, since this species is the main legume plant species of this biome and it is found essentially year-round, except in winter (Boldrini 1993).

Some authors reported a decrease in voluntary intake of plant species containing more than 50 g CT $kg^{-1}$ DM (Frutos et al. 2004). However, all these studies used monocultures; in a diverse native grassland such as in the Pampa biome, animals select and graze different species of plants to meet their nutritional requirements, which may promote interactions among forage chemicals that attenuate such effects (Provenza et al. 2003). By consuming varied species, animals can profit from the plant secondary compounds, minimizing their negative effects and enhancing their potential benefits (Provenza et al. 2007; Provenza 2008; Villalba et al. 2011).

The aims of this study were to evaluate whether sheep graze *D. incanum* in diverse natural pastures in the Pampas and to assess CT concentration across the annual cycle of this plant.

## Material and Methods

### *Study site and experimental design*

This study was approved by the Ethics Committee on Animal Use [Comissão de Ética no Uso de Animais da Universidade Federal do Rio Grande do Sul (CEUA-UFRGS) – project 39705]. It was conducted from December 2020 to May 2021 at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, located 46 m above sea level in southern Brazil (29°13′26″ S, 53°40′45″ W). The climate is classified as subtropical humid (Cfa classification, Köppen) with a long-term average rainfall of 1 440 mm per year distributed evenly throughout the year.

The three seasons where *D. incanum* is found (spring, summer, and fall) were established as treatments in a completely randomized design using six paddocks of 0.13 ha as replications. Evaluations were carried out over periods of 2 weeks in each season (1 to 15 December 2020; 9 to 23 February 2021; and 17 May to 1 June 2021), when the paddocks were grazed by 5 to 6 wethers during each season. The same animals were placed in the same paddocks in each season. In the last season, one animal per group was removed from the study to adjust stocking rate. The area had not been occupied by sheep previously.

Due to an infestation of annoni weed between period 1 and period 2 of evaluation, we used herbicides control with glyphosate (1L diluted in 20L of water) using chemical hoes in one application.

*Animals*

Thirty-six 18-month old sheep, 20 wethers and 16 ewes were used in this study. In the first period of evaluation (spring), animals weighed 34.9 ± 4.8 kg. Means for summer and fall were 41.3 ± 5.2 kg and 45.6 ± 4.9 kg, respectively. Sheep were assigned in six groups of six, with at least two females per group and attaining equivalent stocking rates.

*Pasture evaluations*

Sward height was calculated as the mean of 100 random measures per paddock. A 1-m sward stick was used to measure the highest point of the leaf from the ground (Bircham 1981). Evaluations of herbage mass (HM) were carried using 0.25-m² frames to measure eight sample points per paddock (four at the average pasture height and four at random spots). The samples were cut close to the ground and weighed. Subsequently, they were homogenized, and a subsample was used for evaluation of botanical composition, separating the following herbage components: *D. incannum*, grasses, other species and dead material. The separated subsamples were oven-dried at 60ºC until they reached constant weight, then weighed to a precision of 0.01 to assess dry matter (DM; kg $ha^{-1}$).

The pasture composition showed some seasonal differences (Table 1). Spring was the season with the highest mass of *D. incanum*, which was more than 3.5 times the amount found in summer. Summer was the season with the lowest occurrence of this species: before grazing, values of total pasture height and mass, and the herbage mass (HM) of *D. incanum* and grasses were lower than the other two seasons. However, this was the only season when values of total mass of grasses and dead material did not show a reduction after grazing. Finally, fall was the season with the highest HM, but nearly half of it was dead material. Results for pasture height, total DM, DM of *D. incanum*, grasses, other species and dead material before and after grazing are shown in Table 1.

*Botanical composition and consumption of Desmodium incanum*

Before stocking paddocks with animals for each evaluation period, 45 plants of *D. incanum* were tagged within each paddock using colored phone wires. To identify the plants, squares of 0.5 $m^2$ were used to locate nine different spots, where five plants were tagged. Each spot was identified using two flags at opposite corners of the square. With a sketch indicating the location of each of the tagged plants, it was possible to identify and evaluate the plants individually. After 24, 48 and 72 hours of animal grazing, the tagged plants were assessed for grazing. The number of leaves and development stage of the plant (reproductive or vegetative) were also noted to help detect grazing. Once the plant was grazed in any part, its tag was removed. Plant species cover (botanical composition) was evaluated up to three days before the evaluation period using the Londo scale for visual estimation of cover-abundance (Londo 1976).

*Chemical composition and forage intake*

Samples of pasture were collected using the hand-pluck technique to simulate the process of herbage grazing by the animals (Cook, Stoddart and Harris 1951). Samples were analyzed for content of DM, mineral matter (MM), CP, acid detergent fiber (ADF) and neutral detergent fiber (NDF) according to AOAC (1995), acid detergent lignin (ADL) according to Goering and Van Soest (1970) and organic matter digestibility (OMD) according to Nocek (1988).

Total fecal collection was conducted from two males per paddock for 5 consecutive days within each season, using fecal-collection bags fastened to the animal with a harness. The fecal bags were emptied twice daily, and the fresh feces were weighed and recorded. A sample of approximately 20% of that voided was taken to be dried at 60ºC for DM estimates.

Consumption of DM (g/day) was calculated using the formula: CDM = fecal production in g of DM / (1– forage digestibility). Subsequently, consumption per unit metabolic weight was calculated using the formula: CDM / (live body weight)$^{0.75}$.

Samples of *D. incanum* were also collected from areas surrounding the experimental paddock in all three seasons. They were freeze-dried and evaluated for CP, NDF and ADF as described above. Protein-bound CT (PBCT) was extracted with 10 mL of sodiumdodecyl sulfate (1% w/v) mercaptoethanol (5% v/v) containing Tris hydrochloride (0.01 M), as described by Naumann et al. (2014).

### *Statistical Analyses*

Total DM (%) of *D. incanum*, grasses, other species and dead material were analyzed using nonparametric Kruskal-Wallis test, as well as the mean *D. incanum* cover in the paddocks. Cumulative proportion of plants grazed was analyzed by ANOVA, using day and season as fixed factors and a 5% probability criterion. Means were compared by Tukey test.

DM, MM, CP, NDF, CT and digestibility of grazing simulation samples were analyzed by ANOVA at 5% of probability using season as a fixed factor and paddocks as a random factor. Means were compared by Tukey test. ADF and ADL were analyzed by nonparametric Kruskal-Wallis.

Intakes per metabolic weight of total DM and CP were analyzed by ANOVA for cumulative proportion of plants grazed. All statistical analyses were done using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P \leq 0.05$. Only $P > 0.05$ are reported.

## **Results**

### *Botanical composition*

*Desmodium incanum* cover did not differ among the seasons ($P = 0.182$). Mean cover was 9.17 ± 1.47 %. The species with the highest cover was the native grass *Paspalum notatum*, with values above 35% in all seasons, followed by the invasive grass *Eragrostis plana*, with values that increased from 16.27% in the spring to 25% in autumn. The grass species *Andropogon lateralis* had a mean cover of 9.17%, the same value found for *D. incanum* in the paddock. Other species' cover represented less than 5% of the area. The main species found in each season are illustrated in Figure 1.

### *Consumption of Desmodium incanum tagged plants*

No difference was found between the seasons by the third day of evaluation ($P > 0.05$; spring = 78.17 ± 12.43%; summer = 80.59 ± 5.20%; fall = 70.63 ± 14.29%). There was an effect of day, but no interaction between day and season ($P > 0.05$). Means of cumulative proportion of plants grazed on Days 1, 2 and 3 were 39.48 ± 17.28%, 58.36 ± 15.91% and 76.46 ± 11.51% respectively (Figure 2).

### *Chemical composition and intake of pasture*

Results of DM, MM, CP, NDF, ADF, ADL and OMD of samples from the grazing simulation are shown in Table 2. There was an effect of season on DMI and CPI. Dry matter intake per metabolic weight (MW) was significantly greater in spring (91.60 ± 1.37 g kg$^{-1}$ MW) than in summer (79.65 ± 1.31 g kg$^{-1}$ MW) and fall (67.38 ± 1.26 g kg$^{-1}$ MW). Intake during summer was greater than during fall. Intake of CP per unit metabolic weight was also greater in spring (11.09 ± 0.30 g kg$^{-1}$ MW) relative to summer (9.38 ± 0.52 g kg$^{-1}$ MW) and fall (6.86 ± 0.39 g kg$^{-1}$ MW). Again, intake of CP during summer was greater than in fall.

### *Chemical composition of Desmodium incanum*

Although PBCT increased only 6.29 g kg$^{-1}$ DM from spring to summer, it decreased 44.57 g kg$^{-1}$ DM from summer to fall. Values of PBCT, as well as CP, NDF and ADF are detailed in Table 3.

## Discussion

The tagging method showed that *D. incanum* is grazed throughout its entire cycle. Despite differences in pasture composition among seasons, at least 70% of the tagged plants were used at the end of the 3-day observation period. In addition, mass of *D. incanum* declined after grazing in the three seasons (Table 1). The values of PBCT found in this study varied from 90.89 to 135.45 g kg$^{-1}$ DM, exceeding 50 g kg$^{-1}$ DM, value above which animals would avoid consumption according to previous studies (Frutos et al. 2004). The fact that most of *D. incanum* was eaten in the first 3 days of grazing, when pasture was most abundant and diverse, supports what previous authors have addressed: that in diverse pastures animals are more likely to eat tanniferous plants due to the increased likelihood of chemical interactions occurring among different chemicals present in different forage species (Provenza 2008; Provenza et al. 2007; Villalba et al. 2011).

The species of grass and legume most frequently found in the paddock were *Paspalum notatum* and *Desmodium incanum*, which supports other studies that evaluated species cover in Pampa grassland sites (Heringer and Jacques 2002; Caporal and Boldrini 2007; Ferreira and Setubal 2009; Casanova et al. 2013). The experimental area in this study had a mean cover of 9.17% *D. incanum*, a greater value than those described by other authors: 5.67% (Ferreira and Setubal 2009) and 5.2% (Casanova et al. 2013). Another study conducted within the same experimental station, but in a different area found 9.1% cover of *D. incanum* (Bremm et al. 2012). In that study, the area was also infested by the invasive grass *Eragrostis plana*, which is avoided by sheep and cattle. According to the authors, the predominant species inside the tussocks of *E. plana* was *D. incanum*, which sheep selectively grazed.

The pasture composition showed seasonal differences. The high amount of dead material in the fall could be a consequence of the longer period between evaluations, when the paddocks were left with no animals to graze. Previous studies showed that about 40% of the ungrazed leaves eventually senesce, increasing the proportion of dead material (Frame and Hunt 1971). This explains why OMD was lower for this season, since dead material has lower values than all the other parts of the plant (Wilson 1994). Summer had a noticeable decrease in forage mass as a negative consequence of the herbicide application, which was much likely overdone. These differences can explain the decrease in the intakes of total DM per metabolic weight across seasons. According to Dumont et al. (2005), small ruminants faced with low feed availability, responded by selecting a diet that maintained nutrient values. They differ from large ruminants, which focus more on maintaining adequate DMI. However, our study revealed that CPI decreased progressively across the three seasons, suggesting that the decline in nutritive value was greater than the capacity of sheep to maintain dietary nutritional quality.

A previous study evaluated chemical composition of eight species commonly found in subtropical regions, in the reproductive and vegetative stages (Rossetto 2015). In the reproductive stage, *D. incanum* had the second highest CP content (14.7% of DM), surpassed only by a high-quality temperate legume, *Trifolium repens* (20% of DM). However, in the vegetative stage, *D.*

*incanum* was the plant with the highest CP value (23.5% of DM). Besides plant growth stage, there are other factors that can affect CP concentration, such as soil conditions, year-to-year weather variations, fertilization and sward composition (Herrmann et al. 2005).

One of the properties of CT is the ability to bind to dietary protein, protecting the molecule from rumen microbial degradation, thus increasing the amino acid flow in the intestine. Previous studies showed that *Desmodium* spp. are capable of this biological action through the mechanism of precipitation of proteins by phenol (PPP), as described by Hagerman and Butler (1978). Tontini et al. (2019) found a value of 60.1 g PPP $kg^{-1}$ DM for *D. incanum*, and Cooper et al. (2014) found a value of 94 g PPP $kg^{-1}$ DM for *D. paniculatum*.

Despite having a relatively high concentration of CT, *D. incanum* was consumed by sheep in all seasons. However, it is still unclear whether sheep grazing in the Pampa grasslands would have enough dietary protein for CT to bind, reducing the negative impact of these secondary chemicals, while increasing amino acid flow to the intestines, which enhances animal performance. *Desmodium incanum* has considerable CP when compared to other Species (Rossetto 2015), but further studies are necessary to investigate their effects on animal performance.

**Conclusion**

In spite of the seasonal changes in the pasture, sheep were able to maintain *D. incanum* over time. At least 70% of the tagged plants were eaten within the first 3 grazing days despite the high CT content across seasons. This suggests dietary proteins from the legume and other species contributed by binding and precipitation processes to attenuate the negative impacts of such high concentration of CT. This common legume species is of great interest for further sheep nutrition studies in the Pampas since (1) it is a very common legume species in the Pampa biome, (2) sheep graze it readily in all seasons of its annual growth cycle, (3) it is rich in CT which is biologically activity.

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**Table 1** – Pasture height, total dry matter herbage mass (HM), *Desmodium incanum* dry matter mass (DI), grasses, other species and dead material (mean ± SE). Values are the averages of the six paddocks. Values within a column followed by different lower-case letters differ significantly. Different upper-case letters show difference between before and after grazing evaluations within the same period.

| | Height (cm) | Variable (kg ha $^{-1}$ dry matter) | | | | |
|---|---|---|---|---|---|---|
| | | HM | DI | Grasses | Other species | Dead material |
| Before grazing | | | | | | |
| Spring | 17 ± 1.79a | 1852.56 ± 125.44bA | 69.64 ± 11.36aA | 1166.65 ± 88.41aA | 351.67 ± 43.40aA | 264.60 ± 33.11bA |
| Summer | 11.5 ± 3.09b | 801.71 ± 64.84c | 18.55 ± 3.91bA | 441.29 ± 48.84b | 94.01 ± 17.49bA | 247.85 ± 22.60b |
| Fall | 16 ± 3.16a | 2587.60 ± 155.43aA | 46.54 ± 8.16aA | 1079.48 ± 69.61aA | 181.19 ± 71.45bA | 1280.39 ± 73.90aA |
| After grazing | | | | | | |
| Spring | 14.5 ± 2.89 | 882.64 ± 114.28B | 7.60 ± 1.44aB | 689.42 ± 89.88B | 60.35 ± 10.86B | 125.27 ± 19.30cB |
| Summer | 10.67 ± 2.65 | 956.30 ± 109.99 | 8.56 ±2.03aB | 527.42 ± 67.85 | 44.55 ± 15.57B | 375.87 ± 83.09b |
| Fall | 12 ± 3.89 | 1131.44 ± 132.56B | 4.99 ± 1.70bB | 463.91 ± 59.55B | 70.22 ± 27.87B | 592.30 ± 83.61aB |
| *P* value seasons | | | | | | |
| Before | ** | *** | *** | *** | *** | *** |
| After | ns | ns | ** | ns | ns | *** |
| *P* value before/after | | | | | | |
| Spring | ns | *** | *** | *** | *** | *** |
| Summer | ns | ns | *** | ns | ** | ns |
| Fall | ns | *** | * | *** | ** | *** |

**ns = non-significant (>0.05), * *P* <0.05, ** *P* < 0.01, *** *P* < 0.0001**

**Table 2 –** Dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and organic matter digestibility (OMD) of samples of grazing simulation (mean ± SE). Values are the averages of the six paddocks. Values within a column followed by different letters differ

| | DM (%) | MM (%) | CP (%) | NDF (%) | ADF (%) | ADL (%) | OMD (%) |
|---|---|---|---|---|---|---|---|
| Spring | 91.42 ± 0.14a | 6.28 ± 0.11 | 15.15 ± 0.29a | 72.00 ± 0.83b | 35.38 ± 0.43b | 7.24 ± 0.20 | 63.16 ± 0.44a |
| Summer | 90.57 ± 0.13b | 6.11 ± 0.08 | 11.75 ± 0.37a | 75.90 ± 0.14a | 38.53 ± 1.23a | 7.76 ± 0.95 | 62.12 ± 0.81a |
| Fall | 90.56 ± 0.04b | 6.28 ± 0.16 | 10.32 ± 0.29b | 72.37 ± 0.84b | 35.70 ± 0.54ab | 7.79 ± 0.39 | 57.75 ± 1.19b |
| P value | ** | ns | ** | ** | * | ns | ** |

**ns = non significant (>0.05), * *P* <0.05, ** *P* < 0.01, *** *P* < 0.0001**

**Table 3 –** Protein-bound condensed tannin (PBCT), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of *Desmodium incanum*

| | PBCT (g $kg^{-1}$ DM) | CP (%) | NDF (%) | ADF (%) |
|---|---|---|---|---|
| Spring | 129.17 | 12.62 | 57.35 | 41.64 |
| Summer | 135.46 | 13.19 | 49.89 | 35.37 |
| Fall | 90.89 | 13.94 | 54.29 | 39.02 |

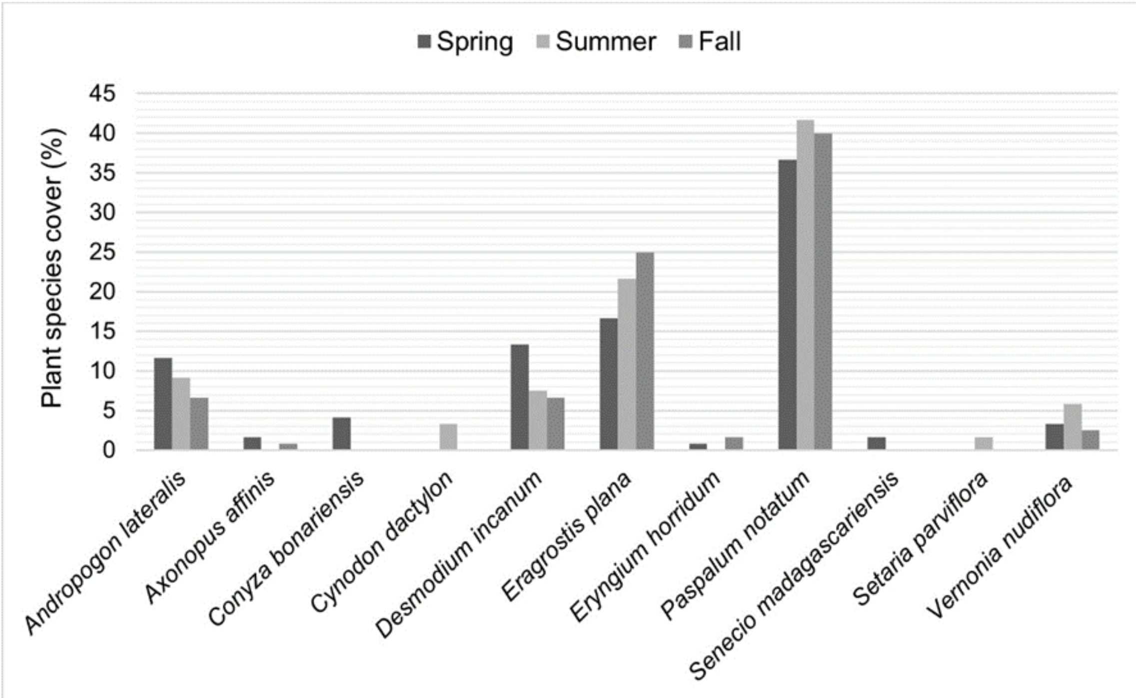


**Figure 1** – Main species found in the experimental area during spring, summer and fall evaluations. Values in representative cover area (%).

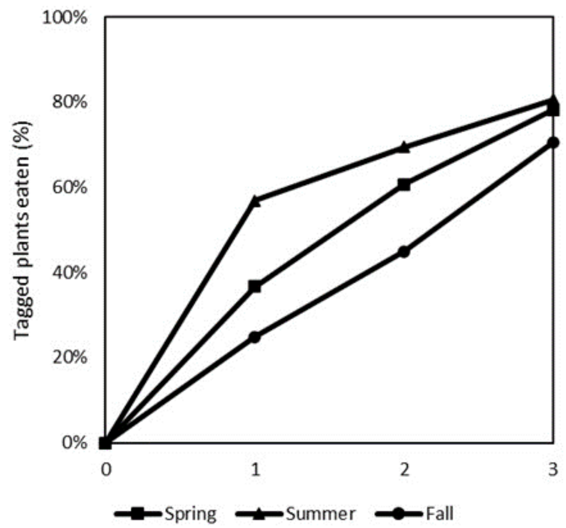


**Figure 2** - Average percentage of the tagged plants (*D. incanum*) grazed in the 3 days of observation.

# CHAPTER III

## Trade-offs between selection of nutrients and tannin extract in feedlot growing lambs

## Trade-offs between selection of nutrients and tannin extract in feedlot growing lambs

### Abstract

Tannins are phenolic compounds that can bring benefits to ruminant meat production due to its action on protein metabolism and antioxidant properties. However, tannins can also have negative effects on the rumen microbiome and host, affect ruminant palatability and thus lower feed intake. This study evaluated the trade-offs between selection of nutrients and tannins, as well as to assess the effects of the diet on performance and blood parameters of growing lambs. Thirty-two lambs, kept in individual pens for 8 weeks, received an *ad libitum* choice between two isoenergetic feeds with variable levels of crude protein (CP) and tannins: high (HP) and low (LP) CP content with (T; 4% dry matter in the feed) or without tannins. Thus, four diets were tested: HP, HP+T, LP and LP+T. Animals were assigned to 4 treatments receiving *ad libitum* amounts of a simultaneous offer of two feeds: LP and HP; LP and HP+T, LP+T and HP; LP+T and HP+T. Feed offer and refusals were weighed daily, and feed intake was calculated as the difference between them. Lambs were weighed every 4 weeks. Blood samples were collected before and after the trials. All four treatments showed preference for the high protein feed, regardless of tannin inclusion. Adding tannins to the preferred high protein feed reduced total feed intake ($P<0.0001$). However, adding tannin to both feeds reduced blood urea nitrogen ($P=0.019$) without compromising total feed intake, feed conversion ratio or weight gain.

### Key words

Blood urea nitrogen, feeding behavior, animal performance, ADG, feed conversion

## 1. Introduction

Tannins are ubiquitous plant metabolites that have been studied in ruminant feeding and nutrition studies since the early 1900s. Their affinity for proteins lowers protein degradation in the rumen, so if dietary protein is available post-ruminally, there can be improvements in production, such as in animal growth, fertility, milk yield, and parasite tolerance (MUELLER-HARVEY *et al*., 2019; WAGHORN, 2008). The initial publications on these chemicals, however, described them as antinutritional due to their detrimental effects to ruminants (BUTLER, 1992; PRICE *et al*., 1979). When binding to salivary proteins, they can cause a sensation of astringency that reduces palatability of the feed and consequently feed intake (FRUTOS *et al*., 2004; JERÓNIMO, *et al*., 2016; WAGHORN, 2008). Other detrimental effects reported for these chemicals are post-ingestive malaise and toxicity – the latter especially associated with hydrolysable tannins (FRUTOS *et al*., 2004).

Because nutritional responses to tannin have been variable, there are contradictory reports about their benefits to ruminants. Whether tannins will have adverse, neutral or favorable effects depends on several factors including tannin type and concentration, species and category of animal and nutrient resources in the diet, particularly proteins (MUELLER-HARVEY *et al*., 2019). Most studies concerning the improvement of ruminant production utilize condensed tannins (CT), because they are less harmful and toxic than hydrolysable tannins. Moderate concentrations of CT in the diet (3-4% of dry matter) can increase protein supply to sheep and, consequently, improve performance. With these concentrations, there is better N-use efficiency, lessening environmental impact by urinary N excretion and greenhouse gasses emission (MIN; SOLAIMAN, 2018).

The benefits of tannins are particularly important for growing lambs, which represent a physiological state where high requirements of CP are present due to growth. Lambs select their diet to meet their CP requirements (KYRIAZAKIS; OLDHAM, 1993), thus satisfying the requirements for maintenance and growth. Likewise, ruminants form preferences for tannins in feeds when these chemicals provide a medicinal effect, e.g., parasitized animals (VILLALBA *et al*., 2014). However, the negative post-ingestive effects of tannins and their astringency may alter food preference, intake and hinder nutrient selection in healthy animals. The objectives of this study were to evaluate the trade-offs between lamb nutrient and CT selection, as well as to assess the effects of the diet selected on performance, digestibility, and nitrogen excretion.

## 2. Materials and Methods

### 2.1. Study site and animals

The study was conducted at the Green Canyon Ecology Center, Utah State University (41°45′59″ N, 111°47′14″ W), in Logan, Utah. The trials took place from 20 April to 14 June, 2022. All procedures were approved by the Utah State University Institutional Animal Care and Use Committee (protocol number 12594).

Thirty-two Rambouillet-Columbia-Suffolk crossbred lambs (3 months of age and initial bodyweight of 22.4 ± 5.8 kg on average) were kept under a protective roof in individual adjacent pens measuring 1.5 × 2.5 m. Animals were familiarized with their pens and feeders for 12 days before the trials. During this period, they received 1.5 kg of a 50% alfalfa pellets, 20% barley, 20% beet pulp and 10% soybean diet daily. All lambs had free access to fresh water and trace-mineralized salt blocks. Before the study, all lambs were dewormed orally with Ivermectin (0.2 mg / kg of BW) and grazed on a 0.85 ha pasture of orchard grass and alfalfa, supplemented with ad libitum alfalfa pellets for 4 days.

### 2.2. Experimental approach

The study lasted 10 weeks: two weeks of adaptation and two trial periods of 4 weeks each. Lambs were assigned to 4 treatments (8 lambs/treatment) to balance weight, gender and breed variation, resulting in similar distributions of these parameters among treatments. Each lamb received a simultaneous offer of two isoenergetic rations of similar particle size. Experimental diets were formulated using a mix of alfalfa, beet pulp, barley and soybean, in order to attain a low protein-energy ratio (LP) or a high protein-energy ratio (HP) (Table 1). Two more rations were formulated with the same characteristics but containing 4% quebracho and chestnut tree tannin extract blend (LP+T and HP+T) (Table 1). The four treatments were: (T1) LP and HP; (T2) HP and LP+T; (T3) LP and HP+T; (T4)

LP+T and HP+T. The tannin extract (Bypro; Silvateam, Indunor S.A., Argentina) was composed of one-third chestnut tannin extract and two-thirds quebracho tannin extract. Both extracts were analyzed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (PIZZI *et al*., 2009). Quebracho tannin composition was (DM basis): 84.3% condensed flavan-3-ols (predominantly profisetinidin), 10.7% oligomers of flavan- 3-ols (catechin and epicatechin dimers), and 5% carbohydrate derivate (dimers of pentose, monocarboxylic acid of hexose, and 6-carbon sugars). Chestnut tannin composition was (DM basis): 7.9% digalloyl glucose, 5.0% trigalloyl glucose, 16.5% pentagalloyl glucose, and 70.6% oligomers of digalloyl glucose, trigalloyl glucose, and pentagalloyl glucose. Three samples of experimental diets were collected for chemical analysis. Dry matter (DM), crude protein (CP) and acid detergent fiber (ADF) were analyzed according to AOAC (1995). Neutral detergent fiber (NDF) and were analyzed according to Mertens (2002).

### 2.3. Feed intake and preference

Lambs were fed daily between 7:30 – 9:00 AM. The feeds were offered ad libitum and adjusted so that there was at least 10% refusals in the feeders before the next feeding. All the refusals and offers were weighed daily, so that daily ration intake was calculated as the difference between the amounts offered and refused. Preference for one diet was calculated as: diet intake/total intake x 100.

### 2.4. Nutrient intake

During the last 5 days of the study, representative (20% of the amount retrieved) daily samples of feed refusals were collected, as well as two samples from the feeds in the same week. The samples were analyzed for chemical composition, and intake of DM, CP, NDF, ADF and tannins were calculated as: [(Offer feed 1 x concentration of nutrient in the offer 1) – (refusal feed 1 x concentration of nutrient in the refusal 1)] + [(Offer feed 2 x concentration of nutrient in the offer 2) – (refusal feed 2 x concentration of nutrient in the refusal 2)]. These samples were analyzed in g feed/kg of body weight (BW).

### 2.5. Fecal output, fecal N excretion and digestibility of DM, CP and NDF

Fecal grab samples (20 to 30 g DM) were collected from the rectum of each lamb during the last 5 days of the study and stored in vacuum plastic bags at -20°C. Subsequently, a composite sample from the feces of each lamb was oven dried at 70°C for 24 hours, and the dried samples were analyzed for chemical composition. Fecal DM output (FO) was determined using the concentration of an internal marker, acid detergent lignin (ADL), in the ration consumed and in feces (VAN SOEST, 1994). Fecal output was then determined using the following formula: Fecal DM Output (g/d) = [DMI (g/d) x ADL in feed (g/g)] / ADL in feces (g/g) (COCHRAN; GALYEAN, 1994). Once fecal output was determined, DM digestibility (DMD) was calculated for each lamb as: DMD (%) = {[DMI (g/d) – FO (g/d)] / DMI (g/d)}x100.

Crude protein digestibility (CPD) and NDF digestibility (NDFD) were calculated as: CPD or NDFD (%) = {[CP or NDF in feed (g/d) − CP or NDF in feces(g/d)]/CP or NDF in feed (g/d)}× 100. The nitrogen excreted through the feces (g/lamb) was calculated by multiplying the fecal output by the nitrogen concentration in feces.

### 2.6. Average daily gain and feed conversion ratio

Animals were weighed before the beginning of the trials and every 4 weeks. Average daily gain (ADG) was calculated by dividing the weight gain by the number of days between the weighing. Feed conversion ratio (FCR) was calculated by dividing daily food intake by the ADG of the whole experimental period.

### 2.7. Blood Urea Nitrogen (BUN)

Blood samples were collected from each lamb using Ethylenediaminetetraacetic acid (EDTA) tubes via jugular venous puncture in the beginning and in the end of the study. After the samples were centrifuged (1500 rpm for 15 min), the plasma was extracted and stored at -80°C. The samples from the dry tubes were later submitted to the Utah Veterinary Diagnostic Laboratory (Logan, UT) for blood urea nitrogen (BUN) analysis using the method described by Lagrange and Villalba (2019).

### 2.8. Statistical Analyses

Results were analyzed as completely randomized design with lambs as the experimental units. Statistical analyses of total intake (g), feed intake (g), feed preference (%), ADG (kg/d), FCR (g/g), nutrient intake (g/kg BW), DMD CPD NDFD (%), fecal output (g) and fecal N excretion (g) were carried out using the Mixed Procedure of SAS (SAS® 9.4 Foundation for Microsoft Windows for x64, Cary, NC: SAS Institute Inc.), with treatment as fixed effect and animal as random effect. Animal liveweight (kg) and blood urea nitrogen (mg/dL) were analyzed before and after the trials, also using Mixed Procedure with treatment as fixed effect and animal as random effect. Intake of each diet was analyzed within treatments and comparing treatments with the same diet. All data were tested for normality (Proc Univariate; Shapiro-Wilk $P > 0.05$).

## 3. Results

### 3.1. Feed intake and preference

Total feed intake (g) and DMI (g/kg BW) were lowest in T3 and highest in T1 and T4 ($P < 0.0001$, Table 2). All treatment lambs ingested more of the high protein diet, regardless of tannin extract inclusion (Table 3). Preference for high protein diets in T1, T2, T3 and T4 were respectively: 60.30%, 60.77%, 51.39% and 56.54% (Figure 2A). Preference for HP over the 8 weeks of the study is illustrated in Figure 2B.

Lambs in T3 had greater daily intake of LP than T1 ($P$=0.028). Animals in T1 and T2 had similar daily intake of HP (P=0.18). Lambs in T4 had greater daily intake of LP+T than T2 ($P$<0.0001). The T4 lambs had greater daily intake of HP+T than T3 ($P$<0.0001). These results are detailed in Table 3.

### 3.2. Nutrient intake

Intake of CP was similar between T1 and T2 lambs and lower in T3 and T4 ($P$<0.0001, Table 2). These values were 9% less in T4 and 20% lower in T3 when compared to control (T1) animals. Intake of NDF was least in T2 and greatest in T4 ($P$=0.0002, Table 2). Intake of ADF was lower in T3 lambs than in the other three treatments (*P*<0.0001, Table 2). As expected, T4 lambs had the greatest intake of tannin extract (*P*<0.0001, Table 2), > 20% greater than in T2 and > 50% greater than in T3.

### 3.3. Fecal output, fecal N excretion and digestibility of DM, CP and NDF

T1 (Control) and T4 lambs had greater fecal output than T2 and T3 (*P*<0.0001, Table 2). Lamb fecal N excretion was T4> T2 = T3 > T1 (*P*<0.0001, Table 2).

Dry matter digestibility was greatest in T2, differing from T3 and T4 (*P*<0.0001, Table 2). Crude protein digestibility of T1 and T2 were higher than T3 and T4 (*P*<0.0001, Table 2). NDF digestibility was highest in T4 and lowest in T1 (*P*<0.0001, Table 2).

### 3.4. Average daily gain and feed conversion ratio

Lamb ADG was similar among treatments (*P*=0.35, Table 2). Lamb FCR was less in T4 than in T2 and T3, and T1 (control) was less than T2 (*P*<0.000, Table 2). Final lamb weight was similar among the treatments (42.66 ± 1.35kg; *P*= 0.64). Weight gain of the four treatments is illustrated in Figure 1.

### 3.5. Blood Urea Nitrogen

Blood urea nitrogen did not differ among treatments in the samples taken before the trials (*P*=0.46). However, samples taken after the study showed that T4 had lower values than T1 (*P*=0.019, Table 2).

## 4. Discussion

### 4.1. Intake and feeding behavior

In all treatments, animals consumed more of the high protein diet. The preference for high protein diets has already been observed in growing lambs (ASKAR *et al.*, 2006; KYRIAZAKIS; OLDHAM, 1993). This is explained by the greater protein requirements observed in growing animals (KYRIAZAKIS; OLDHAM, 1993). Moreover, studies comparing cafeteria feeding systems with control groups receiving nutritionally balanced diets show that the cafeteria lambs consume more CP, surpassing the amounts required by National Research Council (RODRÍGUEZ *et al*., 2007; ŞAHIN *et al*., 2003).

Adding tannin extract to any of the two rations negatively impacted DMFI. The main factors that reduce intake in tanniferous feeds are (1) low palatability, resulted of the complex formed with salivary proteins causing a sensation of astringency and (2) slow evacuation of the digestive tract, related to declines in rate of passage triggered by the antibiotic effects of tannins on some populations (e.g., cellulolytic) of the rumen microbiome that constrain forage fermentability (FRUTOS *et al*., 2004; WAGHORN, 2008; LAMY *et al*., 2011). This negative effect on feed intake was observed especially in T3 where tannin extract was added in the preferred high protein feed. Intakes of DM and CP per kg BW were also less in this treatment. Still, there was no difference (P>0.05) in weight gain across treatments, which could be explained by the ingestion of these bioactives and the potential improvement in dietary protein use relative to control (T1) animals. On the other hand, the lower feed digestibility observed in T3 (despite the lower feed DMI for this treatment and potential concomitant increments in rumen retention times) can be explained by the high intake of tannin extract and low intake of proteins for this treatment. When tannin extract was added in the least preferred, low-protein feed (T2), lambs displayed a similar feeding preference to the control group. In fact, preference for HP by T1 and T2 groups were the same (60%, Figure 2). Both treatments also had similar DMI and CPI, which is explained by the fact that HP intake did not differ between them. Nevertheless, total feed intake was lower in lambs fed T2, the treatment that received tannins with the low-protein feed LP.

When tannin was added to both feeds (T4), total feed intake did not differ from the control treatment and T2. In the T4 group, animals had to ingest tannins as they were present at equal concentrations in both feeds on offer and thus, they had to adapt to a diet with 4% tannin extracts. Even at these levels, feed DMI was not reduced relative to control animals. In fact, the pattern of selection of HP and LP in T4 was similar to T1 and T2 (Figure 2).

### 4.2. Digestibility, efficiency and performance

Lambs fed T1 and T2 had similar feeding behavior choices (Figure 2) and, because T2 had tannin extract included in the least preferred feed, these two treatments had similar DM and CP intakes and digestibility. The lower values of CPD in T3 and T4 diets can be explained by the greater tannin intake and formation of protein-tannin complexes.

As with feed DMI, FCR in T4 animals were similar to T1. However, T4 lambs showed lower values than in T2 and T3, suggesting a better efficiency in converting feed into gain. It is possible that the astringency in only one of the two feeds (T2 or T3) disrupted the selection of nutrients from the different rations relative to when tannins were absent (T1), or present in both feeds (T4). Findings regarding tannin effects on ruminant performance are variable, since it depends on many factors, such as type and concentration of tannin, animal species, physiological state and nutrient supply, among others (MUELLER-HARVEY *et al*., 2019). However, levels of tannins in a ration to a threshold of 5% represent most of the studies with neutral or positive effects on performance (FRUTOS *et al*., 2004). Since tannin extract inclusion in diets was below 5%, this level may have at least prevented a negative impact on FCR or feed intake.

### 4.3. Nitrogen use

As hypothesized, lambs that received tannin extract in both feeds had less BUN and greater fecal N excretion at the end of the experiment. Tanniferous diets reduce rumen proteolysis, which leads to less urinal N excretion and greater fecal N excretion (MUELLER-HARVEY *et al*., 2019; WAGHORN, 2008). A recent study that fed increasing values of CT to lambs (0, 20, 40, 60 and 80 g CT/kg DM) observed linear decreases of BUN as CT increased (COSTA *et al*., 2021). The same study reported a quadratic decrease in urinary N excretion. Diets containing 1.5 or 2.0% of CT also reduce serum urea (DEY *et al*., 2008). A shift from urinary to fecal excretion of nitrogen could reduce nitrogen losses by 25% (MUELLER-HARVEY *et al*., 2019) and it is of great interest from an environmental perspective because less nitrogen excreted by urine represents lower potential contamination of waterways and groundwater.

## 5. Conclusion

Lambs preferred the high-protein feed in all treatments. The inclusion of 4% tannin extracts decreased total feed intake when added to the preferred high-protein feed. When included in both feeds, a diet with 4% tannin extracts showed reductions in blood urea nitrogen and increases in fecal N excretion without compromising feed intake, FCR or weight gain.

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**Table 1 –** Ingredient and chemical composition (% of DM) of experimental feeds.

| | LP | HP | LP+T | HP+T |
|---|---|---|---|---|
| Alfalfa (%) | 23 | 47 | 15 | 35 |
| Barley (%) | 1 | 32 | 15 | 40 |
| Beet Pulp (%) | 75 | 1 | 65 | 1 |
| Soybean (%) | 1 | 20 | 1 | 20 |
| Tannins[1] (%) | 0 | 0 | 4 | 4 |
| DM (%) | 92.8 | 91.47 | 92.33 | 90.93 |
| CP (%) | 11.87 | 25.03 | 12.23 | 22.83 |
| ADF (%) | 25.73 | 20.33 | 22.40 | 16.20 |
| NDF (%) | 38.87 | 27 | 34.9 | 23.1 |
| DE[2] (Mcal/kg) | 3.2 | 3.2 | 3.2 | 3.2 |

**LP = low protein without tannin, HP = high protein without tannin, LP+T = low protein with tannin, HP+T = high protein with tannin, DM= dry matter, CP = crude protein, ADF = acid detergent lignin, NDF = neutral detergent lignin., DE = digestible energy.**

**[1] Bypro; Silvateam, Indunor S.A., Argentina. Two-thirds quebracho tannin: 84.3% condensed flavan-3-ols (predominantly profisetinidin), 10.7% oligomers of flavan- 3-ols (catechin and epicatechin dimers), and 5% carbohydrate derivate (dimers of pentose, monocarboxylic acid of hexose, and 6-carbon sugars). One-third of chestnut tannin: 7.9% digalloyl glucose, 5.0% trigalloyl glucose, 16.5% pentagalloyl glucose, and 70.6% oligomers of digalloyl glucose, trigalloyl glucose, and pentagalloyl glucose.**

**[2]Determined by NRC (1985) tables.**

**Table 2 –** Total feed intake, intake of nutrients, nutrient digestibility and performance results (means ± standard error). Values within a row followed by different letters differ significantly.

| | T1 | T2 | T3 | T4 | SE | *P* value |
|---|---|---|---|---|---|---|
| Total feed intake (g) | 1623a | 1542b | 1394 c | 1605ab | 19 | <0.0001 |
| Intake of DM (g/kg BW) | 41.3a | 42.4a | 39.1b | 41.7a | 0.39 | <0.0001 |
| Intake of CP (g/kg BW) | 10.07a | 10.16a | 7.97c | 9.17b | 0.105 | <.0001 |
| Intake of NDF (g/kg BW) | 14.19ab | 13.56c | 13.75bc | 14.36a | 0.144 | 0.0002 |
| Intake of ADF (g/kg BW) | 9.43a | 9.41a | 8.53b | 9.18a | 0.100 | <.0001 |
| Intake of tannin (g/kg BW) | - | 0.68c | 0.86b | 1.80a | 0.013 | <.0001 |
| Fecal output (g) | 354a | 323b | 316b | 349a | 4 | <0.0001 |
| Fecal nitrogen excretion (g) | 10.63b | 11.36b | 11.59b | 14.06a | 0.48 | <.0001 |
| DM digestibility (%) | 76.49ab | 77.62a | 75.42b | 76.19b | 0.315 | <.0001 |
| CP digestibility (%) | 82.30a | 80.68a | 72.87b | 73.44b | 0.506 | <.0001 |
| NDF digestibility (%) | 62.34c | 63.76bc | 65.16b | 67.75a | 0.665 | <.0001 |
| ADG (kg/d) | 0.32 | 0.28 | 0.26 | 0.32 | 0.028 | 0.35 |
| FCR (g/g) | 5.24bc | 5.61a | 5.44ab | 5.17c | 0.068 | <.0001 |
| Final weight (kg) | 44.36 | 41.60 | 40.25 | 44.42 | 2.754 | 0.64 |
| Blood urea nitrogen (mg/dL) | 24.37a | 19.87ab | 21.25ab | 18.00b | 1.36 | 0.02 |

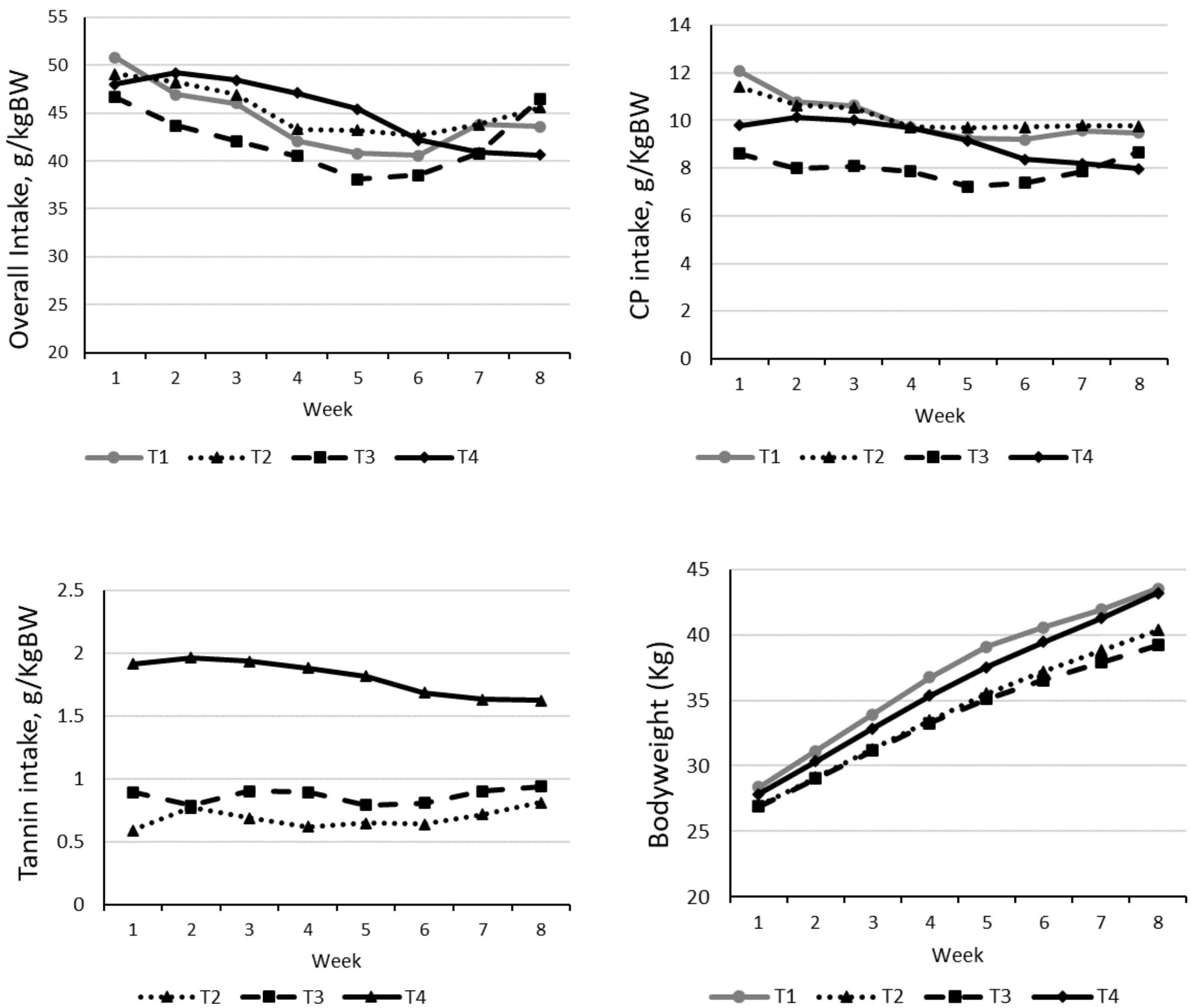
**SE = Standard error, DM = dry matter, CP = crude protein, NDF = neutral detergent lignin, ADF = acid detergent lignin, ADG = average daily gain, FCR = feed conversion ratio, T1 = low protein without tannin (LP) and high protein without tannin (HP), T2 = low protein with tannin (LP+T) and high protein without tannin (HP), T3 = low protein without tannin (LP) and high protein with tannin (HP+T), T4 = low protein with tannin (LP+T) and high protein with tannin (HP+T).**

**Table 3 –** Daily intake of each diet per treatment (means ± SE). Values within a row followed by different lower-case letters differ significantly. Values within a column followed by different upper-case letters differ significantly.

| | T1 | T2 | T3 | T4 | SE | P value |
|---|---|---|---|---|---|---|
| LP intake (g) | 631bB | - | 669aB | - | 12 | 0.028 |
| HP intake (g) | 992.02A | 960.29A | - | - | 16.79 | 0.18 |
| LP+T intake (g) | - | 584.08bB | - | 689.98aB | 11.49 | <0.0001 |
| HP+T intake (g) | - | - | 725.46bA | 917.25aA | 14.46 | <0.0001 |
| SE | 14.32 | 15.18 | 13.66 | 12.48 | - | |
| *P* value | <0.0001 | <0.0001 | 0.003 | <0.0001 | | |

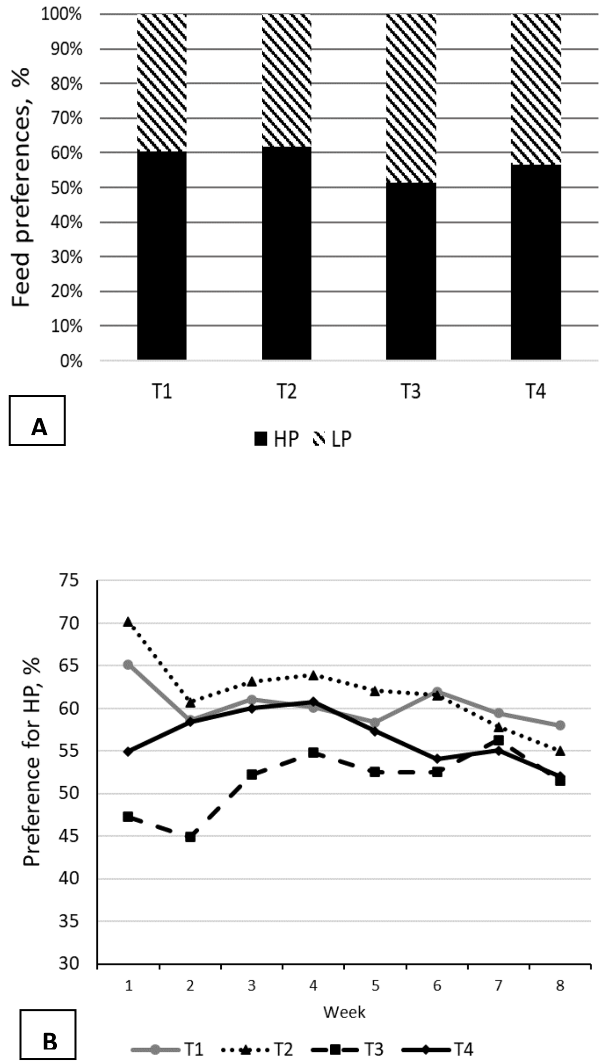
**SE = Standard error, T1 = low protein without tannin (LP) and high protein without tannin (HP), T2 = low protein with tannin (LP+T) and high protein without tannin (HP), T3 = low protein without tannin (LP) and high protein with tannin (HP+T), T4 = low protein with tannin (LP+T) and high protein with tannin (HP+T).**

**Figure 1** – Feeding behavior and weight gain over the 8 weeks.




**T1 = low protein without tannin (LP) and high protein without tannin (HP), T2 = low protein with tannin (LP+T) and high protein without tannin (HP), T3 = low protein without tannin (LP) and high protein with tannin (HP+T), T4 = low protein with tannin (LP+T) and high protein with tannin (HP+T).**

**Figure 2** – Overall feed preference (A) and preference for HP over the 8 weeks (B).




**T1 = low protein without tannin (LP) and high protein without tannin (HP), T2 = low protein with tannin (LP+T) and high protein without tannin (HP), T3 = low protein without tannin (LP) and high protein with tannin (HP+T), T4 = low protein with tannin (LP+T) and high protein with tannin (HP+T).**

# CHAPTER IV

## Sheep diurnal ingestive behavior in a Pampa biome pasture

# Sheep diurnal ingestive behavior in a Pampa biome pasture


## Abstract

The Pampa biome is a biodiverse grassland in South America that has been endangered by mismanagement and row crop cultivation. A better understanding of sheep ingestive behavior is essential for a sustainable use of this native grassland. This study described sheep diurnal ingestive behavior in a native Pampa biome grassland for three seasons: spring, summer and fall. Average of daylight length was 14 h in spring, 13 h in summer and 10 h 24 min in fall. Thirty-six crossbred sheep were divided into six paddocks each with six animals, and two behavior observations per period were carried out. Feeding behavior was evaluated from sunrise to sunset using all animal scan sampling with intervals of 5 minutes. Grazing, ruminating and idling duration were recorded as a percentage in daytime. Grazing time was higher in the fall, while spring and summer showed no difference. Ruminating time decreased with daylight duration: spring value was higher than summer, which was higher than fall. Idling was more frequent in the summer, followed by spring and fall. The behaviors showed patterns along the day typically described for sheep, with most of the grazing around sunrise and sunset. There was a moderate positive correlation between percentage of dead material in the pasture and grazing percentage time ($P$<0.000, r = 0.46), and a moderate negative correlation between dead material and ruminating time| ($P$<0.000, r = -0.49). The Pampa is a diverse and heterogeneous environment and these characteristics, along with seasonal changes, elucidate differences in feeding behavior strategies.



## Key words

Foraging, grazing, native pasture, ruminant

## 1. Introduction

The Pampa is a biodiverse grassland that is recognized as one of Brazil's six official biomes and covers part of the southernmost Brazilian state of Rio Grande do Sul. The pampa is part of the Río de la Plata natural grasslands, the largest grassland in South America, and has been the foundation of domestic herbivorous feeding for centuries (CARVALHO *et al*., 2009). The vegetation richness of the Pampa prairies offers ideal conditions for producing high quality ruminant meat. However, this biome is being ravaged by activities that in the short term bring better economic return (OVERBECK *et al*., 2007; CARVALHO *et al*., 2009). By managing these natural grasslands properly, it should be possible to attain both profitable and sustainable production of high-quality livestock products, but this requires multidisciplinary knowledge.

The study of animal behavior and cognition is a key to understanding their needs. This knowledge allows better management decisions in terms of productivity and animal welfare. Feeding and foraging behavior is influenced by the animal's condition and physiological state. Previous studies reported parasitic burden causing anorexia in sheep (KYRIAZAKIS *et al*., 1998; HUTCHINGS *et al*., 2000). Under free-ranging conditions and ad libitum supply, grazing sheep develop grazing behavior circadian rhythms. Grazing activity occurs mainly during the day, normally with two important periods around sunrise and sunset (ARNOLD, 1984). Seasonal changes can affect grazing duration directly, by daylight duration and weather conditions, and indirectly, by variations in the pasture such as sward structure. When pasture quality decreases, for instance, animals must graze for longer periods to cover their nutritional needs by being more selective (MANTECA; SMITH, 1994). Herbage mass and sward height can also influence grazing duration (da TRINDADE *et al*., 2012). Grazing duration is directly related to forage efficiency, which is why it matters for animal science studies. Finally, grazing also alters the structure of the sward, which impacts management practice decisions.

The objective of this study was to evaluate sheep feeding behavior in a Brazilian Pampa biome grassland in three distinct seasons of the year, correlating with pasture characteristics and fecal egg counts.

## 2. Materials and Methods

### 2.1. Study site and experimental design

The study was carried out at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul, located 46 m above sea level in the state of Rio Grande do Sul, Brazil (29°13′26″ S, 53°40′45″ W). The climate of the region is classified as subtropical humid (Cfa classification, Köppen) with a long-term average rainfall of 1,440 mm distributed evenly throughout the year.

A flock of 36 crossbred sheep aged of 18 months, 20 castrated males and 16 females was kept in an area of 2 ha of *Panicum maximum*. They were randomly assigned to 6 groups of equivalent average weight and at least two females, and for three evaluation periods of 2 weeks they were kept in a 0.8 ha of native grassland area. This area had never been occupied by sheep before and was divided into six paddocks of the same size. Evaluations took place from December 1st to 15th 2020, from February 9th to 23rd 2021 and from May 17th to June 1st 2021, equivalent to early, mid and late growing seasons, respectively. The same animals occupied their respective paddocks in all evaluation periods. In the last period, one animal per group was removed from the study to adjust stocking rate. Means of weights in the three periods were: 34.9 ± 4.8 kg; 41.3 ± 5.2 kg and 45.6 ± 4.9 kg.

### 2.2. Weather

Temperature was recorded by the weather station of Agronomic Experimental Station of the Federal University of Rio Grande do Sul in summer and fall. Data from spring was retrieved from Agrometeorological monitoring system in Arroio dos Ratos, approximately 8 km from the experimental area. Weather data is detailed in Table 1. Summer was 1 hour shorter than spring and 1 hour and 38 minutes longer than fall.

### 2.3. Measurements

Pasture evaluations were carried out the day before animals entered the paddocks and the day after they left, totaling two evaluations per season. A sward stick was used to measure sward height (BIRCHAM, 1981). One hundred random points per paddock were measured do calculate mean sward height. Herbage mass (HM) was calculated from samples cut close to the ground using 0.25 $m^2$ frames. Eight samples were collected per paddock, four at the mean sward height and four at random. The samples were weighed, homogenized and a representative subsample of approximately 50 g was separated into green and dead material. The separated subsamples were oven-dried at 60ºC until they reached constant weight, then weighed on a precision scale to measure DM (kg / ha) of each. Dry matter (%) of the sample was calculated as DM = [(dry weight of the subsample / fresh weight of the subsample) x fresh weight of the sample].

Ingestive behavior was evaluated from sunrise to sunset using all-animal scan sampling (ALTMANN, 1974) with intervals of 5 minutes. The behaviors recorded were: grazing (when the animal is selecting and eating forage, including displacement while selecting the diet), ruminating (when the animal is chewing the bolus), idling (when the animal is neither grazing or ruminating). These were used to calculate percentage of total daylight time spent in each behavior. Two behavior evaluations were conducted per season, the first one approximately 120 hours after the animal entrance in the study area and the second in the last day of the evaluation period. The day before being transferred to the experimental paddocks, animals were weighed after a 12-h fast and had fecal samples collected from their rectum for fecal egg count (GORDON; WHITLOCK, 1939).

### 2.4. Statistical analysis

All statistical analyses were executed using SPSS 22.0 software, (IBM Corp., Armonk, NY, USA). Fecal egg count and percentage of dead materiel were analyzed by non-parametric Kruskal-Wallis test with significance level at ≤ 5%. Pasture height, HM and percentage times of grazing, ruminating and idling were analyzed using one-way ANOVA with period and paddock as fixed effects and day as repetition. Variables were tested for normality (Shapiro−Wilk) and homoscedasticity (Levene). Means were compared by Tukey HSD at a 5% significance level. Spearman’s correlations were carried out between fecal egg count, pasture characteristics (herbage mass, pasture height and dead material percentage) and behavior percentage times (grazing, ruminating and idling).

# 3. Results

## 3.1. Fecal egg count and pasture characteristics

Since many lambs had 0 FEC, the distribution of the values was not normal. Medians for spring, summer and fall were 0, 200 and 300 respectively (Table 2). Pasture height was smaller in summer compared to spring and fall ($P$ < 0.010, Table 2). The greatest herbage mass was found in the fall, but half of this mass was dead material ($P$ < 0.000, Table 2). Details of these values are shown in Table 2.

## 3.2. Ingestive behavior

Animals spent a greater proportion of the day grazing during the fall, while spring and summer were similar ($P$ < 0.000, Table 3). Ruminating was more frequent during the spring, followed by summer and fall ($P$ < 0.000, Table 3). Idling was more frequent in the summer, followed by spring and fall ($P$ < 0.000, Table 3). Proportion of daily time duration spent in each behavior is illustrated in Figure 1.

Frequency of the behaviors along the day is detailed in Figure 2. In all three periods, more than 80% of the animals grazed in the hour that followed sunrise. The grazing peak decreased and reached less than 50% at 10 h in all periods. In the fall, there was a new peak of grazing at noon, reaching 75%. In this period, grazing remained above 50% until sunset, and increased constantly from 14 to 17 h. Spring had a softer peak of grazing from 11 to 12 h, while summer had less than 20% of animals in this activity for the same hours. In the afternoon, grazing behavior increased up to more than 90% at 17 h in spring and summer. Ruminating was more frequent between 10 and 13 h. In the summer, there was an idling peak at 11 h, when 36% of the animals were in this activity and only 16% grazed.

## 3.3. Correlations

There was a moderate positive correlation between percentage of dead material in the pasture and grazing percentage time ($P$ < 0.000, r = 0.462, Figure 3A), and a moderate negative correlation between dead material and ruminating time ($P$ < 0.000, r = -0.488, Figure 3B). All the other correlations were weak or $P$ >0.05 (Table 4).

## 4. Discussion

As the days became shorter, animals spent a greater proportion of daylight time grazing. This confirms our hypothesis, since grazing animals need to spend a minimum time foraging to supply their nutritional needs. Sheep graze mostly during the day, while ruminating is more observed at night (COLQUHOUN, 1970). The difference in daylight duration from spring to summer was only 1 h, while the difference from summer to fall was 2 h 38 min. Shorter fall daylight hours explain why grazing took a greater proportion of the day in fall than in spring and summer. Another explanation for this result is the weather change. When temperatures are warm, sheep tend to spend more time idling during the hot hours and graze around crepuscular hours and even during the night. In this study, idling time in summer was superior to fall and spring, even though spring had longer daylight hours.

Circadian rhythms observed in this study are typical for grazing sheep with free access to pasture. Most sheep grazing occurs immediately after sunrise and before sunset (ARNOLD, 1984). As the days become shorter, these two peaks become naturally closer. In this study, there was a smaller peak of grazing in the middle of the day in summer and fall. Studies of sheep grazing behavior in places with greater difference in the daylight duration between seasons describe greater grazing intensity in the afternoon during the winter (COLQUHOUN, 1970; ARNOLD, 1984).

Ruminating decreased with daylight length. Previous studies described most of this behavior occurring at night (COLQUHOUN, 1970; ROOK; PENNING, 1991). Therefore, as the nights became shorter, it is natural that ruminating occur more during the day. Peaks of ruminating were observed when grazing decreased, which is why its frequency was greater between 10h and 13h; a similar pattern was also described in previous studies (SILVA *et al*., 2020).

Fecal egg count was very low, with many animals having zero or close to zero eggs. These values explain why correlations of FEC with behaviors were non-significant. Pasture height had a weak negative correlation with grazing time. The Pampa is characterized by a high diversity of plant species, resulting in very heterogeneous heights in the same paddock, making it difficult to predict the role of height on grazing. Previous studies on pasture height found an effect on biting rate, but not on grazing duration (SILVA *et al*., 2020).

Correlation between percentage of dead material and grazing time was moderate. When dead matter is abundant in the pasture, sheep will naturally need more time to select against it and eat primarily live material. Poor forage quality can increase time spent grazing (MANTECA; SMITH, 1994). Rumination time is also negatively correlated to forage quality, with a decrease in ruminating time as quality forage increases, which also explains the moderate positive correlation between this behavior and dead material percentage (WELCH; SMITH, 1969).

The Pampa grassland is a diverse and heterogeneous environment. Unlike in most Brazil, seasonal changes induce differences in daylight and temperatures. These differences explain the behaviors observed in this study, with grazing occupying the greater proportion of the day in fall.

## 5. Conclusion

Of the three seasons observed, fall had the shortest daylight length and the greatest proportion spent grazing. Although spring had longer days, summer was the season with greatest idling time, probably due to higher temperatures. Ruminating proportions decreased with daylight length.

Fecal egg count was not correlated to ingestive behaviors. As for sward structure, percentage of dead material was the only measure that correlated moderately with behavior. The Pampa is a diverse and heterogeneous environment and these characteristics along with seasonal changes explain differences in feeding behavior strategies.

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**Table 1 –** Weather data during the behavior evaluation days

| | Date (D/M/Y) | Sunrise | Sunset | Daylight length | Min Temp ºC | Ave Temp ºC | Max Temp ºC |
|---|---|---|---|---|---|---|---|
| Spring[1] | 5/12/2020 | 5:16 | 19:15 | 13 h 59 min | 16.4 | 18.7 | 21.1 |
| | 14/12/2020 | 5:18 | 19:22 | 14 h 4 min | 18.1 | 21.5 | 24.8 |
| | Average | 5:17 | 19:19 | 14 h 2 min | 18.8 | 20.9 | 23.2 |
| Summer[2] | 14/2/2021 | 6:05 | 19:13 | 13 h 8 min | 20.3 | 22.8 | 27.9 |
| | 21/2/2021 | 6:10 | 19:06 | 12 h 56 min | 19.2 | 24.9 | 31.4 |
| | Average | 6:08 | 19:10 | 13 h 2 min | 19.7 | 23.8 | 29.6 |
| Fall[2] | 23/5/2021 | 7:08 | 17:35 | 10 h 27 min | 12.6 | 13.9 | 15.1 |
| | 31/5/2021 | 7:12 | 17:33 | 10 h 21 min | 0.5 | 7.9 | 17.5 |
| | Average | 7:10 | 17:34 | 10 h 24 min | 6.5 | 10.9 | 16.3 |

**[1]Data retrieved from Agrometeorological monitoring system Arroio dos Ratos**
**[2]Data retrieved from Agronomic Experimental Station of the Federal University of Rio Grande do Sul**

**Table 2-** Lambs' fecal egg count (FEC) and pasture characteristics in the three periods of evaluation.

| | FEC[1] | Pasture height (cm)[2] | Herbage mass (kg ha $^{-1}$ DM) [2] | Percentage dead material (%)[1] |
|---|---|---|---|---|
| Spring | 0 (0-100) b | 17 ± 1.79 a | 1852.56 ± 125.44 b | 14.34 (11.72 – 16.28) c |
| Summer | 200 (0-600) ab | 11.5 ± 3.09 b | 801.71 ± 64.84 c | 31.79 (27.80 – 39.19) b |
| Fall | 300 (100-700) a | 16 ± 3.16 a | 2587.60 ± 155.43 a | 50.51 (49.19 – 51.16) a |
| *P*-value | < 0.000 | < 0.010 | < 0.000 | < 0.000 |

**Values within the same row followed by different letters differ significantly (significance level at 5%).**
**[1] Median (min-max); analyzed by Kruskal-Wallis**
**[2] Means ± standard error; analyzed by ANOVA**

**Table 3 –** Diurnal feeding behaviors in the three periods of evaluation as a percentage of daylight time. Values within the same row followed by different letters differ (Tukey $P \leq 0.05$).

| | Spring | Summer | Fall | SE | *P* |
|---|---|---|---|---|---|
| Grazing (%) | 62.5b | 62.9b | 73.7a | 0.7 | <0.000 |
| Ruminating (%) | 29.3a | 25.5b | 21.4c | 0.6 | <0.000 |
| Idling (%) | 7.6b | 11.5a | 4.8c | 0.6 | <0.000 |

**SE = Standard Error.**

**Table 4 –** Correlations between fecal egg count (FEC) and pasture characteristics with daily behavior.

| | Grazing | | Ruminating | | Idling | |
|---|---|---|---|---|---|---|
| | *P*-value | r | *P*-value | r | *P*-value | r |
| FEC | 0.071 | 0.131 | 0.047 | -0.144 | 0.865 | 0.012 |
| Pasture height | 0.001 | -0.235 | <.001 | 0.337 | 0.787 | -0.019 |
| Herbage mass | 0.344 | -0.067 | 0.305 | 0.072 | 0.835 | -0.015 |
| Percentage of dead material | <0.000 | 0.462** | <0.000 | -0.488** | 0.002 | -0.221 |

**** Moderate correlation (r > 0.399)**

**Figure 1** – Proportional duration of diurnal behaviors per season

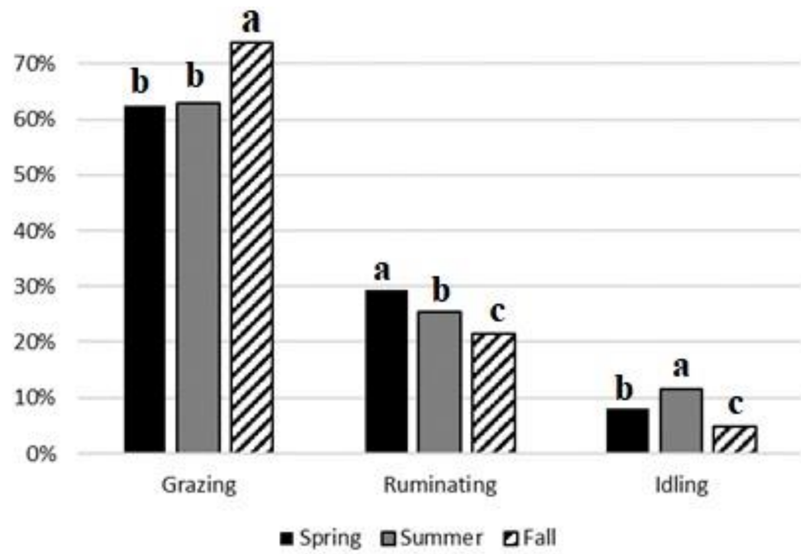
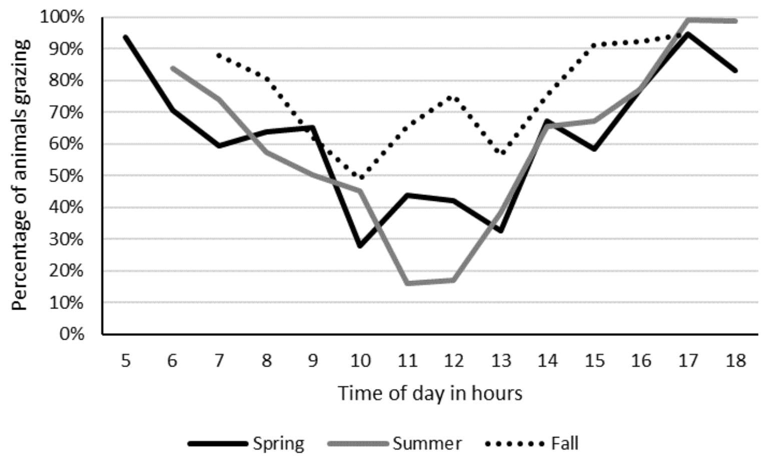
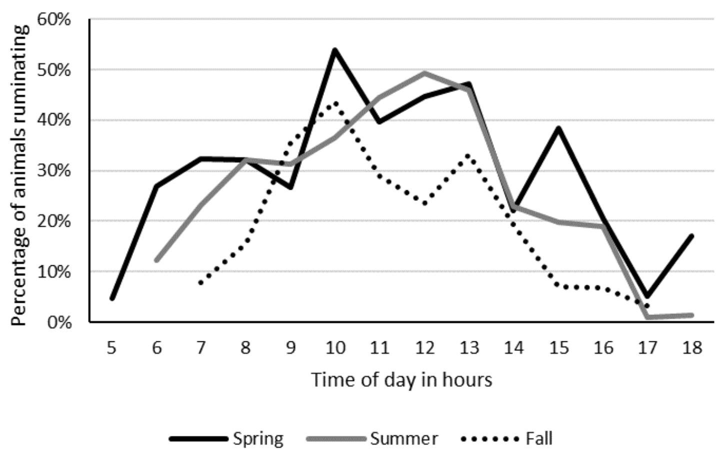


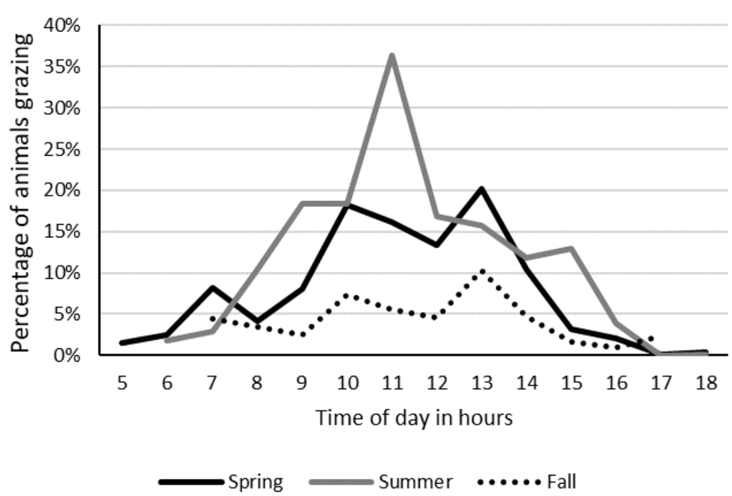
**Figure 2** – Proportional of animals grazing (A), ruminating (B) and idling (C) through the day.




A

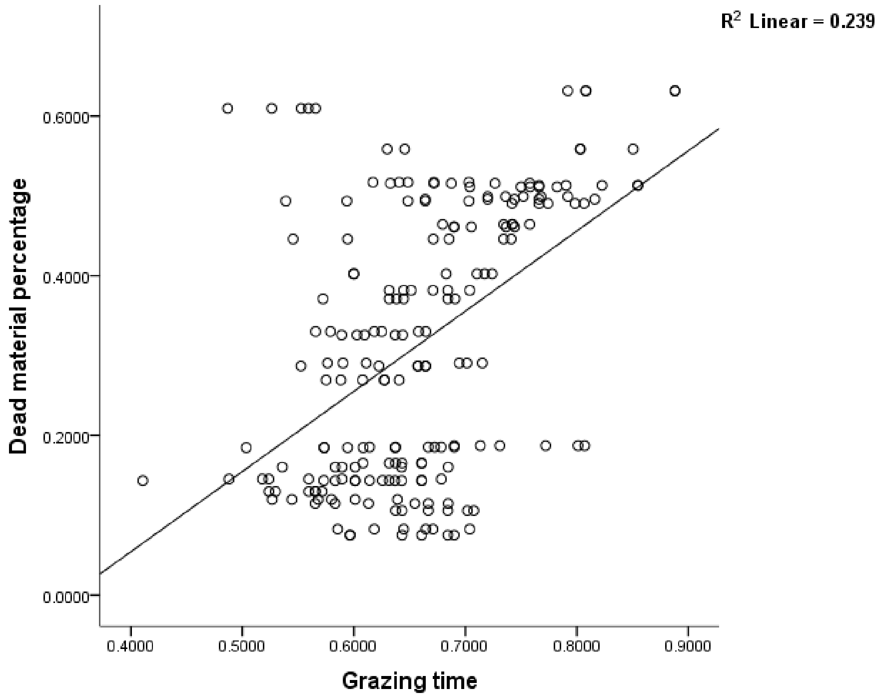



B

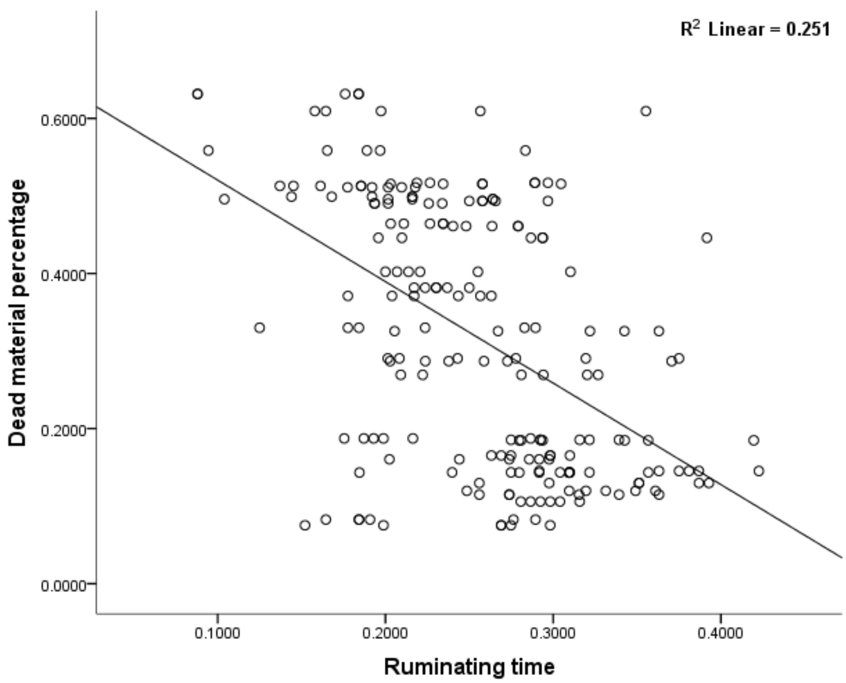



C

**Figure 3** – Correlations between dead material percentage and grazing time or ruminating time.




**A**




**B**

# CHAPTER V

# 4. FINAL CONSIDERATIONS

The first study observed that sheep grazed the legume *Desmodium incanum* (DI) despite its high concentrations of tannins. The facts that this plant is very common in the Pampa biome grasslands and preferentially grazed by sheep suggest that it is an important source of tannin as well as nutrients for sheep in this environment.

The second study showed that growing lambs prefered high protein diets, and the inclusion of 4% of tannin extract did not reduce total feed intake when there was a tannin-free option. This result could be explained by a disruption in the selection mechanism of sheep, affected by the astringency sensation caused by tannin in only one of the feeds. The second study also observed a shift from urinary to fecal excretion of nitrogen, which has environmental implications. The treatment that received 4% of tannin extract in both diets achieved lower values of blood urea nitrogen (which is related to urinary N excretion) and higher values of fecal N excretion.

The third study demonstrated differences in sheep ingestive behavior in a natural Pampa biome grassland. Behaviors were affected by season and percentage of dead material (which varied within the periods of observation).

If managed correctly, the Pampa biome offers optimal conditions for sheep farming, producing high-quality meat resulting from the intake of tannins in the pasture. Sheep are highly selective animals able to select and attain their required nutrients. In the diverse native Pampa pasture, they are free to select both protein and tannin levels that could improve meat production and quality. Further studies are encouraged to address the effect of DI tannin intake on sheep performance.

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# APPENDICES

U F R G S

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CEUA UFRGS

## *CARTA DE APROVAÇÃO*

**Comissão De Ética No Uso De Animais** analisou o projeto:

**Número:** 39705

**Titulo:** Ingestão voluntária de tanino condensado por ovinos

**Vigência:** 01/10/2020 à 31/07/2024

**Pesquisadores:**

**Equipe UFRGS:**

CESAR HENRIQUE ESPIRITO CANDAL POLI - coordenador desde 01/10/2020
Henrique Jonatha Tavares - desde 01/10/2020
MARINA TERRA BRAGA - desde 01/10/2020

***Comissão De Ética No Uso De Animais** aprovou o mesmo em seus aspectos éticos e metodológicos, para a utilização de 36 cordeiros, 20 ovinos machos castrados e 16 ovinos fêmeas, provenientes de Estação Experimental Agronômica da UFRGS, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.*

Porto Alegre, Sexta-Feira, 22 de Janeiro de 2021

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética

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Bell RHV. 1982. The effect of soil nutrient availability on community structure in African ecosystems. In: Huntley BJ, Walker BH (eds), *Ecology of tropical savannas.* Berlin: Springer-Verlag. pp 193–216.

Hoffman MT, Cowling RM. 1990. Desertification in the lower Sundays River Valley, South Africa. *Journal of Arid Environments* 19: 105–117.

Leng RA. 1986. *Drought feeding strategies: theory and practice.* Armidale: Penambul Books.

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Sales J, Britz PJ. 2001. Research on abalone (*Haliotis midae* L.) cultivation in South Africa. *Aquaculture Research* 32: 863–874.

### Journal article, more than two authors
Ellender BR, Weyl OLF, Shanyengange MK, Cowley PD. 2008. Juvenile population dynamics of *Oreochromis mossambicus* in an intermittently open estuary at the limit of its natural distribution. *African Zoology* 43: 277–283.

**Note**: Issue numbers are omitted from citations.

### Abstract as original source [not to be encouraged]
Botha CEJ, Liu L, Cross RHM. 2005. Functionality within plasmodesmata: roles for callose, actin and myosin? [Abstract]. *South African Journal of Botany* 71: 261.

### Abstract from a secondary source [not to be encouraged]
Szumilas M, Kutcher SP. 2008. Youth and suicide. *Canadian Medical Association Journal* 178: 286–286. Abstract obtained from *Journal of Child & Adolescent Mental Health* 20: 70.

### Book, one author
Mackenzie J. 2004. *The editor's companion*. Cambridge: Cambridge University Press.

Zar JH. 1984. *Biostatistical analysis* (2nd edn). Englewood Cliffs: Prentice-Hall.

**Note**: '(2nd edn)' is not italicised.

### Book, two authors
Milroy L, Gordon M. 2003. *Sociolinguistics: method and interpretation*. Oxford: Blackwell.

### Edited book
Huntley BJ, Walker BH (eds). 1982. *Ecology of tropical savannas*. Berlin: Springer-Verlag.

### Chapter in a book
de Villiers C, Hodgson A, Forbes AT. 1999. Studies on estuarine macrobenthos. In: Allanson BR, Baird D (eds), *Estuaries of South Africa*. Cambridge: Cambridge University Press. pp 167–207.

### Non-English book
Makatsch W. 1974. *Die eier der vögel Europas*, vol. 1. Berlin: Neumann.

### Magazine/newsletter article, one author
Burger S. 2001. Indigenous invaders of the winter-rainfall region. *Farmer's Weekly* 21/28 December: 8.

### Newspaper article, no author
Anon. 2002, January 19. Why target the good guys, Mr Moosa? *The Herald*. p 4.

### Contract research document (published)
Whitfield AK, Bate GC (eds). 2007. *A review of information on temporarily open/closed estuaries in the warm and cool temperate biogeographic regions of South Africa, with particular emphasis on the influence of river flow on these systems*. WRC Report No. 1581/1/07. Pretoria: Water Research Commission.

### Government legislation (upper case used for formal documents)
DEAT (Department of Environmental Affairs and Tourism). 2006. Publication of draft policies for the allocation and management of medium-term small-scale commercial fishing rights and for the allocation and management of medium-term subsistence fishery rights. *Government Gazette, South Africa* 497(29391).

RSA (Republic of South Africa). 1998. Marine Living Resources Act (Act No. 18 of 1998). *Government Gazette, South Africa* 395(18930).

### Series
Govender A. 2000. Garrick (*Lichia amia*). In: Mann BQ (ed.), *Southern African marine linefish status reports. Special Publication* No. 7. Durban: Oceanographic Research Institute. pp 16–17.

McKenzie CL, Burrell VG, Rosenfield A, Hobart WL. 1997. The history, present condition, and future of the molluscan fisheries of North and Central America and Europe. Vol. 1: Atlantic and Gulf Coasts. *NOAA Technical Report* 127.

Rolando CA, Hitchins M, Olivier S. 2006. Methods to improve late season planting of *Pinus patula*. *ICFR Bulletin Series* No. 08/2006. Pietermaritzburg: Institute for Commercial Forestry Research.

### Dissertation/thesis
Vorwerk PD. 2006. A preliminary examination of selected biological links between four Eastern Cape estuaries and the inshore marine environment. PhD thesis, Rhodes University, South Africa.

### Unpublished paper presented at a meeting [not to be encouraged; content should be accessible]
Gibbons M. 2006. Engagement as a core value in a Mode 2 society. Paper presented at the CHEHEQC/JET-CHESP Conference on Community Engagement in Higher Education, Cape Town, 3–5 September 2006.

### Conference/workshop proceedings
Tibbits WN, Boomsma DB, Jarvis S. 1997. Distribution, biology, genetics and improvement programmes for *Eucalyptus globulus* and *E. nitens* around the world. In: White T, Huber D, Powell G (eds), *Proceedings of the 24th Biennial Southern Tree Improvement Conference, 9–12 June, Orlando, Florida*. Orlando: Southern Tree Improvement Committee. pp 1–15.

### Unpublished reports
Booth A. 2005. South African monkfish (*Lophius vomerinus*) stock assessment. Report No. WG/05/04/D:A:07. Marine and Coastal Management, Cape Town.

# Figure Guidelines for Authors:
## format, style and technical considerations

Authors are expected to provide final figures that conform to the journal style and meet the technical requirements for online and print publication. Minor adjustments are often needed to suit the journal style and for this reason we prefer to receive illustrations in a format that allows us to adjust figure elements, such as line thickness, symbols, fill colours and fonts, if necessary. However, costs of reworking substandard figures will be charged to authors.

**Figure size**

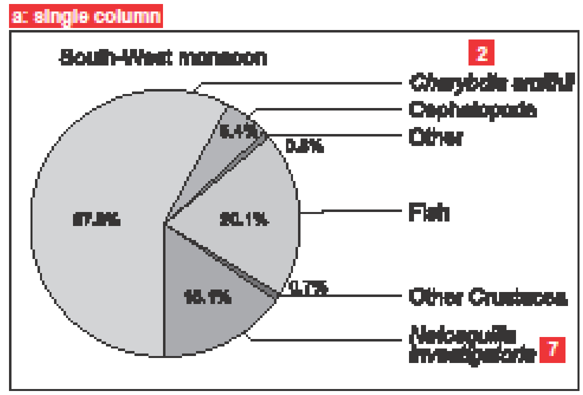
- For larger format journals (210 × 275 mm page size), figures should be prepared as (a) single (84 mm) or (b) double (176 mm) column width, with a maximum depth of 225 mm to allow space for the figure caption.
- For smaller format journals (170 × 240 mm page size), figures should be prepared as no wider than 140 mm and maximum depth should be 190 mm.

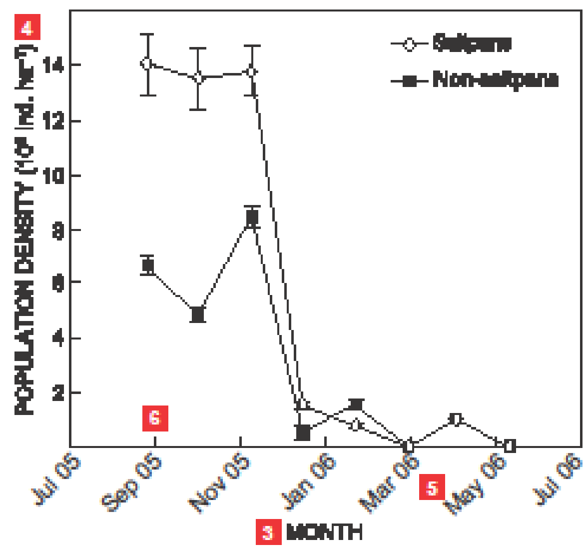
**Graphs and pie charts**

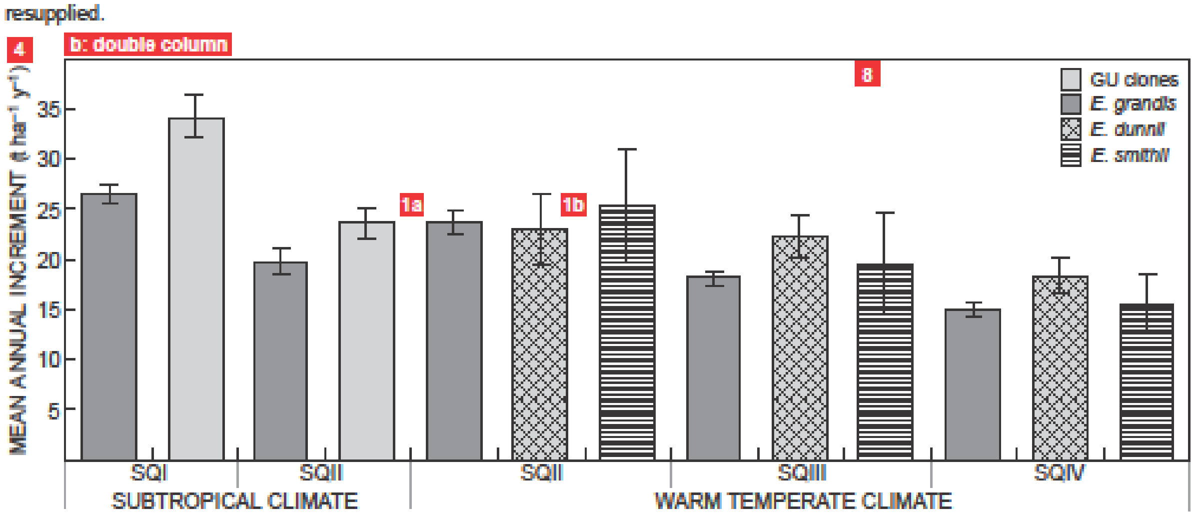
1. Grey shading is preferable (1a) but patterns may be used where necessary (1b)
2. Labels taken out of the pie chart to reduce clutter
3. Axis labels 9 point Arial, upper case
4. Units in lower case, in parentheses
5. Axis label slanting where appropriate
6. Tick marks 0.5 point, inwards facing (for clarity)
7. Text in graphics usually 8 point Arial
8. Charts to be boxed with 0.5 point line

Graphs, pie charts and flow charts can be generated in MS Excel, MS Powerpoint or Adobe Illustrator and supplied as XLSX, PPTX or AI format files, respectively. Where a specialist drawing or statistics program is used to generate graphs, export these as AI, EPS, EMF, PDF, SVG or WMF files to preserve the vector format and allow for minor changes to be made, where necessary. DO NOT embed, paste or insert illustrations into an MS Word document as this can render them uneditable and they may need to be resupplied.

a: single column



# VITA

Marina Terra Braga nasceu em Porto Alegre (RS) em 8 de fevereiro de 1989, filha de Ana Lúcia Terra Braga e Marcelo Cardoso Braga. Cursou a graduação em Medicina Veterinária na Universidade Federal do Rio Grande do Sul, tendo colado grau em 13 de dezembro de 2014. Durante a graduação, integrou o Centro de Ensino e Pesquisa em Ovinocultura e escreveu o TCC sobre comportamento ingestivo de cordeiros, sob a orientação do professor César Poli. Realizou o estágio curricular da graduação no departamento de estudo de comportamento do Zoológico de Saint Louis, nos Estados Unidos. Em 2016 ingressou no mestrado em Comportamento Animal e Humano na Universidade de Rennes 1, na França. Em abril e maio de 2017 realizou estágio curricular na Universidade de Évora, em Portugal. Em 2017 transferiu-se para a Universidade Montpellier SupAgro, na França, para cursar o segundo ano do mestrado em Sistemas de Produção Animal. Realizou o estágio obrigatório na empresa LabToField, atuante em pesquisa na nutrição equina, onde desenvolveu estudo de comportamento alimentar com diferentes formas de polpa de beterraba. Em setembro de 2018 defendeu sua dissertação (sobre o mesmo estudo) e obteve o grau de mestre com menção máxima (“très bien”). Em 2019 retorna a Universidade Federal do Rio Grande do Sul ingressando no doutorado em Zootecnia, sob orientação do professor César Poli. Durante o período de dezembro de 2021 a maio de 2022 realizou doutorado sanduíche na Universidade de Utah, nos Estados Unidos, sob orientação do professor Juan Villalba, que também foi coorientador do doutorado. Foi bolsista CNPq durante todo o período de seu doutorado, exceto no período sanduíche, quando era bolsista CAPES Print.