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***ROTATINUOUS STOCKING*: AN INNOVATION IN GRAZING MANAGEMENT BASED ON ANIMAL BEHAVIOUR AND IMPLICATIONS TO PASTURE PRODUCTION, FORAGING BEHAVIOUR, HERBAGE INTAKE AND METHANE EMISSION BY GRAZING SHEEP**

JEAN VÍCTOR SAVIAN

Zootecnista/UFSM
Mestre em Zootecnia/UFRGS

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JEAN VICTOR SAVIAN
Zootecnista e Mestre em Zootecnia

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Pela Banca Examinadora

PAULO CÉSAR DE FACCIO CARVALHO
PPG Zootecnia/UFRGS
Orientador

SILA CARNEIRO DA SILVA
USP/ESALQ

R. Mark Sulc

REUBEN MARK SULC
Ohio State University/EUA

JÉRÔME BINDELLE
University of Liège/Bélgica

Homologado em: 19.04.2017
Por

PAULO CÉSAR DE FACCIO CARVALHO
Coordenador do Programa de Pós-Graduação em Zootecnia

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

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Rotatinuous stocking: an innovation in grazing management based on animal behaviour and implications to pasture production, foraging behaviour, herbage intake and methane emission by grazing sheep[1]

Author: Jean Víctor Savian
Advisor: Paulo César de Faccio Carvalho

Abstract: The aim of this thesis was to investigate contrasting pasture management strategies on the foraging behaviour, pasture production, carcass characteristics, herbage intake, faecal gas emissions and methane emission by sheep grazing Italian ryegrass (*Lolium multiflorum*) pastures. The experiment was carried out in 2014 and 2015. The experimental design was a randomized complete block with four replicates and two grazing strategy treatments (traditional rotational stocking method – RT meaning pre- and post-grazing target heights of 25 and 5 cm, respectively and, "rotatinuous stocking" – RN with pre- and post-grazing target heights of 18 and 11 cm, respectively). The grazing management was based on a 1-day strip-grazing regime. Male castrated sheep were used. The actual average sward heights for the RN treatment were 17.9 and 11.1 cm (pre- and post-grazing, respectively) and 27.1 and 7.8 cm for the RT (pre- and post-grazing, respectively). The stocking period in 2014 was 146 and 140 days (RN and RT, respectively) and in 2015 it was 155 and 146 days (RN and RT, respectively). The diurnal animal activities (grazing, ruminating and resting time) did not differ between treatments, with average of 439.6, 166.9 and 85.0 minutes, respectively. The bite rate, feeding station per min and steps per min were greater at the RN than the RT treatment. Grazing time and bite rate were greater in the afternoon than morning in both treatments. Therefore, the daily herbage intake by sheep grazing Italian ryegrass was greater for the RN than the RT treatment (CHAPTER II). The herbage production in the RN was 28% higher than the RT treatment. Individual average daily gain and live weight gain per hectare were greater in the RN than the RT treatment (CHAPTER III). RN treatment presented greater final live weight, carcass and commercial cut weights from grazing sheep than RT treatment (CHAPTER IV). RN treatment had a faecal chemical quality greater than RT treatment, resulting in a greater daily nitrogen excretion per animal and greater faecal CH_4 and N_2O emissions (CHAPTER VI). The "rotatinuous stocking" (RN) was the better grazing management strategy for mitigation of CH_4 emissions by sheep grazing Italian ryegrass, emitting 64% less CH_4 per unit area and 170% less CH_4 per unit of animal product than the traditional rotational stocking method (RT) (CHAPTER V).

Key words: animal behaviour, carcass characteristics, organic matter intake, greenhouse gases, rotational stocking, sward heights.

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1. CHAPTER I

1.1. GENERAL INTRODUCTION

According to UNGAR & NOY-MEIR (1988), the grazing process consists in the searching and handling of forage by the animal. Thus, in a pastoral environment, the animals use disposal strategies to optimise the intake of nutrients (FORBES & GREGORINI, 2015), mainly of metabolizable energy (BOVAL & DIXON, 2012). The forage intake is the result of a dynamic combination of animal, rumen, and plant factors, that can be negative or positive stimuli (SODER et al., 2009). According to CARVALHO (2013) the herbivores select plants and their morphological components in order to optimize nutrient intake. Therefore, the diet selection by herbivores in pastoral systems is a key point to achieve high levels of herbage intake (PRACHE et al., 1998). Nonetheless, the traditional grazing management proposes from the rotational stocking method, which prioritize the maximum herbage mass accumulation to start grazing with high herbage harvested, is based on the plant growth associated with large resting periods. Thus, there is a challenge to be achieve and surpassed, which is to manage the sward based on the animal, which prioritize the high forage intake.

Therefore, a new pasture management concept has been proposed that considers the animal as the main agent to define grazing targets. In other words, the ideal sward structure that maximizes and keeps the highest animal forage intake per unit of grazing time. This grazing management was called “rotatinuous stocking”, which is based on the principle of “*take the best and leave the rest*” from the pasture (CARVALHO, 2013). Previous studies were performed to assess the ideal pre-grazing height, including native grassland (GONÇALVES et al., 2009), *Sorghum bicolor* (FONSECA et al., 2012), *Pennisetum glaucun* (MEZZALIRA et al., 2013), *Lolium multiflorum* (AMARAL et al., 2013), *Cynodon* sp. and *Avena strigosa* (MEZZALIRA et al., 2014). These studies concluded that each forage species has an optimum sward structure to be grazed (i.e., sward height). For example, Italian ryegrass (the plant model in this thesis) has the ideal height at 18 cm at which animal herbage intake rate is maximized (AMARAL et al., 2013; DA SILVA, 2013). In order to maintain the highest forage intake rate, sequential experiments demonstrated that the optimum post-grazing sward height was a reduction of no more than 40% of pre-grazing sward height for tropical (FONSECA et al., 2012; MEZZALIRA et al., 2014) and temperate species (MEZZALIRA et al., 2014).

A consequence of this proposal is animals having a high herbage intake rate because they are eating the top stratum of the pasture, predominantly leaves (FONSECA et al., 2012; MEZZALIRA et al., 2014). The residue post-grazing is high, which hypothetically can also result in higher herbage regrowth (BARKER et al., 2010). Thus, based on the necessity for the development of sound pastoral systems, this new pasture management concept based on high grazing frequency and low grazing intensity, has the aim to minimize the trade-offs between forage production, animal production (individual and per area) and greenhouse gases from grazing livestock. According to HERRERO et al. (2016) improving animal reproductive rates, feed availability and average daily gain rates, may result in the reduction of herd size and reduced greenhouse gas emissions. Therefore, production systems soundly managed, besides being able to reduce the number of ruminants, can also increase animal production, which is a necessity to satisfy future global demands for milk and meat (CAMPBELL et al., 2014) with quality and safety.

According to this contextualization, this thesis presents a conceptual model with hypothesis and objectives that show the main issues in five chapters.

1.2. CONCEPTUAL MODEL

The conceptual model presented in Figure 1 shows the effect of grazing management (pre- and post-grazing sward heights) under sward structure and their implications at herbage production, herbage chemical quality, herbage intake, animal performance, carcass characteristics and greenhouse gas emissions.

The **pasture** component shows the main effects of pre- and post-grazing sward heights under the sward structure modification and their implications on the sward light interception, that is the key central to the tillers dynamics, such as population density and natality and mortality of tillers (SBRISSIA et al., 2001). The sward light interception is the variable that drives the herbage accumulation rate. This process is a consequence mainly of the herbage residue (post-grazing), which controls the herbage accumulation rate and therefore the total herbage production in grazing systems.

The animal behaviour includes the smaller scale (bite) to the biggest scales, as for example, grazing and ruminating time. This is the main interaction point between **pasture** and **animal** components of this conceptual model. The sward height is an important regulator of feeding behaviour, mainly of bite mass and bite rate by grazing animals (FONSECA et al., 2013; MEZZALIRA et al., 2014). Thus, the velocity of forage ingestion by animals is also related to the plant parts that the animal has access to, such as leaves and stems in a grazing event.

Nonetheless, the central piece in this model is the forage intake (strong red box) by animals that represent the foundation to build an adequate pastoral environment. Nutrient intake by animals is the main component that regulates the average daily gain affecting carcass characteristics and meat quality from grazing animals. Therefore, the combination between average daily gain and stocking rate results in the animal production per area.

Lastly, the nutrient intake affects the amount of gases produced by the enteric fermentation (methane, mainly by eructation and respiration) and by animal excreta, mainly nitrogen (faeces and urine) in part result in methane and nitrous oxide emissions. Thus, an understanding of the grazing process is very important to minimize the environmental impact and maximize the animal production.

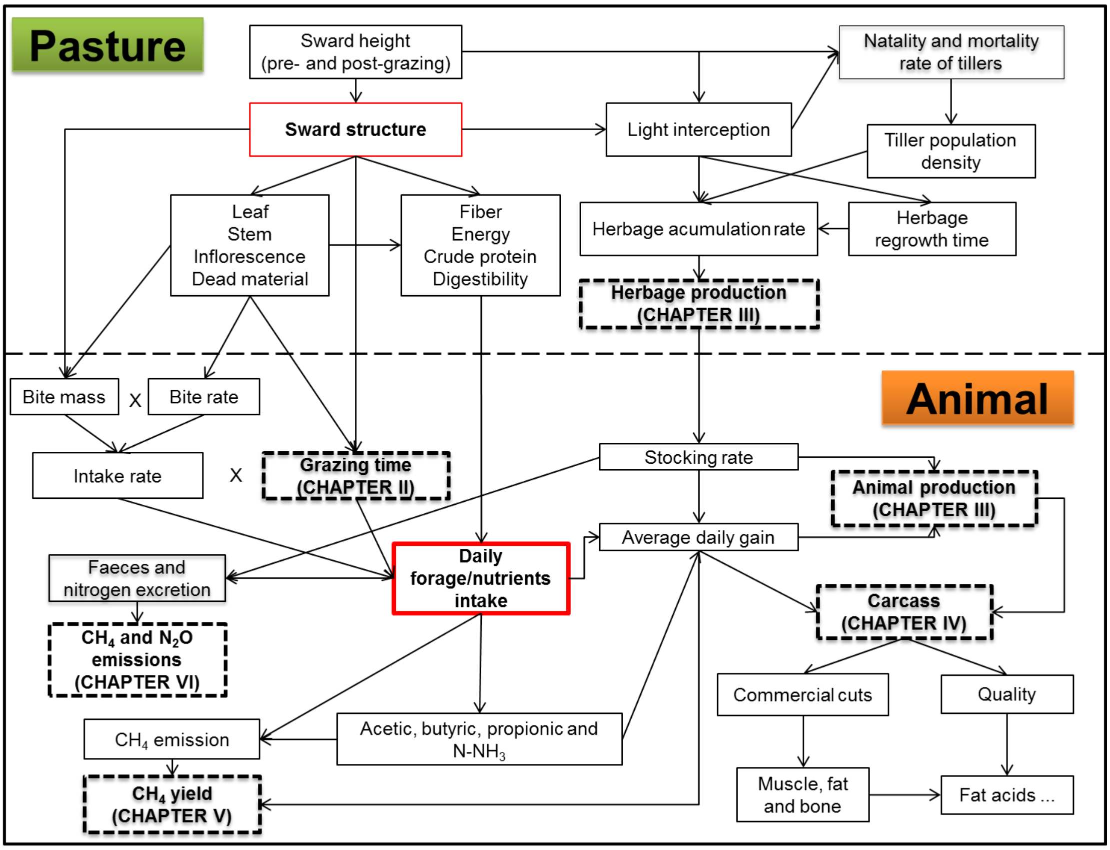

Figure 1. Conceptual model of the effects of grazing management and sward structure (pre- and post-grazing sward heights) on the response variables of herbage production, animal production, animal behaviour, herbage intake and their implications on greenhouse gas emission by grazing sheep.

1.3. HYPOTHESIS

The hypothesis presented in this thesis is tested using two grazing management strategies in rotational stocking. The traditional rotational stocking method (RT), defined by large resting periods to favour the amount of herbage accumulation (pre-grazing mass) and by high grazing pressure to harvest all herbage in the strip (low post-grazing mass), represents the concept of herbage harvesting efficiency (*sensu* HODGSON, 1979) that classically drives pasture management. Conversely, “rotatinuous stocking” (RN) (CARVALHO, 2013) is proposed as a concept in pasture management in order to maintain the grazing animals at their highest herbage intake rates. In this case, pre- and post-grazing masses are merely a consequence of animal ingestive behaviour. Hence, this strategy represents the concept of herbage conversion efficiency (*sensu* HODGSON, 1979), but focusing only at the animal component. The consequence of this proposal, in terms of grazing management applied to rotational stocking, is short resting periods (high grazing frequency) and low grazing intensity (CARVALHO, 2013).

We hypothesized that the classical concept of herbage harvesting efficiency based mainly on sward growth and harvesting processes would not drive pasture management. On the contrary, animal ingestive behaviour would drive because it influences both pasture production that rewards the livestock operation (i.e. animal production) and environmental footprints (C and N coupling and decoupling).

To test this hypothesis, we contrasted two grazing strategies as presented above, and we assessed the performance of many parameters presented in the conceptual model. After the contextualization of this subject by a review of literature, we present our findings as follows:

Chapter II: Sward structure (pre- and post-grazing) according to the “rotatinuous stocking” (RN) strategy results in better herbage chemical composition, higher grazing time and higher herbage intake by sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT).

Chapter III: Sward structure (pre- and post-grazing) in the “rotatinuous stocking” (RN) results in higher herbage harvested and higher performance of sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT).

Chapter IV: Sward structure (pre- and post-grazing) in the “rotatinuous stocking” (RN) results in better carcass characteristics and higher meat quality of sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT).

Chapter V: Sward structure (pre- and post-grazing) in the “rotatinuous stocking” (RN) results in higher nutrient intake and lower methane emission and intensity by sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT).

Chapter VI: Sward structure (pre- and post-grazing) in the “rotatinuous stocking” (RN) results in higher amount of faeces and nitrogen excretion and higher gas emission by faeces from sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT).

1.4. LITERATURE REVIEW

1.4.1. Grazing management

According to ALLEN et al. (2011) the terminology “grazing management” is defined by *“the manipulation of grazing in pursuit of a specific objective or set of objectives”.* Grazing management has been proposed based on concepts of plant growth such as leaf area index and canopy light interception (BROUGHAM, 1956; 1957) or concepts of amount of plant tissue available to the animal, such as forage allowance (GIBB & TREACHER, 1978) and leaf allowance (BIRCHAM & SHEATH, 1986).

Grazing management has its foundation on plant morphogenesis (leaf elongation, appearance and lifespan) which means ultimately how herbage accumulates in time and space. These variables define sward structure characteristics, such as leaf size, tiller density and leaves per tiller that may change the leaf area index (CHAPMAN & LEMAIRE, 1993).

The predominant basis of pasture management are the concepts of leaf area index and canopy light interception (BROUGHAM, 1956; 1957), that focus on the plant component in order to propose grazing management protocols. In general, maximum net herbage accumulation is the goal, and sward light interception of 95% is the reference to start grazing for temperate (BROUGHAM, 1957; PARSONS & PENNING, 1988) and tropical pastures (MELLO & PEDREIRA, 2004; CARNEVALLI et al., 2006; BARBOSA et al., 2007; DA SILVA & NASCIMENTO JUNIOR, 2007; DA SILVEIRA et al., 2013). For example, BARBOSA et al. (2007) reported greater herbage accumulation of capim tanzania (*Panicum maximum* Jacq.) pastures with sward light interception of 95% (pre-grazing) combined with residue of the 25 cm (post-grazing) compared to sward light interception of 90% or 100% and residue of 50 cm.

Therefore, grazing management can be viewed in different ways, but which grazing management is more adequate? Could the ideal grazing management be that which maintains a sward structure that maximizes the forage intake and individual gain by animals? If so, would that be achieved with different targets, as for example, light interception, herbage allowance or sward height? In this rationale, would herbage production be only a consequence of this process? For example, higher herbage intake rate is achieved when the animal eats mainly the top stratum of the pasture, predominantly leaves (FONSECA et al., 2012; MEZZALIRA et al., 2014). In that scenario, the defoliation by the animal is low and the post-grazing residue is high, which can also result in greater herbage regrowth. In this sense, swards would be managed based on animal behaviour and not prioritizing only the plant, but indirectly having positive impacts on plant growth. According to BROUGHAM (1956), moderate grazing intensities result in higher residual leaf, and higher sward photosynthetic efficiency after each grazing event. Moreover, the defoliation intensity is the pivotal factor affecting natality and mortality rate of tillers (SBRISSIA et al., 2001).

1.4.2. Forage intake and animal behaviour as a grazing management target

Forage intake is the result of a dynamic combination of animal, rumen, and plant factors, that can be negative or positive stimuli (SODER et al., 2009). Forage intake by grazing animals is controlled to satisfy the nutritional requirements (FORBES, 1987) for growth, maintenance and reproduction (GORDON, 1995). MERTENS (1994) stated that the diet fibrous carbohydrates are the main elements limiting forage intake; neutral detergent fibre being the best predictor of voluntary intake by ruminants. Fibre is related to plant maturity (HODGSON, 1990), sward structure (CARVALHO, 2013) and the plant part that the animal eats (e.g. leaves on the top or stems on the bottom stratum) (BENVENUTTI et al., 2015). Forage intake is controlled also by digestible factors such as passage rate, gastrointestinal capacity and non-nutritional factors such as thermoregulation, socialization and vigilance (LACA & DEMMENT, 1992). Thus, forage intake by herbivores in pastoral ecosystems is complex and is a key factor for achieving high levels of animal production (PRACHE et al., 1998). CARVALHO (2013) claimed that herbivores select plants and their morphological components to optimize nutrient intake. Thus, the ultimate objective would be to achieve the highest possible intake of metabolizable energy (BOVAL & DIXON, 2012).

According to HODGSON (1981), forage intake is a product of bite weight, bite rate and grazing time. Accordingly, the challenge of the grazing process is to identify sward structures that can maximize forage and nutrient intake, with lower grazing time (i.e. energy costs) and higher opportunity to select a balanced diet (VILLALBA & PROVENZA, 2009). An understanding of the fundamental processes of grazing behaviour is a prerequisite for the design of efficient grazing management systems (SODER et al., 2009).

In this context, a new strategy has been proposed based on the concept that considers the animal as the agent that defines better grazing management. In others words, the ideal sward structure in one that maximizes and maintains the highest animal forage intake per unit of grazing time. This strategy was called “rotatinuous stocking”, which is based on the principle of *“take the best and leave the rest”* from the pasture (CARVALHO 2013).

“Rotatinuous stocking” was proposed for different forage species, such as native grassland (GONÇALVES et al., 2009), *Sorghum bicolor* (FONSECA et al., 2012), *Pennisetum glaucun* (MEZZALIRA et al., 2013), *Lolium multiflorum* (AMARAL et al., 2013), *Cynodon* sp. and *Avena strigosa* (MEZZALIRA et al., 2014). These authors investigated the ideal sward height that maximized the short-term herbage intake rate by animals and concluded that the different pasture species had different ideal sward heights.

Once the ideal sward height is known, a new question arises: What is the ideal post-grazing sward height to keep herbage intake rate at its maximum? Several experiments were devoted to this question, and concluded that the limit for sward depletion (grazing down) was 40% of pre-grazing sward height for tropical (FONSECA et al., 2012; MEZZALIRA et al., 2014) and temperate forages (MEZZALIRA et al., 2014) (Figure 2).

Another important information presented by MEZZALIRA et al. (2014) is that when sward pre-grazing height was reduced by 40%, the amount of surface area non-grazed at that point was 23 and 24% in *Cynodon* sp. and

Avena strigosa pastures, respectively. After a sward depletion of 40%, the nutrient harvested per unit time of bite formation decreases (CARVALHO, 2013). CARVALHO (2013) also proposed that herbage intake rate is constant until the animal graze two-thirds of the uppermost stratum of the pasture surface layer, when intake rate tends to drop.

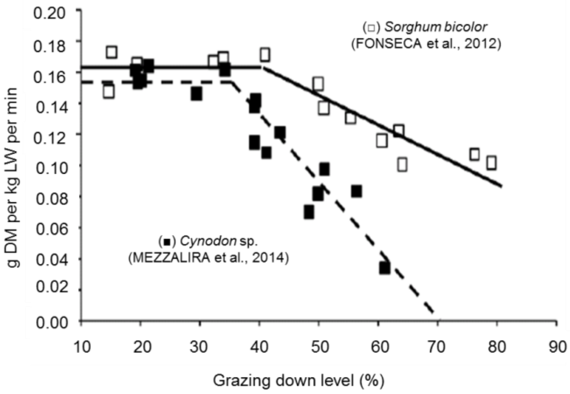

Figure 2. Short-term herbage intake rate during the grazing down (% reduction of initial sward height) (Adapted from CARVALHO, 2013).

Thus, we can see that forage intake is directly related to sward structure (height) and that the bite is a main part of this process (see CARVALHO et al., 2015). The studies mentioned above also show that bite mass is the variable that drives herbage intake rate; these variables had a similar response when the sward height was modified.

The ideal sward height for a high bite mass also is a consequence of higher leaf/stem ratio. AMARAL et al. (2013) observed that when the sward height (Italian ryegrass) was reduced from 17.3 to 10.7 cm (ideal grazing management), the leaf/stem ratio decreased from 1.05 to 0.69. When the sward height was reduced from 26.0 to 8.2 cm, the leaf/stem ratio decreased from 0.85 to 0.34. FONSECA et al. (2012) also found that in *Sorghum bicolour* pastures the post-grazing leaf mass decreased from 388 to 29 kg DM/ha when the defoliation increased from 36 to 63 cm of the sward pre-grazing height. Both studies demonstrated that bite mass was higher when the leaf/stem ratio was also higher.

BENVENUTTI et al. (2008) reported that when the stem density of the canopy increased, the animals spent more time in herbage manipulation; consequently, the time per bite also was higher. According to FONSECA et al. (2013), when the herbage depletion was higher than 40% of pre-grazing sward height, bite rate decreases linearly. When the animal spend more time per bite, the time to herbage selection was reduced (SHIPLEY et al., 1996). BENVENUTTI et al. (2015) described that diet quality was greater when the animals had access to the top leaf stratum of the sward, and declined when the animal grazed the bottom sward strata with a high proportion of stems. This is a very important topic in plant-animal interface because, given the choice, the animal eats only the plant parts (leaves) of better quality.

1.4.3. Implications of grazing management on animal performance

The success of animal production in grazing systems is dependent mainly on grazing management. Variation in sward structure is the main component that modifies the forage intake by grazing animals and consequently the individual animal performance in grasslands (HODGSON et al. 1994).

According to BOVAL & DIXON (2012) a management priority to increase output of animal products from grasslands should be based on the objective to achieve the highest possible intakes of metabolizable energy. These authors point out that the dry matter intake, or preferably intake of digestible nutrients, is an excellent indicator of the performance of grazing animals.

BOVAL et al. (2010) showed that the digestible organic matter intake by ewes was related to production of milk. Therefore, to evaluate the better diet and improve the sward management, variables such as digestible organic matter intake should be assessed.

In temperate pastures, SAVIAN et al. (2014) reported that growing lambs grazing Italian ryegrass pastures in continuous stocking had a greater herbage intake than rotational stocking (1345 and 1075 g DM/animal/day, respectively) and consequently greater average daily gain (150 and 90 g/animal, respectively), when both grazing methods had the same herbage allowance (13.4 kg DM/100 kg LW). ROCHA et al. (2011) and KUNRATH et al. (2014) reported that managing oats and annual ryegrass mixed pastures at 20 cm height under continuous stocking lead to overall soil–plant–animal system benefits. An optimum balance between harvesting and utilisation efficiencies was reported compared to pastures managed at 10 or 40 cm.

Native grassland in the south of Brazil managed in continuous stocking with daily forage allowance between 8 and 12% (kg DM/100 kg of LW), during different seasons of the year, resulted in greater average daily gain by beef heifers (345 g/animal) compared to 4% forage allowance (22 g/animal) (MEZZALIRA et al., 2012). Results are a consequence of the higher dry matter intake by animals in sward structures managed with daily forage allowance of 12% (DA TRINDADE et al., 2016).

In tropical pastures, EUCLIDES et al. (2016) found a greater average daily gain by steers grazing *Panicum maximum* (cv. Mombaça) in rotational stocking with 50 cm post-grazing sward height (655 g/animal) than 30 cm post-grazing sward height (392 g/animal). These authors commented that this result was mainly a consequence of higher leaf content on post-grazing mass, greater nutritive value and greater herbage intake by animals at 50 cm post-grazing sward height. The same response was found by DIFANTE et al. (2010), where individual daily gain by steers grazing *Panicum maximum* Jacq. cv. Tanzânia was of 801 g/animal at post-grazing sward height of 50 cm and 664 g/animal at post-grazing sward height of 25 cm.

As shown above, grazing management is central for achieving high individual animal performance. However, individual gain can be negatively related to stocking rate, as reported by lambs grazing perennial ryegrass (ARMSTRONG et al., 1995), steers grazing Italian ryegrass and oat mixed pastures (AGUINAGA et al., 2006; KUNRATH et al., 2014) and *Panicum maximum* (cv. Mombaça) (EUCLIDES et al., 2016). This phenomenon concerns the classic trade-off between individual gain and gain per area (PENNING et al., 1991).

According to PENNING et al. (1991), the highest live weight gain per hectare was achieved on perennial ryegrass managed at 3 cm height in continuous stocking. However, the lambs in that management had the lowest individual daily gains and experienced losses in body condition score, which were twice that observed on swards managed at 6 and 9 cm heights. According to the authors, results reflect the severe restriction in herbage intake per animal in pastures managed at 3 cm height in spring.

As described above, modification of sward structure by grazing management results in different levels of herbage intake and animal performance. To better evaluate the efficiency of a pastoral environment, it is also necessary to know the level of herbage utilization. For example, the amount of forage necessary to produce one kilogram of live weight gain. Do Canto et al. (1999) showed that this value can vary between 10.5 to 28.2 kg DM/kg live weight gain by sheep, depending on grazing management (herbage mass in this case). Therefore, grazing management that results in greater nutrient intake by animals requires lower forage per unit animal product, which makes the production system more efficient.

A consequence of this rationale can be examined on meat production, seeking out smart systems with greater yield of commercial cuts and meat quality. According to CARVALHO et al. (2006), lambs grazing Italian ryegrass pastures managed at 5 cm height presented lower live weight gain and consequently lower carcass yield and lower weight of commercial cuts compared to animals managed at 15 and 20 cm. The same response was reported by LOPES et al. (2008); beef steers finished in oat and Italian ryegrass mixed pastures presented greater live weight gain and better carcass characteristics in pastures managed with heights between 20 and 30 cm than extreme heights (10 and 40 cm). When increasing the nutritional density of the diet, it was possible to obtain well-conformed and heavy carcasses of goat, with no excessive fattening (LIMÉA et al., 2009). Hence, an adequate sward management has not only a consequence on individual live weight gain, but also improves carcasses and commercial cuts yield.

Another important topic is the meat quality that is incorporated in human diets and has been considered to be beneficial to human health (FREITAS et al., 2014). For example, meat derived from steers reared on native pastures in south of Brazil had higher concentrations of n−3 fatty acid, resulting in lower n−6/n−3 ratio compared to steers finished with concentrate (feedlot) (FREITAS et al., 2014). AUROUSSEAU et al. (2004) reported that meat lipids from grass fed lambs (grazing) had a composition potentially beneficial for human health, accumulating less C16:0, and more (n-3) polyunsaturated fatty acids and 9 *cis*,11 *trans* conjugated linoleic acid compared to lambs fed concentrate (indoor). In grazing systems, LOURENÇO et al. (2007) reported that lambs grazing legume rich pastures, with better quality (23.5% of crude protein), presented higher polyunsaturated fatty acid proportions in abomasum, subcutaneous and intramuscular fat than lambs grazing ryegrass or diverse pastures with lower chemical quality (15% or 10% of crude protein, respectively). However, SANTOS-SILVA et al. (2002) concluded that the feeding system (pasture, supplemented pasture and feedlot) did not affect meat quality of lambs. PRACHE et al. (2011) evaluated the composition of meat and carcass quality of lambs in conventional or organic grazing systems and concluded that similar results for live weight gain, carcass weight, conformation, fatness and fatty acid distribution or content were found for both grazing systems.

Therefore, animal production in pastoral ecosystems should be based on grazing management targets that seek the equilibrium between forage production and animal production per animal and per area. Thus, a sward management strategy based on optimizing the forage intake by animals, as proposed by “rotatinuous stocking” has a greater capacity to improve animal production in the long-term. It is necessary to always respect the optimal residue post-grazing, which may maximize the herbage regrowth and a high diet quality selection by animals. Consequently, one can expect high animal performance with high carcass and commercial cuts yield and meat quality, while reducing the risks of soil degradation (because of soil cover). Our hypothesis is that higher grazing frequency combined with low grazing intensity proposed by the “rotatinuous stocking” will conciliate the trade-off between forage production, animal production (individual and per area) and greenhouse gases from grazing livestock.

1.4.4. Greenhouse gas emissions from livestock and agriculture

According to the IPCC (2014) the atmospheric concentration of greenhouse gases (GHGs), mainly carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have been increasing in recent years. Approximately 10-12% of total anthropogenic GHG emissions are derived from the agriculture sector (SMITH et al. 2007). Livestock contributes around 80% of global agricultural GHG emissions (FAO, 2006). BEAUCHEMIN et al. (2010) using life cycle assessments, estimated the GHG emissions (CO_2eq, % of total emissions) from a beef farm in Canada, to be 63% of gas emission derived from enteric fermentation (CH_4), followed by manure (N_2O and CH_4, 28%), energy CO_2 (5%) and soil N_2O (4%). In Brazil, approximately 56% of GHGs from agriculture have origin on CH_4 emission (MCT, 2010). The contribution of different processes to total GHG emissions from the global cattle sector has estimated that enteric fermentation (CH_4) represents 46.6% and 42.6% for dairy and beef cattle, respectively. Already, small ruminants presented greater percentage for enteric fermentation (CH_4), with an average of 57.2% and 54.9% for dairy and meat, respectively (OPIO et al., 2013). This shows that the enteric fermentation (CH_4 emission) is the main source of gases from livestock.

According to the Food and Agriculture Organization of the United Nations (FAO), the greater source of gas emissions (CO_2eq) are derived from beef cattle (2.5 Gt CO_2eq/year), followed by dairy cattle herd (2.1 Gt CO_2eq/year), pig (0.7 Gt CO_2eq/year), poultry (0.7 Gt CO_2eq/year), buffalo (0.6 Gt CO_2eq/year) and small ruminants (0.5 Gt CO_2eq/year) (OPIO et al., 2013). These authors also reported that the average emission intensities for beef produced in grazing farming systems were estimated at 38.4 kg CO_2eq/kg carcass weight. However, small ruminant meat was more efficient with average emission intensity of 24.0 kg CO_2eq/kg carcass weight in grazing systems. The same response was reported by SAVIAN et al. (2014) when comparing emission intensities for grazing lambs with data described by PHETTEPLACE et al. (2001) for grazing cattle. The average emission for lambs was of 5 kg CO_2eq/kg live weight gain and for cattle was of 6.4 kg CO_2eq/kg live weight gain.

According to FAO (2015), demand for ruminant meat is projected to increase globally at almost the same rate as milk (+90%). In other words, it is necessary to produce food with low environmental impact, both by area and per unit of animal production (e.g. meat and milk). Therefore, it is important to know the processes involved in production systems, mainly in grazing systems that are the base of animal production worldwide. Grazing management has an essential role in this process, being very important in reducing the environmental impact and promoting climate smart livestock/agriculture.

1.4.5. Animal excreta and their implications for gas emission

The nitrogen excreted in urine and faeces by animals is conducive to nitrogen losses to the environment as ammonia (NH_3), nitrous oxide (N_2O), nitric oxide (NO), di-nitrogen (N_2) and nitrate (NO_3^-) in nitrification and denitrification processes (RICHARDSON et al., 2009).

There is a positive relationship between nitrogen intake and nitrogen excretion by animals (DIJKSTRA et al., 2011; BANNINK et al., 2013; GARDINER et al., 2016). Thus, many studies have shown this response with different forage and animal species. LUO et al. (2015) reported a higher nitrogen concentration in forage rape than in Perennial ryegrass, resulted in daily nitrogen intake by sheep fed forage rape 23% higher compared with perennial ryegrass. HÜNERBERG et al. (2013) showed also that the greater nitrogen intake by growing beef cattle resulted in greater nitrogen excretion in the faeces and urine. Lastly, OENEMA et al. (2005) point out that nitrogen excretion by dairy cows increases nearly linearly with milk production and protein content of the animal feed.

According to DIJKSTRA et al (2011), the major dietary strategies to reduce urinary nitrogen losses are to decrease the concentration of dietary protein, and consequently increase non-protein substrates in the diet. However, this goal may be achieved in more controlled systems, such as feedlots, being much more complicated in grazing systems. Manipulating the crude protein intake by animals grazing Italian ryegrass of high quality, for example containing 24% of crude protein as described by AZEVEDO et al. (2014), is not an easy target. BANNINK et al. (2013) point out that reducing herbage nitrogen content by lowering nitrogen fertiliser or harvesting at a more mature stage, may be more effective in reducing nitrogen excretion, especially in urine. However, focusing on reduction of one pollutant (e.g. nitrogen) may have implications for emissions of other pollutants (e.g. enteric CH_4) (DIJKSTRA et al., 2011). For example, if the sward structure is modified, mainly with use of more mature forage as described above, the intake of nutrients by animals may be prejudiced, which has a direct impact on animal performance and CH_4 emission.

The trade-off between manure N_2O emission and enteric CH_4 emission by beef cattle was reported by HÜNERBERG et al. (2014). These authors showed that high-fat distiller grains in the diet of feedlot cattle may decrease enteric CH_4 emissions, but at high dietary levels it increases nitrogen excretion and results in a net increase in GHG emissions; which was increased mainly due to higher emission of N_2O from manure. Nutritional measures to reduce nitrogen excretion and related nitrogen emissions do not necessarily also reduce GHG emission (BANNINK et al., 2013; GARDINER et al., 2016). DIJKSTRA et al. (2011) point out that dietary measures to reduce total nitrogen excretion by cattle are expected to lead to increased CH_4 production in some dietary situations.

Therefore, it is important to understand the effect of grazing management strategies and their implications and trade-offs under nitrogen excretions and gas emissions in pastoral systems. Aiming for equilibrium of the system is the better way to improve the indices of animal production and mitigate pollution from livestock, and it is necessary to know it for all production systems, because sometimes nitrogen excretion may be important for different systems within a farm. An example that supports this rationale can be illustrated by a study with an integrated crop-livestock system proposed by DA SILVA et al. (2014). These authors showed that dung from grazing beef cattle in the pasture phase (livestock) increased soybean grain yield (crop phase) by 23% in relation to the absence of dung; increasing also soil availability of phosphorus and potassium in such areas reflecting an improvement in nutrient cycling. The decoupling of carbon and nitrogen of tissue plants by animal intake provokes the return of such elements to the system in the form of dung and urine. However, high grazing intensity can affect the system cycling dynamics by excessive carbon and nitrogen decoupling (SOUSSANA & LEMAIRE, 2014). ASSMANN et al. (2015) found that carbon and nitrogen release rates from pasture and dung were greater in soybean-beef cattle integration under moderate grazing than low and high grazing intensities and in non-grazed areas.

It is important to know what should be priority. For example, the GHG emission from beef production in Canada was derived mainly from enteric CH_4 emission (63%). Thus, reducing enteric CH_4 production may be more important than reducing the N_2O from dung and urine, mainly because these excreta may be important to the system, which can reduce the application of mineral fertilizers in the medium and long-term.

It is known that inputs of animal excreta (dung and urine) in grazing systems results in the increase of soil NH_4^+ and NO_3^- content, and consequently increases N_2O production and fluxes (LUO et al., 2013). According to PRIANO et al. (2014), steers accessing pastures (oat) in the morning presented better herbage and faecal chemical composition than animals accessing pastures in the afternoon. Consequently, faecal CH_4 emission in the morning is six times more than in the afternoon. LUO et al. (2015) also reported that faeces from sheep fed forage rape, with higher quality presented greater emission factor (N_2O-N) (0.08%) than faeces from sheep fed lower quality ryegrass (0.03%).

In different countries, research showed lower N_2O-N emission factor values from excreta (1% and 2% for sheep and cattle, respectively) compared to that proposed by the Intergovernmental Panel on Climate Change, IPCC (2013). TOMAZI et al. (2015) (Brazil) and LUO et al. (2013) (New Zealand) reported faecal emission factor of 0.06% for sheep. Similar value (0.09%) was reported in Germany (HOEFT et al., 2012). HOEFT et al. (2012) reported emission factor similar for cattle faeces (0.05%) in temperate grassland, however, SORDI et al. (2014) reported values slightly higher also for cattle faeces (0.15%) in subtropical Brazilian.

The urine emission factor is greater compared with faeces (HOEFT et al., 2012; KELLIHER et al., 2014; LUO et al., 2015; TOMAZI et al., 2015). Faecal emissions mainly of N_2O are lower compared to urinary emissions (HOEFT et al., 2012). It is also important to highlight that 70-80% of nitrogen is excreted as urine (SOUSSANA & LEMAIRE, 2014).

KELLIHER et al. (2014) reported in a statistical analysis from New Zealand data from lowland and hill country soils, at low slope position, a mean emission factor by sheep urine of 0.48%. The same value (0.48%) was reported by HOEFT et al. (2012). However these authors presented lower values (0.39%) for cattle urine. Nonetheless, lower emission factor values for urine from sheep fed ryegrass (0.27%), forage rape (0.11%) (LUO et al., 2015) and Italian ryegrass (0.25%) (TOMAZI et al., 2015) were found in New Zealand and Brazil, respectively.

TOMAZI et al. (2015) have suggested that urinary and faecal excreta should be addressed separately in national GHG inventories. There is also a great need to generate such data in different parts of the world, considering that, in general, the IPCC overestimates these values.

1.4.6. Strategies to reduce CH_4 emission by ruminants

CH_4 emission is a natural process of ruminants, being essential to maintain optimal health conditions in the rumen. During carbohydrate fermentation, short chain fatty acids are formed (mainly acetic, propionic and butyric), which are the main source of energy for ruminants (FRANCE & DIJKSTRA, 2005). The bacteria, archaea, protozoa and fungi are anaerobic species that are able to ferment the carbohydrates of plants and grains. Thus, H_2 and CO_2 are the main gases produced during rumen fermentation of structural carbohydrates that are used by methanogens bacteria to produce CH_4 and H_2O (BUCCIONI et al., 2015). Rumen H_2 elimination is essential for animals, reducing the pressure of hydrogen in the rumen and favouring efficient fermentation (SONG et al., 2011).

The loss of gross energy intake in the form of CH_4 varies between 2 and 12% (JOHNSON & JOHNSON, 1995). IPCC reported mean values of 6.5% for mature cattle, buffalo and sheep (IPCC, 2006). Therefore, this loss of energy through CH_4 emission can vary mainly according to the forage intake level, forage quality and animal category. While growing sheep grazing Italian ryegrass and pearl millet presented values of 5.8% (SAVIAN et al., 2014) and 5.0% (AMARAL et al., 2016), respectively, lactating ewes (SAVIAN et al., 2014) and lactating dairy cows grazing Italian ryegrass (DALL-ORSOLETTA et al., 2016) presented values of 7.3% and 9.2%, respectively.

The literature has also shown that daily CH_4 emissions can vary from 12.2 (HAMMOND et al., 2011) to 41.7 g/animal (SAVIAN et al., 2014) for sheep, from 322 (WARNER et al., 2015) to 656 g/animal (DALL-ORSOLETTA et al., 2016) for lactating dairy cows, and from 86 (DERAMUS et al., 2003) to 304 g/animal (MC GEOUGH et al., 2010) for beef cattle. However, daily emission can vary mainly according to the live weight of animal, animal category, forage intake level and forage species (quality) used for feed. Some studies have shown a classic relationship between daily forage intake and CH_4 emission. When the forage intake increases, CH_4 emission also increases (KURIHARA et al., 1999; HAMMOND et al., 2013; SAVIAN et al., 2014; MOORBY et al., 2015; AMARAL et al., 2016; CHARMLEY et al., 2016; ZHAO et al., 2016). However, this response is more consistent in controlled trials as that proposed by ZHAO et al. (2016) ($y=16.7x+3.1$; $R^2=0.86$; $P<0.001$) and KURIHARA et al. (1999) than grazing trials, such as showed by SAVIAN et al. (2014) ($y=0.008x+25.2$; $R^2=0.23$; $P<0.001$), AMARAL et al. (2016) ($y=0.0057x+8.86$; $R^2=0.44$; $P=0.039$) and RICHMOND et al. (2014) ($R^2=0.042$), which are less controlled. RICHMOND et al. (2014) stated that other factors, such as variable weather conditions (wind, rain, etc.) may affect the efficiency of capturing breath samples and thereby contribute to a greater variance in the determination of CH_4 emissions by the SF_6 technique. In addition, another factor that is very important and may affect this relationship is the forage intake estimates, because in grazing systems this measurement is not simple and the techniques for this purpose also have many constraints.

HAMMOND et al. (2013) reported that when the feeding level increases from 0.8 to 2.5 x MEm (requirements for maintenance), the daily herbage intake increases from 490 to 1510 g DM/animal, daily CH_4 emission also increases from 13.1 to 31.9 g CH_4/animal, however, daily CH_4 yield decreases from 27 to 23.9 g CH_4/kg DM intake by sheep fed perennial ryegrass.

The difference in the daily CH_4 emission is not common for similar animals with similar live weight, eating the same forage species (WIMS et al., 2010; BOLAND et al., 2013; NASCIMENTO et al., 2016) and may vary for animals eating different forage species (DINI et al., 2012; WILLIAMS et al., 2016) or pure versus mixed pastures (ENRIQUEZ-HIDALGO et al., 2014a), except when the forage allowance level contrasts, with a large difference in forage intake as was shown by HAMMOND et al. (2013).

Many strategies are important and necessary to reduce emissions of livestock GHG, such as use of tannin-rich plants (ARCHIMÈDE et al., 2015), legumes (ARCHIMÈDE et al., 2011), concentrate diets (DOREAU et al., 2011), additives, such as nitrate (HULSHOF et al., 2012; LEE et al., 2015), saponin and ropadiar (WANG et al., 2009), sunflower oil and monensin (MCGINN et al., 2004). These strategies for CH_4 mitigation in the livestock sector can be assessed in a review of different articles, as reported by BOADI et al. (2004); ECKARD et al. (2010); MARTIN et al. (2010); COTTLE et al. (2011); BERNDT & TOMKINS (2013); HRISTOV et al. (2013a, 2013b); KNAPP et al. (2014); CARO et al. (2016) and HERRERO et al. (2016).

For example, WILLIAMS et al. (2016) reported that forage with better chemical quality (forage brassica) resulted in more forage intake, higher milk production and lower CH_4 emission, lower rumen pH and acetic/propionic ratio in the rumen of dairy cows than forage with lower chemical quality (reproductive chicory). However, HÜNERBERG et al. (2015) point out that rumen pH by itself is also not a strategy for CH_4 mitigation and not the main determining factor for

CH_4 reduction. MCGINN et al. (2004) also showed that changes in short-chain fatty acids were found with specific diets, using monensin. AGUERRE et al. (2011) studied the CH_4 emission by dairy cows, and concluded that the results failed to confirm that CH_4 emission could be predicted from ruminal short-chain fatty acid patterns alone. In other words, the forage-to-concentrate ratio was modified from 47:53 to 68:32 and the acetate and propionate acids in the rumen were not modified. Another study proposed by ENRIQUEZ-HIDALGO et al. (2014a) also showed that acetate and propionate acids in the rumen of dairy cows grazing pure perennial ryegrass or mixed perennial ryegrass and white clover pastures were not altered. Even diets with nitrate did not interfere with the acetic and propionic acid concentration of beef cattle (HULSHOF et al., 2012). Different growth stages of *Urochloa brizantha* harvested for hay did not affect rumen pH, acetic and propionic acids rumen concentration of steers, even with differences in hay chemical quality, that varied between treatments from 4.2% and 77% to 10.7% and 70% (crude protein and neutral detergent fiber, respectively) (NASCIMENTO et al., 2016).

Although, these studies have not supported many changes in rumen parameters, CH_4 emission in some cases may be reduced with the use of these additives and/or diets. For example, decreases in forage/concentrate ratio from 68/32 to 47/53 resulted in decreased of daily CH_4 emission from 648 to 538 g/cow and from 37.1 to 29.5 g/kg of OM intake (AGUERRE et al., 2011), which also resulted in the same response for CH_4 emission per kg of milk, decreasing from 17.8 to 14.0 g/kg of milk. ARCHIMÈDE et al. (2015) showed the potential of some tannin-rich plants to reduce CH_4 production by sheep; the values can be reduced from 47.1 to 33.5 g/kg digestible organic matter intake, without tannin and with tannin (*M. esculentain*) in the diet, respectively. Another study proposed by HULSHOF et al. (2012) conclude that CH_4 emission by beef cattle was 27% less on the nitrate diet (13.3 g CH_4/kg DM intake) than on the control diet (18.2 g CH_4/kg DM intake).

Thus, considering that there are different forms to mitigate the CH_4 emission by ruminants, we need to understand the process and grazing systems must be further investigated and targets for grazing management need to be adopted. Grazing management is the main goal before any other strategy, because the pasture (grazing systems) is the basis of ruminant production worldwide. Firstly, we must do the basics (good grazing management adoption), and then we can make the systems more complex, diversifying them with the inclusion of other species, such as legumes and using concentrate levels and additives in the concentrate when the feed availability and quality is low. Little attention to sward management is reported when reviewing papers about this topic.

1.4.7. How can grazing management mitigate the methane emission by ruminants?

Unfortunately, grazing management is only cited in a few papers and reviewed as an alternative to reduce CH_4 emission from livestock. This topic should be among the main points in manuscripts concerning enteric CH_4 mitigation, because ruminants worldwide are reared primarily in grazing systems; however, most of the research targets other strategies as mentioned above.

According to HERRERO et al. (2016), improving animal reproductive rates, feed availability and average daily gain (ADG) rates have as consequence the reduction of herd size and are effective approaches for reducing GHG emissions. In pastoral systems this may be achieved mainly by improvements in grazing management.

DERAMUS et al. (2003) showed that when proper grazing management practices are implemented, the animals had increased animal productivity and decreased CH_4 emission per kg of animal product. Another study proposed by SAVIAN et al. (2014) confirmed that grazing management had high potential to reduce CH_4 emission by sheep grazing Italian ryegrass, in that the continuous stocking method had greater herbage intake and ADG by animals and consequently presented 35% less CH_4 per kg of ADG than the rotational stocking method. Beef cattle grazing improved on lowland grasslands providing greater forage intake (9.55 kg/animal/day), ADG (1.08 kg/animal) and lower CH_4 yield (197 g/kg ADG) than semi-natural upland (8.68 kg/animal/day; 0.73 kg/animal; 261 g/kg ADG, respectively) (RICHMOND et al., 2014). These authors also point out that grazing management with lower ADG would be expected to take longer to reach an acceptable finishing weight. Reducing the number of unproductive animals on a farm can also potentially both improve profitability and reduce CH_4 production (ECKARD et al., 2010).

Thus, grazing livestock systems that improve the forage intake and ADG can be considered efficient (smart livestock, *sensu* FAO, 2013). Smart livestock should be based on processes that reduce the trade-off between forage production and animal production. According to SOUSSANA & LEMAIRE (2014), grassland intensification needs to overcome trade-offs between production and environmental goals. These authors emphasized also that the organic carbon in grassland soil increases with the net primary production and the high stocking density impairs soil carbon sequestration. HENDERSON et al. (2015) pointed out that the impacts of animal would fully offset all of the carbon sequestration gains from improved grazing management.

There is a necessity to investigate grazing systems that may mitigate the emission per unit of animal production (milk, meat), but it is also necessary to reduce the total emission per area. Sound management of pastures, for example, by using ideal pre- and post-grazing sward heights in rotational stocking, and ideal forage allowance in other stocking methods, in addition to adequate soil cover, besides reducing CH_4 emissions, also has the potential to accumulate more carbon in the soil. According to BRAZ et al. (2013), more productive pastures can accumulate more soil carbon than degraded pastures.

CARDOSO et al. (2016) investigated the impact of increasing animal productivity using fertilizers, forage legumes, supplements and concentrates, on the emissions of GHG in five scenarios for beef production in Brazil (scenario 1 was without intensification and 5 with high intensification). This study showed that in scenario 1 (only *Brachiaria* sp. without fertilizers) the production was 31.2 kg of carcass per hectare and the carbon footprint was 58.3 kg CO_2eq per kg carcass. However, when better management was used (scenario 3, mixed grass legume fertilized with P and K) the production was 140.2 kg of carcass per hectare and the carbon footprint was 29.6 kg CO_2eq per kg carcass. It is important to emphasize that on farm practices aimed at CH_4 mitigation are more likely to target emission intensities (e.g. kg GHG/kg beef yield) rather than individual animal emissions (BERNDT & TOMKINS, 2013).

The use of integrated well managed crop-livestock systems may also contribute to improvements in the relationship between soil-plant-animal-environment. According to THORNTON & HERRERO (2015) the introduction of cropping into the grazing system improves overall land management and reduces overgrazing. The integrated systems may lead to greater farm efficiency, productivity or sustainability by enhancing nutrient cycling, preserving natural resources and the environment, improving soil quality, and enhancing biodiversity (LEMAIRE et al., 2014). However, grazing management is one of the main variables affecting the success of integrated crop-livestock systems using no-tillage, where crop development is partially due to conditions created by grazing management (CARVALHO et al., 2010). For example, according to DA SILVA et al. (2014b), an integrated crop–livestock system acts as source or a sink of atmospheric carbon depending on the grazing intensity. High grazing intensity results in a carbon source (0.04 Mg/ha/year), while low grazing intensity results in a carbon sink (ranging from 0.25 to 0.37 Mg/ha/year). Thus, integrated systems have an important role to mitigate the GHG from livestock and agriculture, hence the CH_4 emitted by animals may be compensated by the soil carbon sequestration.

Thus, based on the necessity for the development of more adequate production systems, with high potential to reduce the trade-offs between forage and animal production and GHG production, this present thesis has the goal to investigate a grazing management proposal based on animal behaviour (“rotatinuous stocking”), focusing the animal as the main agent of the production system. We believe that CH_4 emission can be reduced, mainly by the greater animal production and lower stocking rate. Well managed systems may reduce the number of ruminants and increase the animal production needed to satisfy future global demands for milk and meat (CAMPBELL et al., 2014).

2. CHAPTER II[1]

Effect of grazing management strategies on sward structure, foraging behaviour and herbage intake by grazing sheep

[1]Prepared in accordance with the standards of the Applied Animal Behaviour Science.

Effect of grazing management strategies on sward structure, foraging behaviour and herbage intake by grazing sheep

Abstract: The objective of this study was to evaluate the effect of different grazing management strategies (pre- and post-grazing sward heights) on the foraging behaviour and herbage intake by sheep grazing Italian ryegrass pastures in rotational stocking. The experiment was carried out in 2015, in the south of Brazil. The experimental design was a randomized complete block with two grazing management strategies and four replicates. The grazing management treatments were: RT- traditional rotational stocking method, with pre- and post-grazing sward heights of 25 and 5 cm, respectively and; RN – “rotatinuous stocking” with pre- and post-grazing sward heights of 18 and 11 cm, respectively. Male sheep with average live weight of 32±2.3 kg were used. The real average sward heights were 17.2 and 11.9 cm (pre- and post-grazing, respectively) for the RN treatment and 26.1 and 7.8 cm (pre- and post-grazing, respectively) for the RT treatment. The herbage chemical composition was better for the RN than the RT treatment, with greater crude protein and lower acid and neutral detergent fiber content for the RN treatment. Total time of diurnal animal activities (grazing, ruminating and resting time) did not differ between treatments ($P>0.05$), with average of 439.6, 166.9 and 85.0 minutes, respectively. The bite rate, feeding station per min and steps per min by sheep were greater ($P<0.05$) at the RN than the RT treatment. Grazing time per hour and bite rate by sheep were greater ($P<0.05$) in the afternoon than morning in both treatments. However, the grazing time per hour also was greater ($P<0.05$) for the RN than the RT treatment. The daily herbage intake by sheep grazing

Italian ryegrass was greater ($P<0.05$) for the RN than the RT (843.7 and 707.8 g OM animal^{-1} day^{-1}, respectively). In conclusion, this study demonstrated that the new grazing management strategy (“rotatinuous stocking”, RN) resulted in a better sward structure, greater herbage chemical composition and greater grazing time per hour than the traditional rotational stocking method (RT). Consequently, the daily organic matter intake by sheep grazing Italian ryegrass pastures was greater for the “rotatinuous stocking” (RN) than the traditional rotational stocking method (RT).

Keywords: lambs, grazing behaviour, organic matter intake, sward heights, sward management, rotational stocking.

2.1. Introduction

The grazing process consists in the searching and handling of forage by the animal (Ungar and Noy-Meir, 1988), that uses the disposal strategies in the pastoral environment to optimise the intake of nutrients (Forbes and Gregorini, 2015) and total daily herbage intake. The components of grazing behaviour are the result of sward structure (Carvalho, 2013). According to Chapman et al. (2007), when searching costs are low, the animal eats a greater proportion of the preferred feed. This is a result of ideal sward structure, that maximizes bite mass (Carvalho, 2013) and may reduce the meal time consequently reducing the daily grazing time. Gregorini et al. (2009) noted that better structure, with more leaves in the middle and top strata, result in greater bite mass and herbage intake rate by grazing steers. Consequently, more stem content in the forage results in less bite mass (Benvenutti et al., 2006, 2009), which has a direct relationship with phenological stages and sward height. Gonçalves et al. (2009) and Mezzalira et al. (2014) observed that the maximum short-term intake rate by sheep and cattle has a high relationship with the ideal sward height. For example, a short sward height of *Cynodon* sp. and *Avena strigosa* resulted in low animal intake rate, however, as did a tall sward, while the maximum intake rate occurred at an intermediate height, specifically at 20 and 30 cm for the two species, respectively (Mezzalira et al., 2014). The authors confirmed that the feed intake rate is low at short sward height, because the animal has a small bite size and needs to take many bites, while and in a tall sward, the animal spends more time on bite formation, so the bite rate decreases. Consequently, the animal needs to have more grazing time per day to meet the daily feed intake. Furthermore, in rotational stocking, the grazing down level should not exceed 40% of optimal sward height (pre-grazing) for maintenance of high forage intake rate (Fonseca et al., 2012; Mezzalira et al., 2014). Fonseca et al. (2013) observed that the bite mass was reduced from 3.98 mg to 2.90 mg of dry matter per kg of body weight when the herbage depletion changed from 40% to 80% of the pre-grazing sward height.

In the traditional rotational stocking method, the parameters cited above, related to animal foraging behaviour are not considered. In that method, the time to start grazing is defined by maximum herbage accumulation and the end of grazing is defined by greater herbage harvested. According to Romera and Doole (2015), the maximum pasture intake per area resulted in low pasture intake per cow, and the pasture intake per hectare must have greater priority than intake per cow. Therefore, the objective of this study was to evaluate two grazing management strategies on the foraging behaviour and herbage intake by sheep grazing Italian ryegrass pastures: a new grazing management strategy, denominated “rotatinuous stocking” (RN) (Carvalho, 2013), defined by optimal sward height (pre-grazing), when the animal herbage intake per unit of grazing time is maximized (Gonçalves et al., 2009; Fonseca et al., 2012; Amaral et al., 2013; Mezzalira et al., 2013 and Mezzalira et al., 2014) with depletion of pre-grazing herbage height to 40% (Fonseca et al., 2012; Mezzalira et al., 2014) and the traditional rotational stocking method (RT), based on the maximum herbage accumulation and herbage harvested.

2.2. Material and Methods

2.2.1. Experimental conditions and treatments

The experiment was conducted at the experimental station of the Universidade Federal do Rio Grande do Sul, near Eldorado do Sul city, Rio Grande do Sul State, Brazil (30°05´S, 51°39´W), between May and September, 2015. The climate zone is the humid subtropics and the average ambient temperature during the last 30 years was 16.0 °C.

The experimental design was a randomized complete block with two grazing management targets and four replicates (paddocks). The grazing management treatments were: RT) traditional rotational stocking method, with pre- and post-grazing target heights of 25 and 5 cm, respectively and RN) “rotatinuous stocking” (Carvalho, 2013) with pre- and post-grazing target heights of 18 and 11 cm, respectively (Amaral et al., 2013). In the RT treatment, the moment to start grazing was defined by maximum herbage accumulation and by high grazing pressure to harvest all herbage. The new sward management strategy (RN) was defined by optimal sward height (pre-grazing), when the herbage intake per unit of grazing time is maximized (Amaral et al., 2013) and reduction of pre-grazing sward height to 40% of the initial height (Fonseca et al., 2012; Mezzalira et al., 2014).

2.2.2. Pasture

The experimental area of 1.76 hectares was divided into eight equal experimental units (paddocks). The pastures consisted of pure stands of Italian ryegrass (*Lolium multiflorum*). The Italian ryegrass was sown in April, 2015 (30 kg of seed per ha). Three hundred kg per ha of fertilizer (NPK, 5-30-15) and 140 kg ha^{-1} of nitrogen (urea source) were applied at seeding and before the beginning of the stocking period, respectively. The stocking period started on 25 May 2015.

2.2.2.1. Sward management

The grazing management was based on a 1-day strip-grazing regime. The animals were moved into a new strip between 14:00 h and 15:00 h every day for both treatments. The number and size of the strips for each experimental unit were variable depending on the herbage mass. Each stocking cycle started when the first strip had reached the target height for each treatment. The stocking period consisted of 155 and 146 days for the RN and RT treatment, respectively.

2.2.2.2. Sward measurements

One hundred random readings of the sward (canopy) were taken per strip before and after each grazing event (pre- and post-grazing) using a sward stick (Barthram, 1985). In the post-grazing recordings were made of grazed and un-grazed area using the sward stick. Herbage mass was measured by cutting three herbage samples (0.25 m^2) at ground level in each strip (pre- and post-grazing). These samples were dried at 55°C for 72 h and a sub-sample was taken and separated for estimating the leaf/stem ratio.

Herbage hand-plucking samples (Johnson, 1978) were taken in each strip-grazing on two occasions: just after strip grazing allocation at 16:00 h (afternoon) and the following morning at 09:00 h (morning). These herbage hand-plucking samples were analysed to determine their chemical composition as an indicator of their nutritive value, which included organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP) by a NIRS method. These predictions were performed by The Walloon Agricultural Research Centre, Belgium (*sensu* Decruyenaere et al., 2009).

2.2.3. Animal

The experimental animals were crossbreed Texel and Ideal (Polwarth) growing lambs. The animals had average age of 12 months and average live weight of 32 ± 2.3 kg. Each experimental unit had four test-animals (permanent animals in all stocking period) and variable animals (put-and-take technique, Mott and Lucas, 1952).

2.2.3.1. Behaviour measurements

The animal behaviour measurements were performed during the stocking period (after adaptation period of 60 days) in all experimental units for both treatments on two occasions: August and September. The measurement started when the animals changed strip-grazing at 14:00 h until dusk (approximately 19:00 h) (afternoon) and continued the following day after dawn (approximately 06:00 h) until 14:00 h (morning). Measurements were always performed by blocks; on the same day, with one or two blocks evaluated, thus the other blocks were evaluated the following days. All animals to be evaluated were identified, five days before measurement. The behavioural activities (grazing, ruminating and resting) were assessed visually and recorded every five minutes (Hirata et al., 2002), for all animals in each experimental unit. Three animals in each experimental unit were used to evaluate the time spent per bite (Laca et al., 1992) (time per 20 bites) and per feeding station (time per 10 feeding stations) and the number of steps taken per feeding station; these measurements were performed during the afternoon and morning. The data was then used to calculate the time per feeding station (min), bite rate per min and the total number of bites per day. These visual measurements were performed by trained observers.

2.2.3.2. Herbage intake

The faecal crude protein technique (Penning, 2004) was used to measure daily OM intake, which was estimated using the equation proposed for Italian ryegrass pastures by Azevedo et al. (2014) (OM intake=111,33+18,33*faecal crude protein). The measurements were performed during the stocking period on two occasions: August and September. Faecal collection bags were fitted to three test animals per experimental unit. This measurement had a duration of five consecutive days; bags were emptied once a day and faeces were weighed and homogenized. A faecal sample per animal of 20% of the total faecal collection was then taken and dried at 55°C for 72 h. The daily samples were then pooled per animal, ground and analyzed for DM, OM and total nitrogen (AOAC, 1975).

2.2.4. Statistical analysis

Data were analyzed with analysis of variance at 5% level of significance for interpretation of statistics analyses. The analyses were performed using the R software for statistical computing version 2.12.0 (R Development Core Team, 2010). Linear models and mixed linear models (lme) were tested and the best model was selected by likelihood ratio test e Akaike's Information Criterion (AIC). The treatments were considered a fixed effect and the animal behaviour measurements, blocks and animals were considered random effects. The behaviour measurements were considered to be repeated measures. Data were transformed to improve the normality and homogeneity of the residuals if necessary.

2.3. Results

2.3.1. Sward characteristics

The sward height (Table 1) of the RT was greater ($P<0.05$) than the RN treatment in the pre-grazing, did not differ ($P>0.05$) in the mid-grazing and was lower ($P<0.05$) in the post-grazing measurement. In the pre-grazing, the herbage mass (Table 1) was greater ($P<0.05$) for the RT than the RN treatment and in the post-grazing herbage mass did not differ among treatments ($P>0.05$). The pre-grazing leaf/stem ratio of Italian ryegrass pasture (Table 1) did not differ between treatments ($P>0.05$), however in the post-grazing the leaf/stem ratio was greater ($P<0.05$) for the RN than the RT treatment (pre- and post-grazing). The percentage of non-grazed area in the mid- and post-grazing (Table 1) were greater ($P<0.05$) for the RT than the RN treatment.

In general, herbage chemical composition was better for the RN than the RT treatment (Table 2). Herbage crude protein was greater (P<0.05) and acid detergent fiber and neutral detergent fiber were lower (P<0.05) for the RN than the RT treatment. However, herbage organic matter did not differ between treatments (P>0.05).

The herbage acid detergent fiber and neutral detergent fiber were lower (P<0.05) in the afternoon than morning in both treatments. However, there was interaction between treatment and day-shift for herbage crude protein, where RN treatment did not differ (P>0.05) between afternoon and morning, but in the RT treatment, the crude protein was lower (P<0.05) in the morning than afternoon.

2.3.2. Foraging behaviour and herbage intake

Total time of diurnal animal activity (grazing, ruminating and resting time) (Table 3) did not differ between treatments (P>0.05). The bite rate, feeding station per min and steps per min by sheep (Table 3) were greater (P<0.05) for the RN than the RT treatment. Time per bite and time per feeding station (Table 3) were greater (P<0.05) for the RT than the RN treatment. The steps per feeding station by sheep (Table 3) did not differ between treatments (P>0.05).

However, the grazing time per hour was greater (P<0.05) for the RN than the RT treatment (Table 4). Grazing time per hour and bite rate by sheep (Table 4) were greater (P<0.05) in the afternoon than morning in both treatments. Ruminating time and resting time per hour by sheep (Table 4) were greater ($P<0.05$) in the morning than afternoon in both treatments.

Daily OM intake by sheep grazing Italian ryegrass (Table 3) was greater ($P<0.05$; MSE=19.6) for the RN than the RT treatment, with an average of 843.7 and 707.8 g OM animal^{-1} day^{-1}, respectively. This difference was confirmed also for percentage of live weight ($P<0.05$; MSE=0.04), with average of 2.53% and 2.25% (RN and RT, respectively).

2.4. Discussion

This study showed that the grazing management strategy based on animal behaviour (“rotatinuous stocking” - RN) resulted in greater herbage chemical composition, grazing time per hour and consequently greater daily herbage intake by sheep grazing Italian ryegrass than traditional rotational stocking method (RT).

2.4.1. Sward structure and herbage quality

The pre- and post-grazing heights defined in the different grazing treatments had direct influence on the herbage mass, morphological composition and chemical content of forage. In the RN treatment, the sward height reduction was not drastic, because this was the central objective of the RN treatment which was to defoliate around 40% of the sward height; on average the pasture height decreased from 17.2 cm to 11.9 cm during the grazing down process (Table 1). The RT treatment achieved higher levels of defoliation, with more than 70% of the sward height removed from 26.1 cm to

7.8 cm (Table 1). This severe defoliation in RT treatment resulted in lower leaf/stem ratio in the post-grazing than RN treatment (Table 1). Amaral et al. (2013) observed the same response in Italian ryegrass pastures, when the sward height was reduced from 17.3 to 10.7 cm the leaf/stem ratio decreased from 1.05 to 0.69 and when the sward height was grazed from 26.0 to 8.2 cm the leaf/stem ratio decreased from 0.85 to 0.34. Fonseca et al. (2012) also found that in *Sorghum bicolour* pastures, post-grazing leaf mass decreased from 388 to 29 kg DM ha^{-1} when the intensity of defoliation (grazing down level) increased from 36 to 63% of the sward height pre-grazing.

The changes in herbage chemical composition were a consequence of the grazing process and driven by the proportion of leaves in the grazing stratum. In general, the animals always started a new strip grazing (afternoon) with better herbage chemical composition in RN treatment and maintained this good quality until the end of grazing (morning) (e.g. crude protein). Although, the fiber content changed from the afternoon to morning in both treatments; the average values shown in this study were low compared with other studies. Azevedo et al. (2014) found an average value of 43% for neutral detergent fiber just in vegetative Italian ryegrass and higher values for pre-flowering (55%) and flowering (60%). The better chemical composition in the RN treatment may be explained by the greater leaf/stem ratio pre- and post-grazing for RN treatment. This result can support the argument that the animals in the RN treatment had access mainly to the first grazing stratum with higher quality, composed mainly by leaves. The hypothesis is that in the RT treatment the animals had access to the second and the third grazing strata with lower chemical quality. This response was described by Benvenutti et al. (2015), showing that the crude protein content in *Axonopus catarinensis* pastures decreased from the top to the bottom stratum.

2.4.2. Animal behaviour

Despite the pre- and post-grazing sward structure were different in both treatments (RN and RT), the animals had a similar pattern of diurnal activities with average grazing, ruminating and resting times of 439.6, 166.9 and 85.0 minutes, respectively, in both treatments (Table 3). However, the grazing time per hour was greater for the RN than the RT treatment. Pérez-Prieto et al. (2011) showed that the daily grazing time by cows grazing perennial ryegrass/white clover pastures was not affected by pasture allowance with an average of 430 minutes per day despite the post-grazing sward structure was different between treatments. According to Tharmaraj et al. (2003), the forage allowance and sward height did not affect the grazing time by lactating dairy cows with an average of 487 minutes per day across treatments.

The animals started a new strip grazing in the afternoon with high grazing time and low ruminating and resting time, until dusk (Table 4). However, the grazing time was lower the following day, from dawn until the next strip grazing allocation in both treatments. This response was expected, because when the animals started a new strip grazing in the afternoon, the grazing time is longer and intensive, mainly in the evening, when the herbage had greater nutritive value (Gregorini et al., 2007). Abrahamse et al. (2009) also observed that the grazing time, bites and chews were greater in the period immediately after moving the dairy cattle to a fresh paddock of grass.

Bite rate was greater for the RN than the RT treatment, with effect also for the day-shift (afternoon and morning) (Table 4). Different hypothesis can be proposed here; short swards can result in high bite rate than taller swards, because the animals need to compensate for the smaller bite mass (Da Silva et al., 2013) or in the other situation with tall sward height (e.g. RT treatment) the animals need longer time for manipulation of swards (non-biting jaw movements) during grazing (Nadin et al., 2012) and/or spending more time searching for a better sward structure resulting in lower bite rate than medium swards (e.g. RN treatment). Nadin et al. (2012) showed that the bite rate by calves grazing oat pastures was greater for medium (26 cm) than tall (52 cm) and short (15 cm) sward heights. Therefore, this last hypothesis is more acceptable for this present study.

Another point that may help explain this result is the bite fracture force by animals. As the stem participation (post-grazing) was greater for the RT treatment it is expected that the animals need more force per bite, resulting in low bite rate. Tharmaraj et al. (2003) showed that in taller swards the animals had lower bite rate and more bite fracture force than shorter swards.

Bite rate was reduced from afternoon to morning (Table 4) in both treatments. This was likely due to the reduction of leaf/stem ratio in the treatments which may have impaired the manipulation of the plant material by the animals resulting in more time spent per bite. There was a greater proportion of no-grazed leaf area in RN than RT treatment, which had values of

42% and 23% in the mid-grazing, respectively, reducing to 30% and 4% in the post-grazing, respectively (Table 1). Benvenutti et al. (2008) found that when the stem density of the canopy increased, the animals spent more time manipulating the herbage; consequently the time per bite also was longer. Fonseca et al. (2013) found that when the herbage depletion was greater than 40% of pre-grazing height, such as in the RT treatment, the bite rate decreased linearly.

The animals spent more time per feeding station in the RT treatment, which could be explained by the greater leaf availability in RN pastures as defoliation progressed (Table 1), thus the animal had greater selection opportunities. In the RT, the animal grazed deeper into the sward with higher stem proportion (Table 1). The more selection opportunity by animals can be confirmed with the greater number of feeding station per minute in the RN than the RT treatment (Table 3). In the RT treatment, the time per feeding station was greater, because the animal spent more time per bite, resulting in the decrease of time for herbage selection (Shipley et al., 1996).

2.4.3. Herbage intake and diet quality

The animals in the RN treatment had a significantly higher OM intake than the RT treatment (Table 3). This response can be explained by the difference between treatments in the pre-grazing sward structure and the achieved level of depletion of the top grazing stratum.

Since grazing time was the same for both treatments after the strip allocation in the afternoon, the difference in daily OM intake could only arise from a greater intake rate for the RN treatment. The lower daily OM intake in the RT treatment could have resulted from the lower bite rate due to tall swards in the afternoon. The greater time of herbage manipulation in the RT treatment resulted in lower foraging velocity. As a result the total number of bites of the RN treatment was twice the amount of diurnal bites taken in the RT treatment (~22579 and ~11772 bites for RN and RT respectively). This greater number of bites helps to explain the possible greater herbage intake in the RN treatment (Table 3). Mezzalira et al. (2014) observed that the short-term intake rate by heifers grazing *Cynodon* sp. and *Avena strigosa* was low (79 to 91 mg per min per kg of LW) in tall swards (35 to 50 cm,) and high (130 and 154 mg per min per kg of LW) in shorter/ideal swards (20 to 30 cm), respectively.

Further decline of herbage intake in the RT treatment could be explained by the greater level of depletion of the top grazing stratum achieved in this treatment (Table 1). Four and 30% of the area of the pasture remained non-grazed after grazing for the RT and RN treatments, respectively (Table 1). Previous studies showed that intake rate decreased at high levels of depletion in the top grazing stratum (Fonseca et al., 2012; Mezzalira et al., 2014). Fonseca et al. (2013) observed that the bite mass decreased linearly after 40% of the sward height was removed. Carvalho (2013) proposed that intake rate is constant until the animals graze two-thirds of the uppermost stratum of the pasture surface layer, afterwards the intake rate decreased.

The diet quality was greater for RN treatment when the animals started the grazing in a new strip (afternoon), as evidenced by higher crude protein and lower fiber values (Table 2). As defoliation progressed the crude protein content decreased in the RT as the availability of leaves declined, which did not occur in the RN treatment (Table 1). Benvenutti et al. (2015) described that the diet quality was greater when the animals had access to the top leafy stratum of the sward, and declined when the animal grazed the bottom sward strata with a high proportion of stems. Consequently bite mass and nutrient intake were greater when the animal consumed predominantly leaves from the top grazing stratum.

2.5. Conclusion

The new grazing management strategy denominated “rotatinuous stocking” (RN) resulted in a better sward structure, with greater chemical composition of Italian ryegrass pastures grazed by sheep than the traditional rotational stocking method (RT).

The different sward management strategies did not interfere with the total diurnal animal activity (grazing, ruminating and resting time); however, the bite rate and number of feeding stations were influenced by sward structure, with greater values for the RN than the RT treatment. Grazing time per hour was greater for the RN than the RT treatment and was greater in the afternoon than morning for both grazing management targets.

Daily organic matter intake by sheep grazing Italian ryegrass pastures was greater for the “rotatinuous stocking” (RN) than the traditional rotational stocking method (RT), indicating that this new grazing management is a strategy that promotes better results in foraging behaviour and herbage intake by grazing animals.

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Table 1. Characteristics of Italian ryegrass pastures grazed by sheep under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
Sward height (cm)				
Pre-grazing	17.2	26.1	0.16	<0.001
Mid-grazing*	12.6	13.9	0.12	0.253
Post-grazing	11.9	7.8	2.07	<0.001
Herbage mass (kg DM ha^{-1})				
Pre-grazing	2011	2532	107	<0.001
Post-grazing	1376	1262	68.9	0.147
Leaf/stem ratio				
Pre-grazing	3.11	2.64	0.26	0.585
Post-grazing	1.59	0.76	0.12	<0.001
Non-grazed area (%)				
Mid-grazing*	42.3	22.9	3.95	0.007
Post-grazing	29.7	3.49	3.88	<0.001

*Measurements taken before dusk.

Table 2. Chemical composition (g kg DM^{-1}) of Italian ryegrass pastures grazed by sheep under different grazing management strategies (RN and RT) and day-shift (A, afternoon and M, morning).

Variables	RN		RT		MSE	P_T	P_{AM}	P_{TxAM}
	A	M	A	M				
OM	871.4	865.8	875.4	871.4	1.22	0.088	0.084	0.717
CP	249.0a	247.7a	202.4b	174.0c	0.72	<0.001	<0.001	<0.001
ADF	206.6	227.7	238.1	272.3	0.48	<0.001	<0.001	0.259
NDF	410.2	430.8	436.0	469.2	0.53	<0.001	<0.001	0.324

DM = dry matter; OM = organic matter; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; MSE = mean standard error; P_T = probability for treatment (RN and RT); P_{AM} = probability for day-shift (afternoon and morning); P_{TxAM} = probability for interaction between treatment and day-shift.
Means followed by lowercase letters on line differ by Tukey test (P < 0.05).

Table 3. Foraging behavior and herbage intake by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
Diurnal animal activity (min)				
Grazing time	454.3	425.0	14.6	0.258
Ruminating time	159.0	174.7	7.81	0.258
Resting time	79.31	90.71	5.50	0.522
Bite and feeding station behaviour				
Bite rate per min	49.7	27.7	11.0	0.014
Time per bite (s)	1.35	2.39	0.52	0.014
Feeding station per min	8.92	6.04	1.44	<0.001
Time per feeding station (s)	6.96	10.4	1.71	<0.001
Steps per feeding station	1.69	1.88	0.10	0.374
Steps per min	15.6	10.7	2.46	0.017
Organic matter intake				
g animal^{-1} day^{-1}	843.7	707.8	19.6	<0.001
% live weight	2.53	2.25	0.04	0.001

Table 4. Animal activity (time per hour) and bite rate by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT) and day-shift (A, afternoon and M, morning).

Variables	RN		RT		MSE	P_T	P_{AM}	P_{TxAM}
	A	M	A	M				
Animal activity								
Grazing time	52.5	30.4	52.0	25.5	1.26	0.024	<0.001	0.142
Ruminating time	3.64b	19.9a	2.25b	22.3a	0.92	0.138	<0.001	0.003
Resting time	4.33	8.66	4.95	9.35	0.47	0.575	<0.001	0.969
Bite rate per min	54.8	44.2	29.7	24.5	0.83	0.007	<0.001	0.768

MSE = mean standard error; P_T = probability for treatment (RN and RT); P_{AM} = probability for day-shift (afternoon and morning); P_{TxAM} = probability for interaction between treatment and day-shift.

Means followed by lowercase letters on line differ by Tukey test (P<0.05).

3. CHAPTER III[2]

Rotatinuous stocking: a new grazing management strategy based on animal behaviour to improve both herbage harvested and performance by sheep grazing Italian ryegrass in rotational stocking

[2]Prepared in accordance with the standards of the Animal Feed Science and Technology.

Rotatinuous stocking: a new grazing management strategy based on animal behaviour to improve both herbage harvested and performance by sheep grazing Italian ryegrass in rotational stocking

Abstract: The objective of this study was to evaluate the effect of different grazing management strategies on the herbage production, herbage harvested, herbage conversion efficiency and performance of sheep grazing Italian ryegrass (*Lolium multiflorum*) pastures in rotational stocking. The experiment was carried out in 2014 and 2015, in the south of Brazil. The experimental design was a randomized complete block with four replicates and two grazing strategy treatments. Traditional rotational stocking method (RT) with pre- and post-grazing target heights of 25 and 5 cm, respectively and, “rotatinuous stocking” (RN) with pre- and post-grazing target heights of 18 and 11 cm, respectively. Male sheep were used with average live weight of 26.2±0.9 kg (2014) and 22.1±1.8 kg (2015). The real average pre- and post-grazing sward heights for RN treatment were 17.9 and 11.1 cm and 27.1 and 7.8 cm for RT treatment. The number of stocking cycles was greater in the RN than the RT treatment (12 and 4, respectively), with an average of 12.5 and 35.3 days per stocking cycle, respectively. Herbage production in the RN was 28% higher than the RT treatment. The total harvested herbage mass and the herbage conversion efficiency were greater ($P<0.05$) in the RN than the RT treatment. Individual average daily gain and live weight gain per hectare were greater ($P<0.05$) in the RN than the RT treatment. In conclusion, Italian ryegrass pastures managed based on animal behaviour (“rotatinuous stocking”, RN) had greater herbage harvested, herbage production and animal performance than traditional rotational stocking method (RT).

Keywords: Animal production, herbage production, herbage conversion, lamb, sward height, sward management.

3.1. Introduction

Grazing management targets to optimize the maximum forage accumulation has been clearly established. The target indicates the ideal moment to start the grazing event as the point when light interception is near 95% (Brougham, 1957; Parsons and Penning, 1988; Lemaire and Chapman, 1996) with greater forage harvested to reduce the waste. This strategy of maximizing harvest of available forage followed by long rest periods to reach maximal light interception has become the traditional rotational stocking method (Brougham, 1957; Parsons and Penning, 1988; Mello and Pedreira, 2004; Carnevalli et al., 2006; Barbosa et al., 2007; Da Silva and Nascimento Junior, 2007 and Da Silveira et al., 2013). Grazing management based on the animal behaviour, for example animal herbage intake rate maximization, has received less attention.

Recent studies have shown that this approach, denominated “rotatinuous stocking” (RN) (Carvalho, 2013), is designed to maximize animal intake per unit of grazing time; it is based on optimum pre-grazing sward height as found in previous studies on native grassland (Gonçalves et al., 2009), *Sorghum bicolor* (Fonseca et al., 2012), *Pennisetum glaucun* (Mezzalira et al., 2013), *Lolium multiflorum* (Amaral et al., 2013), *Cynodon* sp. and *Avena strigosa* (Mezzalira et al., 2014). It is also based on an optimum post-grazing sward height that maintains the maximum forage intake rate with a sward height reduction limit of 40% as shown in previous studies (Fonseca et al., 2013; Mezzalira et al., 2014). Amaral et al. (2013) concluded that herbage intake rate was higher for animal grazing Italian ryegrass pastures when pre-grazing height was 18 cm and sward height was grazed down by only 40% of initial value. Thus, we hypothesized that the sward structure created by the “rotatinuous stocking” (RN), with a more grazing frequency and less grazing intensity, will result in more herbage harvested and greater performance by sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT). The goals of this study were: i) to evaluate the effect of different grazing management strategies (RN and RT) in rotational stocking on herbage production, herbage harvested, herbage conversion efficiency and performance by sheep grazing Italian ryegrass pastures; ii) to minimize the typical trade-off between forage production and animal production using the animal as the main agent of the production system.

3.2. Material and Methods

3.2.1. Location, treatments and experimental conditions

The experiment was conducted at the Agronomic Experimental Station of the Universidade Federal do Rio Grande do Sul, Rio Grande do Sul State, Brazil (30°05´S, 51°39´W). The climate in the region is classified as subtropical humid (Cfa classification) with climatic average temperature and rainfall between April and October (30-yers) of 17.2°C and 936 mm, respectively. The soil at the experimental site was classified as a Typic Paleudult (USDA, 1999) (17.5% clay, 20.0% silt and 62.5% sand, 0-20 cm). The chemical soil characteristics for the horizon 0-20 cm are listed as follows: pH = 4.05; SMP index = 6.05; P = 23.2 mg dm^{-3}; K = 105.5 mg dm^{-3}; OM = 22.7 g kg^{-}

[1]; Al^{3+} = 0.60 cmolc dm^{-3}; Ca = 2.0 cmolc dm^{-3}; Mg = 0.90 cmolc dm^{-3}; cation exchange capacity = 3.77 cmolc dm^{-3} and base saturation = 39.5%.

The experimental design of this study was a randomized complete block with two grazing management strategies (treatments) and four replicates (paddocks). The two treatments in rotational stocking were: RT- traditional rotational stocking method, with pre- and post-grazing target heights of 25 and 5 cm, respectively and RN - “rotatinuous stocking” (Carvalho, 2013) with pre- and post-grazing target heights of 18 and 11 cm, respectively (Amaral et al., 2013). In the RT treatment, the moment to start grazing was defined by maximum herbage accumulation and by high grazing pressure to harvest all herbage. The new sward management strategy (RN) was defined by optimal sward height (pre-grazing), when the herbage intake per unit of grazing time is maximized (Amaral et al., 2013) and reduction of pre-grazing sward height to 40% of the initial height (Fonseca et al., 2012; Mezzalira et al., 2014).

The present experiment was performed during the stocking season of 2014 and 2015. In the RN treatment, the stocking periods were from 20/05/2014 to 13/10/2014 and from 25/05/2015 to 27/10/2015. In the RT treatment, the stocking periods were from 04/06/2014 to 20/10/2014 and from 05/06/2015 to 28/10/2015. Thus, the stocking period was 146 and 140 days (RN and RT, respectively) in 2014 and 155 and 146 days (RN and RT, respectively) in 2015. The stocking period in the RN started before than the RT treatment, because in the RN treatment the sward height target was achieved before than the RT treatment.

3.2.2. Pasture management and assessments

All pasture management was equal for both treatments (RN and RT) and both years (2014 and 2015). The experimental area was divided into eight equal experimental units (paddocks), with 0.22 hectares each. The pasture used was pure Italian ryegrass (*Lolium multiflorum*), which was seeded with 30 kg per hectare in April (2014 and 2015). In both years, the pasture was fertilized with 300 kg ha^{-1} of NPK (5-30-15) together with the seeding and before the start of the stocking period nitrogen fertilization was performed (140 kg ha^{-1}), with urea.

The strip grazing method was used for grazing management; animals started a new strip grazing every day between 14:00 h and 15:00 h. Only in the first stocking cycle, to maintain the pre-grazing height proposed in both treatments, did the animals start grazing with a sward height 10% inferior to the ideal height, but the post-grazing height was maintained as proposed.

The number of strip grazing per experimental unit was defined by the herbage growth; the animals started a new stocking cycle when the first grazing strip had the ideal height proposed for each treatment.

One hundred random readings of the sward (canopy) were taken per strip before and after grazing (pre- and post-grazing) using a sward stick (Barthram, 1985). Three herbage mass samples (0.25 m^2 each) were estimated by random cutting at ground level in the second strip-grazing (pre- and post-grazing), in every stocking cycle, in all experimental units. In RT treatment to achieve more representative herbage sampling, three herbage mass samples (pre- and post-grazing) were determined in the mid and end of each stocking cycle. These samples were dried at 55°C for 72 hours. After, the herbage sub-sample was separated in leaf blades, stem + sheath, inflorescences and dead material.

At the beginning of the stocking period, six random sampling in each experimental unit were performed to estimate the initial herbage mass. In the first stocking cycle to estimate the daily herbage accumulation rate three grazing exclusion cages were used per experimental unit; the difference between the herbage mass in the cages and initial herbage mass divided by number of days was the daily herbage accumulation rate. However, in the next stocking cycles the daily herbage accumulation rate was estimated by the difference between the herbage mass pre-grazing and post-grazing of the stocking cycles (the same cutting for estimating the herbage mass, was as described above). For example, the daily herbage accumulation of the second stocking cycle was the difference between the herbage mass pre-grazing of the third stocking cycle and post-grazing of the second stocking cycle. This was performed for other stocking cycles.

The initial herbage mass added to the daily herbage accumulation rate and multiplied by the number of days in each stocking cycle, resulted in the total herbage production (kg DM ha^{-1}).

The herbage allowance (% live weigh, LW) was calculated according to the average herbage mass of each stocking cycle (kg DM ha^{-1}), number of days of stocking cycle, daily herbage accumulation (kg DM ha^{-1}) and stocking rate of each stocking cycle (kg LW ha^{-1}).

For measurement of canopy light interception, ten measurements above and ten measurements below of the canopy (pre- and post-grazing) in the same strip grazing and in all experimental units were performed. These measurements were performed only at the vegetative stage of pasture; the assessments were performed between 11:00 and 13:00 hours in different times during the stocking period, only in 2014.

3.2.3. Animal management and assessments

All management with the animals was equal in the two treatments (RN and RT) and years (2014 and 2015) but the group of animals was different. The animals used were crossbreed Texel and Ideal (Polwarth) castrated lambs. At the beginning of the experiment the animals had an average age of ~10 months and average live weight of 26.2±0.9 kg (2014) and 22.1±1.8 kg (2015). Four tester-animals were used in each experimental unit (permanent animals in all stocking period) with variable number of animals (put-and-take technique, Mott and Lucas (1952)) to maintain the sward height proposed in each treatment. All animals were dosed with oral vermifuge at the beginning of the stocking period and each 30 days. For control of foot rot, when necessary, a foot bath was performed (5% formalin).

Animals were weighed in the beginning, middle (each stocking cycle of RT treatment) and end of stocking period, always after 12 hours of fasting from solids and liquids.

The average daily gain (g animal^{-1} day^{-1}) was calculated by the difference between the final and initial weights of the test-animals in each RT stocking cycle for both treatments, divided by number of days of the stocking cycle. Stocking rate (kg LW per hectare) was calculated by average LW of test-animals and the LW of the variable animals, multiplied by number of days that they remained on the experimental unit. To calculate the LW gain (kg per hectare) the multiplication of the number of animals per hectare by average daily gain (test-animals) and by the number of stocking period days was used.

The herbage mass harvested by animals per stocking cycle (kg DM per hectare) was calculated by the difference between herbage mass pre- and post-grazing. The same assessments of the herbage mass cutting described above were used. The total herbage mass harvested (kg DM per hectare) of the stocking period was obtained by sum of herbage mass harvested per each stocking cycle. The herbage harvest efficiency percentage was calculated by division between total herbage mass harvested and total herbage production. The herbage conversion efficiency (kg DM per kg LW gain) was calculated by division between total herbage mass harvested and LW gain per hectare.

The amount of eggs per gram of faeces of gastrointestinal parasites of animals was measured according to Gordon and Whitlock (1939). Faeces were collected from the rectum of each test-animal, three times during the stocking period in 2014 and 2015.

3.2.4. Statistical analysis

Data were subjected to analysis of variance at 5% level of significance for interpretation of statistics analyses. The R software for statistical computing version 2.12.0 (R Development Core Team, 2010) was used. Linear models and mixed linear models (lme) were tested and the best model was selected by likelihood ratio test e Akaike's Information Criterion (AIC). Treatments were considered as a fixed effect and blocks, stocking cycles and years were considered as random effect. The variables were analysed per stocking cycle, including the days after seeding in the model as repeated measures. Data were transformed to normalize and homogenize the residuals if necessary.

3.3. Results

3.3.1. Pasture

The sward characteristics of Italian ryegrass pastures are shown in Table 1. The sward height (pre- and post-grazing) varied according to treatments, with greater ($P<0.05$) sward height for the RT than the RN treatment in the pre-grazing and lower ($P<0.05$) sward height for the RT than the RN in the post-grazing.

The pre-grazing herbage mass (kg DM^{-1} ha^{-1}) was greater ($P<0.05$) for the RT than the RN treatment and post-grazing herbage mass was similar ($P>0.05$) (Table 1). The canopy light interception (Table 1) was greater ($P<0.05$) for the RT than the RN treatment in the pre-grazing and was lower ($P<0.05$) for the RT than the RN treatment in the post-grazing.

In the pre- and post-grazing, the percentage of leaf was greater ($P<0.05$) for the RN than the RT treatment, while the percentage of stem and dead material were lower ($P<0.05$) for the RN than the RT treatment. The percentage of inflorescences did not differ between treatments (P>0.05) (pre- and post-grazing) (Fig. 1).

The number of stocking cycles (Table 2) was greater for the RN than the RT treatment (12 and 4, respectively), with an average of 12.5 and 35.3 days per stocking cycle, respectively.

Herbage and leaf allowance (% LW) were greater for the RN than the RT treatment (Table 2). Daily herbage accumulation (kg DM ha^{-1} day^{-1}), total herbage production (kg DM ha^{-1}) and residual herbage mass (kg DM ha^{-1}) were greater (P<0.05) for the RN than the RT treatment (Table 2).

3.3.2. Animal

The herbage mass harvested per stocking cycle (Table 3) was greater (P<0.05) for the RT than the RN treatment, however, the total harvested herbage mass was greater (P<0.05) for the RN than the RT treatment. The herbage harvest efficiency did not differ between treatments (P>0.05), with an average of 72%. The herbage conversion efficiency was greater (P<0.05) for the RN than the RT treatment (Table 3).

The animal variables are shown in Table 4. Average daily gain (g $animal^{-1}$ day^{-1}) and LW gain per hectare were greater (P<0.05) for the RN than the RT treatment. The stocking rate (kg LW ha^{-1}) was greater in the RT than the RN treatment.

The amount of gastrointestinal parasites of animals was greater (P<0.05) for the RT than the RN treatment (Table 4).

3.4. Discussion

This study showed that the grazing management influenced the forage production and the performance of sheep grazing Italian ryegrass in the rotational stocking method. “Rotatinuous stocking” (RN) conciliates the greater forage production with greater animal production compared with the traditional rotational stocking method (RT).

3.4.1. Pasture

The pre- and post-grazing sward heights in the RN treatment resulted in greater daily herbage accumulation than the RT treatment (52.1 and 33.2 kg DM ha^{-1} day^{-1}, respectively) and consequently greater total herbage production (8714 and 6822 kg DM ha^{-1}, respectively) (Table 2), in other words, the herbage production in the RN was 28% higher than the RT treatment. This response can be supported mainly by the lower level of sward depletion in the RN treatment (38% of pre-grazing height) compared with the high level of sward depletion in the RT treatment (72% of pre-grazing height). The greater sward height post-grazing in the RN treatment had greater percentage of leaf than the RT treatment (46 and 28%, respectively) (Fig. 1); consequently in the post-grazing the light interception of the canopy also was greater (78% and 63%, respectively) (Table 1). Ganche et al. (2014) demonstrated the importance of post-grazing residue in perennial ryegrass pastures; severe defoliation negatively affected the sward regrowth and forage production. Another study proposed by Sousa et al. (2013) demonstrated that Elephant grass (*Pennisetum* *purpureum* Schum.) with 50 cm post-grazing height had a greater forage accumulation rate than pastures managed with lower residue (height of 30 cm).

According to Gastal and Lemaire (2015), the key sward parameter that drives the herbage production is the average leaf area index at which the sward is maintained. Thus, when the grazing is severe (e.g. RT treatment) the leaf area index is lower, consequently the net growth rate is limited by light interception. On the other hands, when the leaf area index is higher, the net growth rate is limited by the burden of respiration and by the greater sward senescence. The greater herbage growing rate in RN treatment (Table 2) resulted in greater total herbage production and more stocking cycles than RT treatment (12 and 4, respectively) (Table 2); consequently the animals returned to the first strip-grazing in 12.5 and 35.3 days for RN and RT treatment, respectively. This was a consequence of the greater grazing frequency and lower grazing intensity, which maintained high leaf participation in the post-grazing (Fig. 1).

The target of the animals to start grazing has traditionally been proposed to be 95% canopy light interception for temperate pastures (Brougham, 1962; Parsons and Penning, 1988) and tropical pastures (Mello and Pedreira, 2004; Carnevalli et al., 2006; Barbosa et al., 2007; Da Silva and Nascimento Junior, 2007 and Da Silveira et al., 2013), in other words, the basis for grazing management has been a sward characteristic and rarely has the animal behaviour been used as the main agent for determining the management of the pasture, as proposed in the present study. However, recent studies also show that *Panicum maximum* cv. Aruana swards managed with

85% canopy light interception had lower stem mass, lower dead material and greater leaf/stem ratio than swards managed with 95% canopy light interception (Da Silva et al., 2015). Lower canopy light interception (91%) was also proposed by Amaral et al. (2013) for Italian ryegrass pastures. These authors reported greater pre- and post-grazing leaf/stem ratio in swards managed with 91% than 95% of canopy light interception. Another important topic is the post-grazing residue that can modify both the leaf content and herbage chemical characteristics (Euclides et al., 2016). These authors showed that *Panicum maximum* cv. Mombaça swards managed with a height of 30 cm (post-grazing) had lower leaf/stem ratio and nutritive value than swards managed with a height of 50 cm. Difante et al. (2010) concluded that this same pasture managed with higher post-grazing height (50 cm) had greater herbage accumulation rate (164 kg DM ha^{-1} day^{-1}) than low post-grazing height (25 cm) (90 kg DM ha^{-1} day^{-1}).

3.4.2. Animal

The greater average daily gain by sheep for the RN compared to the RT treatment (119 and 47 g $animal^{-1}$ day^{-1}, respectively) was due to the use of optimal sward pre- and post-grazing height in the RN treatment. The sward height in the RN decreased from 17.9 to 11.1 cm, and in the RT treatment decreased from 27.2 to 7.83 cm (Table 1). Consistently, the RN treatment resulted in greater harvested total herbage mass (5955 *vs.* 4971 kg DM ha^{-1}). In other words, the total harvested herbage mass in the RN was 20% higher than the RT treatment. In the RN treatment, the animals had more opportunity to select the preferred forage due to the leafy sward structure (Fig. 1). Mezzalira et al. (2014) also found that when the herbage was reduced 40% of the sward height pre-grazing, 23% and 24% of area was not grazed in *Cynodon* sp. and *Avena strigosa* pastures, respectively. The RN treatment was similar to the continuous stocking indicated by Briske et al. (2008) allowing for selective grazing. Euclides et al. (2016) found a greater average daily gain by steers grazing *Panicum maximum* cv. Mombaça with high post-grazing swards (50 cm) (655 g animal^{-1} day^{-1}) than shorter post-grazing swards (30 cm) (392 g animal^{-1} day^{-1}). This result is a consequence mainly of greater post-grazing leaf percentage, greater nutritive value and greater herbage intake for the treatment with tall residue (50 cm height) than short residue post-grazing (30 cm height).

Another topic of importance that may interfere with animal performance is the gastrointestinal parasite counts, which also was higher for the RT than the RN treatment (2472 and 704 eggs per gram of faeces, respectively) (Table 4). This was due to the animals in the RT treatment (higher stocking rate) grazing down the pastures closer to the ground. Pegoraro et al. (2008) found higher concentration of helminthic larvae in Italian ryegrass pastures in the bottom stratum. Molento et al. (2016) point out that high stocking rates can be responsible for enhancing parasite transmission by forcing animals to eat portions of L3 contaminated pasture (bottom stratum of sward).

Although, the stocking rate was greater for the RT than the RN treatment (1235 and 850 kg LW ha^{-1}, respectively), the LW gain per hectare was greater in the RN than the RT treatment (401 and 279 kg LW ha^{-1}, respectively). In other words, the LW gain per area in the RN was 44% higher than the RT treatment. This result was a consequence of greater individual average daily gain by animals in the RN treatment that was 2.5 times higher than the RT treatment.

These results presented in this paper agree with the literature. In general, the individual gain is negatively related to stocking rate, being proved for lambs grazing perennial ryegrass (Armstrong et al., 1995), steers grazing Italian ryegrass and oat mixed (Aguinaga et al., 2006; Kunrath et al., 2014) and *Panicum maximum* cv. Mombaça (Euclides et al., 2016). Therefore, similar values of RN treatment were reported by Do Canto et al. (1999) for sheep grazing Italian ryegrass and white clover mixed, with animal performance and gain per area of 120 g $lamb^{-1}$ day^{-1} and 497 kg LW ha^{-1}, respectively.

A trade-off is usually found between individual gain and gain per area (Penning et al., 1991). Kunrath et al. (2014) described an increase in individual gain and a decrease in gain per area by steers grazing a mixture of Italian ryegrass and oats with an increase in sward height (from 10 to 40 cm). However, sheep grazing mixed Italian ryegrass and white clover (Do Canto et al., 1999) and steers grazing *Panicum maximum* cv. Mombaça (Euclides et al., 2016) had the same response of individual gain and gain per area; increase of average daily gain resulted in increase of gain per hectare.

3.4.3. Herbage production *versus* animal production

Pastoral ecosystems are complex, involving interaction between plant and animal in the same environment; the animal needs the nutrients from the plants for maintenance, growth and reproduction. On the other hand, the plants need to maintain leaf area to have high growth rate.

This study showed that the pastoral system can be adjusted to favour plants and animals at the same time, as shown in the RN treatment. The production system should have the objective to achieve high herbage production and animal production in the short-term, with grassland intensification that can increase the trade-off between gross primary productivity and animal production; but it should maintain the productivity over the long-term.

The main point in grazing systems management is to have high herbage harvested by the animals. This study demonstrated that the herbage harvested per stocking cycle was 2.5 times higher in the RT than the RN treatment (1242 and 497 kg DM ha^{-1}, respectively). However, the total herbage harvested in the RN treatment was 984 kg DM ha^{-1} greater than the RT treatment. This was due to the high grazing frequency and low grazing intensity which resulted in a greater number of stocking cycles in the RN than the RT treatment (12 and 4, respectively). High post-grazing residue is viewed as wasted forage that should have been eaten by animals; however, this residue is very important to maximize the forage regrowth rate and to increase the grazing frequency. Consequently, this favours the gain by the animal, as it is possible for them to eat leaves with high quality and high herbage intake rate. Additionally, the high levels of herbage harvested per grazing event in grazing systems (e.g. RT treatment) are associated with higher levels of nutrient leaching (Romera and Doole, 2015).

Therefore, the greater efficiency of “rotatinuous stocking” (RN) also may be confirmed by the higher herbage conversion efficiency (40% more) than

RT treatment (15.1 and 21.1 kg DM per kg LW gain, respectively). Do Canto et al. (1999) showed that this value can vary between 10.5 to 28.2 kg DM per kg LW gain by sheep, depending on grazing management.

Additionally, another very important point in a conservationist grazing system over the long term is the amount of residual herbage mass that was greater for the RN than the RT treatment (2418 and 1552 kg DM ha^{-1}, respectively). For example, Kunrath et al. (2014) showed similar results (2398 kg DM ha^{-1}) and concluded that this residual amount is sufficient for a no-till integrated crop-livestock system. In this system, it is normal to finish the pasture phase (livestock) and immediately start another grain crop phase (Kunrath et al., 2014). This short interval between crop and livestock may be a problem more accentuated for the RT than the RN treatment, because not only is the residue lower in RT treatment, the last strips grazed have very little residue, which may impair the soil and the subsequent crop. Maintenance of plant residues on the soil surface is essential for system sustainability (Kunrath et al., 2014) in the long-term.

3.5. Conclusions

“Rotatinuous stocking” based on animals behaviour resulted in greater herbage harvested by sheep grazing Italian ryegrass than traditional rotational stocking method, without consequences for the herbage harvest efficiency.

Italian ryegrass pastures management based on optimal pre- and post-grazing sward heights (“rotatinuous stocking”) resulted in greater animal production, per sheep and per area, and also greater herbage production per day and per area than traditional rotational stocking method.

As a consequence of greater forage harvested and animal production in the “rotatinuous stocking”, the herbage conversion efficiency was also greater than the traditional rotational stocking method. Thus, in the “rotatinuous stocking” the animals needed less forage to produce one kg of live weight gain, confirming the greater efficiency of this new grazing management strategy.

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Table 1. Sward height, herbage mass and light interception of Italian ryegrass pastures grazed by sheep under different grazing management strategies in rotational stocking (RN and RT).

Variables	RN	RT	P	MSE
Sward height (cm)				
Pre-grazing	17.9	27.1	<0.001	0.02
Post-grazing	11.1	7.8	<0.001	0.01
Herbage mass (kg DM ha^{-1})				
Pre-grazing	1822	2605	<0.001	53.1
Post-grazing	1325	1362	0.223	33.2
*Light interception (%)**				
Pre-grazing	90.6	95.1	<0.001	0.46
Post-grazing	77.6	62.7	<0.001	1.99

DM=dry matter; P=probability; MSE=mean standard error; *vegetative period.

Table 2. Stocking cycles and variables of Italian ryegrass pastures grazed by sheep under different grazing management strategies in rotational stocking (RN and RT).

Variables	RN	RT	P	MSE
Number of stocking cycles	12	4	-	-
Days per stocking cycle	12.5	35.3	-	-
Herbage allowance (% LW)	24.3	9.1	<0.001	0.81
Leaf allowance (% LW)	10.7	4.78	<0.001	0.48
Herbage accumulation (kg DM ha^{-1} day^{-1})	52.1	33.2	<0.001	2.91
Total herbage production (kg DM ha^{-1})	8714	6822	0.001	330
Residual herbage mass (kg DM ha^{-1})	2418	1552	<0.001	130

LW=live weight; DM=dry matter; P=probability; MSE=mean standard error.

Table 3. Herbage harvested (kg DM per ha) and herbage conversion efficiency (kg DM per kg LW gain) by sheep grazing Italian ryegrass pastures under different grazing management strategies in rotational stocking (RN and RT).

Variables	RN	RT	P	MSE
Herbage harvested per stoking cycles	497	1242	<0.001	36.8
Total herbage harvested	5955	4971	0.014	213
Herbage harvest efficiency (%)	68.8	74.7	0.119	0.02
Herbage conversion efficiency	15.1	21.1	0.028	1.71

LW=live weight; DM=dry matter; P=probability; MSE=mean standard error.

Table 4. Animal production and gastrointestinal parasites of sheep grazing Italian ryegrass pastures under different grazing management strategies in rotational stocking (RN and RT).

Variables	RN	RT	P	MSE
Average daily gain (kg animal^{-1} day^{-1})	0.119	0.047	<0.001	0.01
Live weight gain (kg ha^{-1})	401	279	0.002	26.4
Stocking rate (kg LW ha^{-1})	850	1235	<0.001	76.2
Internal parasites (eggs per g of faeces)	704	2472	<0.001	163

LW=live weight; P=probability; MSE=mean standard error.

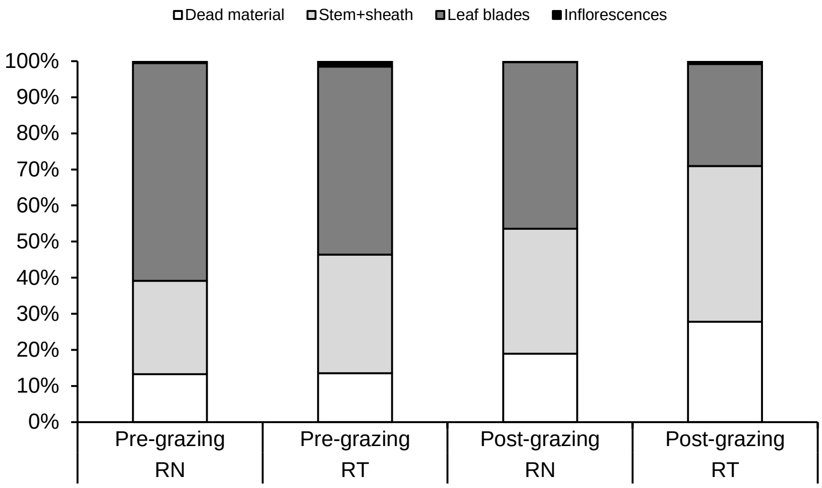

Figure 1. Morphological composition of Italian ryegrass pastures (pre- and post-grazing) grazed by sheep under different grazing management strategies in rotational stocking (RN and RT).

4. CHAPTER IV[3]

Implication of different grazing management strategies on carcass characteristics and meat quality of sheep grazing Italian ryegrass pastures

[3]Prepared in accordance with the standards of the Meat Science.

Implication of different grazing management strategies on carcass characteristics and meat quality of sheep grazing Italian ryegrass pastures

Abstract: The objective of this study was to evaluate the effect of different grazing management strategies in rotational stocking on the carcass characteristics and meat quality of sheep grazing Italian ryegrass pastures. The experiment was carried out in 2015, in the south of Brazil. The experimental design was a randomized complete block with two grazing management strategies (traditional rotational stocking method – RT, with pre- and post-grazing sward heights of 25 and 5 cm, respectively and; “rotatinuous stocking” – RN with pre- and post-grazing sward heights of 18 and 11 cm, respectively) and four replicates. Male castrated lambs crossbred Texel and Ideal (Polwarth) with an average age ~10 months old and average live weight of 22 ± 1.8 kg, were used. The body conformation (*in vivo*) of sheep (leg length, width of croup, thoracic perimeter and posterior height) presented greater values (P<0.05) for the RN than the RT treatment. The carcass conformation of sheep (internal and external length, leg length and width and shoulder length and width) did not differ between treatments (P>0.05). Final live weight, hot and cold carcass weight and all commercial cuts (shoulder, loin, ribs, leg and neck) presented greater values (P<0.05) for the RN than the RT treatment. The *Longissimus dorsi* muscle marbling fat was greater for the RN than the RT treatment. The percentage of muscle, bone and fat did not differ between treatments (P>0.05), with average of 59.0, 25.5 and 15.8%, respectively. Meat colour (L*, a* and b*) did not differ between treatments (P>0.05), with average of 48.6, 15.0 and 5.65, respectively. In conclusion, the better sward structure for the grazing management based on animal behaviour (“rotatinuous stocking” - RN) resulted in greater final live weight, carcass weight and weight of commercial cuts of grazing sheep than traditional rotational stocking method (RT). However, although the marbling fat was greater for the “rotatinuous stocking”, the tissue composition (muscle, bone and fat) and meat colour of grazing sheep were not influenced by different grazing management strategies.

Keywords: carcass conformation, commercial cuts, lambs, rotational stocking, sward heights.

4.1. Introduction

Success in animal production in grazing systems should be based on effective grazing management strategies to improve the individual animal performance and consequently the carcass yield and quality, associated with reduced finishing age of the animals on the farm. Although the human nutritional quality of meat was considered better for grazing animals than housed animals (Aurousseau et al., 2007; Prache et al., 2011; Scerra et al., 2011; Freitas et al., 2014; Wang et al., 2015; Fruet et al., 2016), adequate grazing management is a key point in maintaining a sustainable farm system, seeking the balance between productivity and meat quality in the medium and long term, with capacity to maintain in a competitive sector without impairing the environment.

According to Carvalho et al. (2006), lambs grazing Italian ryegrass pastures in continuous stocking presented greater carcass and commercial cut weights when the daily live weight gain by lambs was also higher. Lopes et al. (2008) showed also that beef steers grazing mixed Italian ryegrass and oat in continuous stocking managed with heights between 25 and 30 cm, presented greater daily live weight gain, carcass weight and subcutaneous fat thickness than swards managed with height of 10 cm.

Therefore, an understanding of grazing behaviour is essential to support grassland management and innovative grazing systems (Carvalho, 2013). Thus, grazing management strategies based on maximization of forage accumulation and forage harvested by the animal, as has been proposed in the traditional rotational stocking method, could be replaced by strategies based on animal behaviour, being who define the ideal grazing management is the animal itself, with opportunity to select their diet, without being forced to eat non-preferred parts of plants, prioritizing the maximization and maintenance of the animal herbage intake per unit of grazing time . This new grazing management called “rotatinuous stocking” (Carvalho, 2013) is based in optimum sward heights of pre- and post-grazing, combining low grazing intensity and high grazing frequency. Therefore, this current study hypothesized that the best sward structure (pre- and post-grazing) and consequently better herbage chemical composition in the “rotatinuous stocking” (RN), results in greater carcass yield, commercial cuts and meat quality of sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT). Thus, the aim of this study was to evaluate the effect of different grazing management strategies in rotational stocking on the carcass characteristics and meat quality of sheep grazing Italian ryegrass pastures.

4.2. Material and Methods

4.2.1. Site, design and treatments

The experiment was conducted at the experimental field of the Federal University of Rio Grande do Sul, Rio Grande do Sul State, in the south of Brazil, with geographic coordinates of 30°05´S, 51°39´W. The climate in the region is subtropical humid and the soil at the experimental site was classified as a Typic Paleudult (USDA, 1999) with 17.5% clay.

The experimental design was a randomized complete block with two grazing management targets and four replicates (paddocks). The grazing management strategies studied were: RT - traditional rotational stocking method, with pre- and post-grazing target heights of 25 and 5 cm, respectively and RN - “rotatinuous stocking” (Carvalho, 2013) with pre- and post-grazing target heights of 18 and 11 cm, respectively (Amaral et al., 2013). In the RT treatment, the moment to start grazing was defined by maximum herbage accumulation and by high grazing pressure to harvest all herbage. The new sward management strategy (RN) was defined by optimal sward height (pre-grazing), when the animal herbage intake per unit of grazing time is maximized (Amaral et al., 2013) and reduction of pre-grazing sward height to 40% of the initial height (Fonseca et al., 2012; Mezzalira et al., 2014).

4.2.2. Herbage and animal management

The experimental area had eight equal experimental units (paddocks), with 0.22 hectare each. Italian ryegrass (*Lolium multiflorum*) pastures were seeded in April, 2015. The grazing management was based on a 1-day strip-grazing regime. Every day, the animals started a new strip-grazing between 14:00 h and 15:00 h in both treatments. The number of strip-grazing per experimental unit was defined by the herbage growth; the animals started a new stoking cycle when the first strip grazing had the ideal height proposed for each treatment. To maintain the sward height for both treatments a sward stick as proposed by Barthram (1985) was used; every two days 100 measurements per strip-grazing (pre- and post-grazing) were taken in all experimental units. Three herbage mass samples (0.25 m^2) were taken at ground level every second strip-grazing (pre- and post-grazing), every stocking cycle. These samples were dried at 55°C for 72 h to estimate the dry matter herbage mass per area.

During the stocking period three measurements of herbage hand-plucked (Johnson, 1978) were performed. The samples were taken in a strip-grazing on two occasions: just after strip grazing allocation (16:00 h) and the following morning at 09:00 h. These herbage hand-plucking samples were dried at 55°C for 72 h and ground for determination of organic matter, crude protein, neutral detergent fiber and acid detergent fiber by a NIRS method. These predictions were performed by The Walloon Agricultural Research Centre, Belgium (*sensu* Decruyenaere et al., 2009).

Experimental animals were castrated lambs crossbred Texel and Ideal (Polwarth). At the beginning of the stocking period the animals had an average age of ten months and average live weight (LW) of 22.1±1.8 kg. Four tester-animals per experimental unit and variable animals (put-and-take technique, Mott and Lucas, 1952) were used. All animals were sheared before the start of the stocking period and dosed with oral vermifuge at the beginning of the stocking period and each 30 days. The animals received water and mineral supplementation ad libitum during the whole stocking period.

At the beginning and end of the stocking period, animals were submitted to a fasting period from solids and liquids for 12 hours before weighing. The stocking period was 155 and 146 days for RN and RT, respectively.

4.2.3. Biometric measurements and carcass characteristics

On the last day of the stocking period biometric measurements were performed on the animals. Linear body conformation measurements were performed, such as body length, leg length and circumference, chest width, width of croup, thoracic perimeter and previous and posterior height (Osório et al., 1998).

Following humanitarian practices, 30 tester-animals (16 and 14 animals, RN and RT treatment, respectively) were slaughter after fasting of 14 hours. All lambs were electrically stunned (head only). Carcasses were trimmed and hot carcass weights were recorded. After slaughter, non-carcass components (tongue, head and red viscera) were weighed. Red viscera were included kidney, lung, heart and liver. Carcasses were chilled at a mean temperature of 4-5°C for 24 hours and cold carcass weights were recorded.

According to Osório et al. (1998), carcass conformation was taken to measure the internal and external length, leg length and depth and shoulder length and depth.

After cooling, the carcasses were separated in commercial cuts (neck, shoulder, ribs, loin and leg). According to Fisher & De Boer (1994), dissection of the shoulder was performed to measure the tissue composition (muscle, bone and fat).

4.2.4. Carcass quality

The carcass pH was measured on the *Longissimus dorsi* muscle (between the eleventh and twelfth rib) at two times: hot (zero time) and cold (24 hours). A pH-meter with a coupled penetration probe (Lutron, PH 208 model) was used.

The marbling was subjectively scored on a five point visual scale of 1 (little or no marbling) to 5 (high marbling) equating to approximately 30% visual intramuscular fat (IMF) on slices of loin taken from the lumbar region (*Longissimus dorsi*) (Osório et al., 1998). Scoring was undertaken by two independent assessors with the values averaged. According to AMSA (1967), the area (cm^2), depth (mm) and width (mm) of loin and subcutaneous fat thickness (mm) were measured. The same loins were used to investigate colour stability using the CIE L* (lightness), CIE a* and (redness) CIE b* (yellowness) scale as according to Centre International de L'Eclairage (1986), using a BYK Gardner GmbH - USA spectrophotometer.

4.2.5. Statistical analysis

The analyses were performed using the R software for statistical computing version 2.12.0 (R Development Core Team, 2010). The treatments were considered as fixed effect and the blocks and paddocks were considered as random effects. Linear models and mixed linear models (lme) were tested and the best model was selected by likelihood ratio test e Akaike's Information Criterion (AIC). Data were transformed to improve the normality and homogeneity of the residuals if necessary.

Pearson correlations (r) between *in vivo* and carcass measurements were performed.

4.3. Results

4.3.1. Herbage characteristics

Italian ryegrass characteristics and chemical composition are shown in Table 1. The sward heights varied according to treatment, with average heights of 17.9 and 11.4 cm for the RN treatment (pre- and post-grazing, respectively) and heights of 25.7 and 8.1 cm for RT treatment (pre- and post-grazing, respectively). Pre-grazing herbage mass was lower ($P<0.05$) for the RN than the RT treatment and the post-grazing herbage mass did not differ between the treatments ($P>0.05$). The chemical composition had better values for the RN than the RT treatment, with greater crude protein and lower acid and neutral detergent fiber ($P<0.05$).

4.3.2. *In vivo* and carcass conformation

The body conformation (*in vivo*) of sheep is shown in Table 2. The variables leg length, width of croup, thoracic perimeter and posterior height were greater ($P<0.05$) for the RN than the RT treatment. However, the variables body length, leg circumference, chest width and previous height did not differ between treatments ($P>0.05$).

The carcass conformation of sheep (internal and external length, leg length and depth and shoulder length and depth) did not differ ($P>0.05$) between treatments (Table 3). However, all commercial cuts (shoulder, loin, ribs, leg and neck) presented greater weight ($P<0.05$) for the RN than the RT treatment (Table 4).

4.3.3. Carcass and non-carcass characteristics

The live weight, carcass and non-carcass characteristics of sheep are shown in Table 5. Final live weight and hot and cold carcass weight were greater for the RN than the RT treatment. The carcass pH in the *Longissimus dorsi* muscle reduced from 7.01 (hot carcass) to 5.65 (cold carcass), but for both times the carcass pH did not differ between treatments (P>0.05).

Eye muscle area and width did not differ between treatments (P>0.05), however the eye muscle height was greater for the RN than the RT treatment. The *Longissimus dorsi* muscle marbling fat was greater for the RN than the RT treatment, however the subcutaneous fat thickness did not differ between treatments (P>0.05). The percentage of muscle, bone and fat did not differ between treatments (P>0.05), with average of 59.0, 25.5 and 15.8%, respectively.

Meat colour (L*, a* and b*) did not differ between treatments (P>0.05), with average of 48.6, 15.0 and 5.65, respectively.

The weight of non-carcass components (tongue and herd) did not differ between treatments (P>0.05), except the red viscera weight was greater (P<0.05) for the RN than the RT treatment.

4.3.4. Correlation between in vivo and carcass measurements

The Pearson correlations (*r*) and probabilities for association between in vivo and carcass measurements are shown in Table 6. The thoracic perimeter (*in vivo*), daily live weight gain and eye muscle area were the variables with more correlation with commercial cuts and carcass weight from grazing sheep. The others variables *in vivo*, such as body length, leg length, leg circumference, chest width and width of croup presented low or no correlation with carcass characteristics. Subcutaneous fat thickness presented a medium correlation with marbling, commercial cuts and carcass weight.

4.4. Discussion

This study showed that the grazing management strategy based on animal behaviour (“rotatinuous stocking” - RN) resulted in better herbage chemical composition with consequently greater final live weight, carcass weight, weight of commercial cuts and marbling fat from sheep grazing Italian ryegrass pastures than traditional rotational stocking method (RT).

4.4.1. *In vivo* and carcass characteristics

The better sheep conformation (*in vivo* and carcass) was a consequence of the greater herbage chemical composition, herbage intake by animals (CHAPTER V) and consequently greater live weight gain for the RN treatment compared with RT treatment (CHAPTER III). The organic matter intake by sheep grazing Italian ryegrass was 23% greater for the RN treatment (CHAPTER V), which is result mainly of ideal sward structure. The herbage short-term intake rate by grazing animals is controlled mainly by the sward structure (sward height) (Gonçalves et al., 2009; Fonseca et al., 2012; Amaral et al., 2013; Mezzalira et al., 2014). In other words, the animals eat more nutrients in this grazing management, because besides the herbage intake has been higher, the herbage chemical composition is also higher (Figure 1).

According to Boval & Dixon (2012) a management priority to increase output of animal products from grasslands should be based on the objective to achieve the highest possible intakes of metabolizable energy. These same authors point out that the dry matter intake or preferably intake of digestible nutrients, is an excellent indicator of the performance of grazing animals. Nonetheless, the grazing management (RN treatment) with greater herbage intake resulted in 2.5 times more animal performance (live weigh gain) than grazing management with lower herbage intake by animals (RT treatment). Nonetheless, the animal performance may be considered the main indicator of carcass performance, which may be proven by the high correlation between live weight gain and carcass weight, for example (Table 6). Prache et al. (2011) evaluated different grazing systems (organic *vs*. convention production) and concluded that the lambs presented the same live weight gain for organic and conventional systems and consequently the same carcass weight. In grazing systems, Carvalho et al. (2006) and Lopes et al. (2008) showed that lambs and steers, respectively, presented greater final live weight and better carcass conformation combined with greater individual live weight gain. These authors concluded that the grazing management was the key factor to achieve good index of carcass yield and conformation. However, it is expected that greater carcass weight for animals finished with greater live weight, because a high correlation between these variables and commercial cuts (loin, shoulder, leg, ribs) was evidenced (Table 6). Galvani et al. (2008) evaluated the carcass characteristics of feedlot crossbred Texel and Ile de France lambs slaughtered at different live weights and concluded that the carcass and cuts weight were linearly correlated with the live weights of slaughter. The correlations between *in vivo* and carcass characteristics are very important to obtain an estimation of carcass yield in production systems. Therefore, for example, the variables with greater correlation with the live weight, carcass weight and commercial cuts were perimeter thoracic and eye muscle area (Table 6). The other *in vivo* variables, such as body length, leg length, leg circumference, chest width and width of croup presented low or no correlation with carcass characteristics. Pinheiro & Jorge (2010) reported that thoracic perimeter and chest and croup width obtained in vivo were highly correlated to the body and cold carcass weights of ewes. Though the subcutaneous fat thickness presented medium correlation with the marbling, commercial cuts and carcass weight (Table 6), this variable may be used to predict the ideal moment to slaughter the animals. In addition to biometric measurements, other measurements *in vivo* may be taken with ultrasound method for example, which aid the farmer to have a final product with better quality, without excess fat or low fat, for example. Silva et al. (2012) showed high correlations between ultrasound measurements (*in vivo*) and post-mortem measurements for *longissimus* area and backfat thickness from Nelore beef cattle.

Nonetheless, more efficient grazing systems such as the RN treatment, mainly by the greater live weight gain, should reduce the time of permanence of these animals on the farm, mainly because the animals reach ideal slaughter weight before. According to Prache et al. (2011) the ideal slaughter time is when the lambs attain a fat class between 2 and 3 (on a scale of 0 to 5), which was approximately 35 kg of live weight for Limousin breed from

France. Similar live weight for slaughter (33 kg) was recommended by Da Silva et al. (2000) for young crossbreed lambs (Texel x Ideal) with an age of 105 days. Thus, assuming that this slaughter live weight is ideal to the crossbreed of Texel and Ideal sheep, the animals in the RN treatment could have been slaughtered 42 days before than animals from RT treatment.

Increasing turn-off weight and reducing turn-off age of lambs on the farm is the key point to improve the production system. As may be visualized in the RN treatment, this may be achieved only with adjustments in grazing management (ideal pre- and post-grazing heights), without increase the production cost with feed supplements and labor, for example. According to Bray et al. (2016), in northern Australia, increase live weight gain of beef cattle and reduce age of turn-off with intensively feeding may return a positive enterprise gross margin.

4.4.2. Meat quality

Although, the ultimate pH in the *Longissimus dorsi* muscle did not differ between both grazing management, the average of 5.65 is considered an ideal value for lambs. Luciano et al. (2012) showed that differences in the final muscle pH between concentrate and pasture-fed lambs do not occur when both feeding systems provide adequate feeding levels and average values of 5.67 presented regular trend of the post mortem glycolysis in muscle. According to Young et al. (2004) the ultimate muscle pH should not exceed 5.7; greater values may result in several defects, mainly on the meat colour, in the other words, the greater the pH the darker the meat. Therefore, as the final pH was equal in both treatments, the meat colour presented low variations and no differences were found, with an average of 48.6 for lightness, 15.0 for redness and 5.65 for yellowness. Thus, the maturity of animals may be a more relevant factor on the meat colour changes. Liméa et al. (2009) evaluated the carcass characteristics of Caribbean goats and concluded that animals slaughtered before 272 days old and live weight of 23.3 kg, presented less red meat (redness = 14.9) than animals slaughtered later, at 333 days old and live weight of 23.6 kg (redness = 16.9). In grazing systems, Fruet et al. (2016) evaluating the carcass characteristics from ewes with 5.8 years old and live weight of 51 kg, found values of 17.9 for redness of meat. On the other hand, Luciano et al. (2012) found values of 12.1 for redness of meat in young lambs (90 days old) with slaughter live weight of 19.5 kg.

Furthermore, the marbling fat in the meat may be the result of feeding level; animals eating a high diet quality (e.g. RN treatment) (Table 1) present greater marbling fat (Table 5) than animals eating a diet with lower quality (e.g. RT treatment) (Table 1). Nonetheless, studies have shown that the intramuscular fat is associated with meat quality. Jiang et al. (2010) concluded that the high concentration of conjugated linoleic acids in meat was associated with higher intramuscular fat in beef cattle. The effect of herbage quality was observed by Lourenço et al. (2007), that studied the different botanical composition diets by lambs and concluded that animals grazing a leguminous rich pasture (high chemical composition, with 23% of crude protein) presented greater live weight gain and consequently higher polyunsaturated fatty acid proportions in abomasum, subcutaneous and intramuscular fat than animals eating a diet with diverse botanical composition (low chemical composition, with 10% of crude protein). On the other hands, Baghurst (2004) point out that red meat consumption is increasing from a low base and a high degree of marbling is preferred because of its cooking qualities and relative tenderness.

Thus, it can be hypothesised that the “rotatinuous stocking” (RN), in addition to presenting greater animal performance and carcass conformation, also may be considered a grazing management that produces meat with high quality and with an important role in human health.

4.5. Conclusions

The better sward structure with consequently better herbage chemical composition for the grazing management strategy based on animal behaviour (“rotatinuous stocking” - RN) resulted in greater final live weight, carcass weight and weight of commercial cuts of sheep grazing Italian ryegrass pastures than traditional rotational stocking method (RT). However, although the marbling fat was greater for the “rotatinuous stocking”, the tissue composition (muscle, bone and fat) and meat colour of grazing sheep were not influenced by different grazing management strategies.

The thoracic perimeter (*in vivo*), daily live weight gain and eye muscle area were the variables with more correlation with commercial cuts and carcass weight from grazing sheep. In general, the subcutaneous fat thickness presented a medium correlation with marbling, commercial cuts and carcass weight.

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Table 1. Characteristics and chemical composition of Italian ryegrass pastures grazed by sheep under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
Sward height (cm)				
Pre-grazing	17.9	25.7	<0.001	0.03
Post-grazing	11.4	8.13	<0.001	0.02
Herbage mass (kg DM ha^{-1})				
Pre-grazing	1783	2448	0.010	61.1
Post-grazing	1349	1137	0.280	43.4
Chemical composition (g kg DM^{-1})				
Organic matter	872.1	879.3	0.066	1.97
Crude protein	231.2	172.7	<0.001	0.80
Acid detergent fiber	226.3	256.8	<0.001	0.42
Neutral detergent fiber	429.2	458.0	<0.001	0.49

P = probability; MSE = mean standard error.

Table 2. Body conformation measurements *in vivo* of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
Body length (cm)	68.7	68.6	0.957	0.78
Leg length (cm)	55.1	53.4	0.035	0.42
Leg circumference (cm)	36.9	35.6	0.307	0.42
Chest width (cm)	13.1	12.5	0.296	0.28
Width of croup (cm)	18.8	17.1	0.004	0.32
Thoracic perimeter (cm)	107.1	97.9	<0.001	1.14
Previous height (cm)	63.5	61.8	0.099	0.52
Posterior height (cm)	67.4	65.3	0.006	0.40

P = probability; MSE = mean standard error.

Table 3. Carcass conformation measurements of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
Internal length (cm)	81.3	77.5	0.089	1.12
External length (cm)	68.4	66.3	0.213	0.57
Leg length (cm)	29.5	29.3	0.723	0.58
Leg depth (cm)	34.6	32.1	0.079	0.87
Shoulder length (cm)	26.4	25.3	0.476	0.51
Shoulder depth (cm)	28.2	27.4	0.340	0.37

P = probability; MSE = mean standard error.

Table 4. Commercial cuts of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Characteristics	RN	RT	P	MSE
Shoulder (kg)	1.76	1.45	0.002	0.04
Loin (kg)	0.47	0.39	0.027	0.01
Ribs (kg)	2.87	2.35	0.005	0.08
Leg (kg)	3.10	2.68	0.018	0.08
Neck (kg)*	0.74	0.64	0.029	0.02

*Primary cut.

P = probability; MSE = mean standard error.

Table 5. Live weight, carcass and non-carcass characteristics of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Characteristics	RN	RT	P	MSE
Live weight				
Initial (kg)	21.2	23.0	0.007	0.34
Final (kg)	39.9	34.9	0.028	0.84
Carcass				
Hot carcass weight (kg)	18.7	15.7	0.006	0.45
Cold carcass weight (kg)	18.2	15.3	0.006	0.44
pH (hot carcass)	6.97	7.06	0.070	0.03
pH (cold carcass)	5.66	5.65	0.792	0.03
Eye muscle area (cm^2)	16.4	15.2	0.284	0.56
Eye muscle height (mm)	31.2	28.3	0.009	0.58
Eye muscle width (mm)	60.4	61.5	0.671	1.30
Marbling	2.5	1.5	<0.001	0.15
Subcutaneous fat thickness (mm)	3.73	3.15	0.467	0.26
Muscle (%)	60.0	58.1	0.169	0.72
Bone (%)	25.3	25.8	0.801	0.55
Fat (%)	15.6	16.1	0.711	0.80
Meat colour (*L*)*	47.3	50.0	0.149	0.91
Meat colour (*a*)*	15.1	15.0	0.807	0.30
Meat colour (*b*)*	7.99	8.06	0.824	0.33
Non-carcass				
Red viscera (kg)	1.95	1.72	0.016	0.04
Tongue (kg)	0.07	0.07	0.962	0.01
Head (kg)	1.72	1.66	0.427	0.03

*Meat colour at 24 h *post mortem* (*L* = lightness; *a* = redness; *b* = yellowness).
P = probability; MSE = mean standard error.

Table 6. Pearson correlations (*r*) and probabilities for associations between *in vivo* and carcass measurements from sheep grazing Italian ryegrass pastures.

Variables	LW	LWG	CWt	Loin	Shoulder	Leg	Ribs	BL	LL	LC	CW	WC	TP	PreH	PostH	SFat	EMA
LWG	0.90***																
CWt	0.83***	0.83***															
Loin	0.48*	0.50**	0.76***														
Shoulder	0.84***	0.83***	0.94***	0.69***													
Leg	0.70***	0.67***	0.86***	0.82***	0.79***												
Ribs	0.79***	0.81***	0.97***	0.73***	0.90***	0.84***											
BL	-0.10^{ns}	-0.08^{ns}	-0.07^{ns}	-0.14^{ns}	-0.15ns	-0.04^{ns}	-0.007^{ns}										
LL	0.25^{ns}	0.27^{ns}	0.36*	0.41*	0.37*	0.33^{ns}	0.32^{ns}	-0.38*									
LC	0.26^{ns}	0.28^{ns}	0.39*	0.47**	0.29^{ns}	0.36*	0.35^{ns}	0.10^{ns}	0.38*								
CW	0.23^{ns}	0.18^{ns}	0.11^{ns}	0.02^{ns}	0.07^{ns}	0.16^{ns}	0.04^{ns}	0.29^{ns}	0.03^{ns}	0.17^{ns}							
WC	0.61***	0.57**	0.39*	0.19^{ns}	0.45*	0.29^{ns}	0.44*	0.06^{ns}	0.19^{ns}	0.03^{ns}	0.02^{ns}						
TP	0.72***	0.83***	0.62***	0.40*	0.69***	0.54**	0.57***	-0.05^{ns}	0.28^{ns}	0.15^{ns}	0.32^{ns}	0.37*					
PreH	0.47*	0.51**	0.38*	0.20^{ns}	0.44*	0.17^{ns}	0.44*	-0.16^{ns}	0.21^{ns}	-0.07^{ns}	-0.43*	0.54**	0.31^{ns}				
PostH	0.59**	0.48*	0.42*	0.24^{ns}	0.50**	0.28^{ns}	0.46**	-0.16^{ns}	0.58***	0.05^{ns}	-0.05^{ns}	0.73***	0.38*	0.62***			
SFat	0.18^{ns}	0.20^{ns}	0.53**	0.32^{ns}	0.42*	0.42*	0.61***	0.19^{ns}	0.08^{ns}	0.23^{ns}	-0.24^{ns}	0.02^{ns}	0.01^{ns}	0.31^{ns}	0.11^{ns}		
EMA	0.48*	0.34^{ns}	0.70***	0.68***	0.62***	0.74***	0.68***	-0.08^{ns}	0.30^{ns}	0.31^{ns}	0.03^{ns}	0.21^{ns}	0.14^{ns}	0.19^{ns}	0.38*	0.52**	
IMF	0.52**	0.58**	0.59***	0.41*	0.61***	0.40*	0.64***	0.02^{ns}	0.21^{ns}	0.04^{ns}	0.09^{ns}	0.34^{ns}	0.46*	0.38*	0.46**	0.37*	0.39*

LW = live weight (kg); LWG = live weight gain (g $animal^{-1}$ day^{-1}); CWt = carcass weight (kg); BL = body length (cm); LL = leg length (cm); LC = leg circumference (cm); CW = chest width (cm); WC = width of croup (cm); TP = thoracic perimeter (cm); PreH = previous height (cm); PostH = posterior height (cm); SFat = subcutaneous fat (mm); EMA = eye muscle area (cm^2); IMF = marbling.

Loin, Shoulder, leg and ribs (kg).

*** = P<0.001; ** = P<0.01; * = P<0.05; ns = non-significant (P>0.05).

5. CHAPTER V[4]

Rotatinuous stocking: new grazing management process has high potential to mitigate methane emissions by grazing sheep

[4]Prepared in accordance with the standards of the Journal of Cleaner Production.

Rotatinuous stocking: new grazing management process has high potential to mitigate methane emissions by grazing sheep

Abstract: The objective of this study was to evaluate the effect of different grazing management strategies on the CH_4 emission by sheep grazing Italian ryegrass (*Lolium multiflorum*) pastures in rotational stocking. The experiment was carried out in 2014 and 2015, in the south of Brazil. The experimental design was a randomized complete block with four replicates and two grazing management strategies; traditional rotational stocking method (RT) with pre- and post-grazing sward target heights of 25 and 5 cm, respectively and, “rotatinuous stocking” (RN) with pre- and post-grazing sward heights of 18 and 11 cm, respectively. Male castrated sheep were used. The herbage chemical composition had better values for quality for the RN than the RT treatment, with greater ($P<0.05$) crude protein and lower neutral and acid detergent fiber. The herbage digestibility and intake (OM and digestible OM) by sheep grazing Italian ryegrass were greater for the RN than the RT treatment. CH_4 emission per animal (g day^{-1}) was greater ($P<0.05$) for the RN than the RT treatment. However, CH_4 emission per kg of digestible OM and OM intake, per hectare per day and per kg of average daily gain by grazing sheep were lower ($P<0.05$) for the RN than the RT treatment. The rumen pH and short-chain fatty acids of sheep grazing Italian ryegrass did not differ ($P>0.05$) between grazing managements. In conclusion, this study demonstrated that the “rotatinuous stocking” (RN) based on animal behaviour, with better diet quality and greater herbage intake was the better grazing management strategy for mitigation of CH_4 emissions by sheep grazing Italian ryegrass, with 64% less CH_4 production

per area and 170% less CH_4 per unit of animal product than the traditional rotational stocking method (RT).

Keywords: greenhouse gases, herbage intake, Italian ryegrass, lambs, rotational stocking, sward height.

5.1. Introduction

Grassland management has important impacts on biodiversity and mitigation of global greenhouse gas emissions (Boval and Dixon, 2012). According to Henderson et al. (2015) adjustments in grazing pressure, with maximization of forage production, can sequester 148.4 Tg CO_2 per year in grazing lands worldwide, indicating also that the animal emissions may be fully offset by the greater carbon sequestration gains from improved grazing management. Another study by Conant et al. (2001) indicated that improving grazing management resulted in an increase of soil carbon stocks of 0.35 Mg per hectare/year. Soussana and Lemaire (2014) point out that in the short term, reducing animal stocking rates would both restore pasture productivity and soil organic carbon stocks, while reducing nitrogen and greenhouse gas losses. The animal stocking reduction is a consequence of good grazing management, such as: ideal sward heights and forage allowance. The equilibrium between grazing and conversion efficiencies was reached on mixed Italian ryegrass and oat pastures managed at 20 cm (moderate grazing intensity), indicating that this sward height target provided enough herbage mass to allow both animal performance and no-till crop demand for soil cover than a sward height managed at 10 cm (high grazing intensity) (Kunrath et al., 2014). DeRamus et al. (2003) showed that when proper grazing management practices are implemented there was an increase in animal productivity and a decrease in CH_4 emission per kg of animal product. Savian et al. (2014) confirmed that grazing management had high potential to reduce CH_4 emission by sheep grazing Italian ryegrass where by continuous stocking method resulted in greater herbage intake by sheep and consequently greater individual daily gain, resulting in 35% less CH_4 per kg of average daily gain than rotational stocking method.

Herrero et al. (2016) suggested that improving animal reproductive rates, feed availability and average daily gain rates, may result in the reduction of herd size and reduced greenhouse gas emissions. In pastoral systems this may be achieved by improving grazing management; however the sward management should be based on the animal. The traditional rotational grazing method is based on maximizing herbage accumulation and herbage harvested rather than on maximizing animal performance.

Thus, a new grazing management strategy, called “rotatinuous stocking” (RN) (Carvalho, 2013) and based on animal behaviour, was developed with the goal of identifying for different forage species the ideal sward heights for pre-grazing (Gonçalves et al., 2009; Fonseca et al., 2012; Amaral et al., 2013; Mezzalira et al., 2014) and post-grazing (Fonseca et al., 2012; Mezzalira et al., 2014) that maximize and maintain the herbage intake per unit of grazing time by grazing ruminants, combining low grazing intensity and high grazing frequency. Therefore, we hypothesized that the best sward structure (pre- and post-grazing) in the “rotatinuous stocking” (RN) results in greater daily herbage intake and lower CH_4 intensity and emission by sheep grazing Italian ryegrass pastures than the traditional rotational stocking method (RT). Thus, the goal of this study was to evaluate the effect of different grazing management strategies on the herbage quality, herbage intake, ruminal parameters and CH_4 emission by sheep grazing Italian ryegrass pastures in rotational stocking.

5.2. Material and Methods

5.2.1. Site and treatments

The experiment was conducted at the experimental field of the Universidade Federal do Rio Grande do Sul, in the south of Brazil (30°05´S, 51°39´W). The data were collected between May and October, during two years (2014 and 2015). The climate in the region is subtropical humid and 18.8°C was the climatic average temperature over the last 30 years.

The experimental design was a randomized complete block with two grazing management strategies and four replicates (paddocks). The grazing management strategies studied were: RT - traditional rotational stocking method, with pre- and post-grazing target heights of 25 and 5 cm, respectively and RN - “rotatinuous stocking” (Carvalho, 2013) with pre- and post-grazing target heights of 18 and 11 cm, respectively (Amaral et al., 2013). In the RT treatment, the moment to start grazing was defined by maximum herbage accumulation and by high grazing pressure to harvest all herbage. The new sward management strategy (RN) was defined by optimal sward height (pre-grazing), when the animal herbage intake per unit of grazing time is maximized (Amaral et al., 2013) and reduction of pre-grazing sward height to 40% of the initial height (Fonseca et al., 2012; Mezzalira et al., 2014).

5.2.2. Pasture

The experimental area had 1.76 hectares divided into eight equal experimental units (paddocks). The pastures used were pure Italian ryegrass (*Lolium multiflorum*) sown in April 2014 and 2015. The pasture received one application of 300 kg/ha of fertilizer (NPK, 5-30-15) together with the Italian ryegrass seeding and 30 days after the pastures were fertilized with 140 kg of nitrogen per hectare. All pasture management and measurements protocols were the same for the two years.

5.2.2.1. Grazing management

The grazing management was based on a 1-day strip-grazing regime. Every day, the animals started a new strip-grazing between 14:00 h and 15:00 h. The sward management was according to the sward heights proposed for both treatments. The number of strip-grazing per experimental unit was defined by the herbage growth; the animals started a new stocking cycle when the first strip-grazing had the target height for each treatment. The stocking period in 2014 was 146 and 140 days for the RN and RT treatment, respectively, and in 2015, the stocking period was 155 and 146 days (RN and RT treatment, respectively).

5.2.2.2. Sward measurements, herbage sampling and analyses

Sward stick was used to measure the sward height (Barthram, 1985). Every two days 100 measurements per strip-grazing (pre- and post-grazing) were taken.

Three herbage mass samples (0.25 m^2) were taken at ground level every second strip-grazing (pre- and post-grazing), every stocking cycle. Hand-plucked samples were taken for the determination of herbage chemical composition, as indicated by Johnson (1978). This measurement was performed always together with each evaluation of herbage intake. Thus, two measurements were performed, after strip-grazing allocation (afternoon) and in the next morning at 09:00 h, always in the same strip-grazing. All herbage samples were dried at 55°C for 72 hours. Hand-plucked herbage samples were ground for determination of organic matter, ash, crude protein, acid detergent lignin, acid detergent fiber and neutral detergent fiber by a NIRS method. These predictions were performed by The Walloon Agricultural Research Centre, Belgium (*sensu* Decruyenaere et al., 2009).

5.2.3. Animal

Experimental animals were crossbreed of Texel and Ideal (Polwarth) castrated lambs. At the beginning of the stocking period the animals were on average of ~10 months old with an average live weight (LW) of 26.2 ± 0.9 kg in 2014 and 22.1 ± 1.8 kg in 2015. Each experimental unit had four test animals (permanent animals in all stocking period) and variable animals (put-and-take technique, Mott and Lucas (1952)). All animal measurements were the same in both years but the group of animals was different.

5.2.3.1. Herbage intake and digestibility

Daily OM intake was estimated using the faecal crude protein technique (Penning, 2004). The equation proposed for Italian ryegrass pastures by Azevedo et al. (2014) was used (OM intake=111.33+18.33*faecal crude protein). Three test animals per experimental unit were used. Total faeces collections were performed during five consecutive days using bags which were emptied once a day. The faeces were weighed and homogenized and a sub sample of 20% of the total was taken. These samples were dried at 55°C for 72 hours, pooled per animal, ground and analyzed for DM (Easley et al., 1965), OM and total nitrogen (AOAC, 1975). OM digestibility was calculated using the following equation: OM digestibility=1-total amount of faeces/OM intake. The digestible OM intake was calculated using the OM intake and OM digestibility. For estimate metabolisable energy (ME) intake, ME was calculated using the model proposed by CSIRO (2007) (ME=0.169*OM digestibility-1.986).

In 2014, four and three daily OM intake measurement periods were performed in the treatments RN and RT, respectively. In 2015, three daily OM intake measurement periods were performed in both treatments. In both years, the first measurement was performed approximately 20 days after starting the stocking period and the other measurements were performed in the middle and end of the stocking period.

5.2.3.2. Methane emission

Daily CH_4 emission was measured using the sulphur hexafluoride (SF_6) tracer technique (Johnson et al., 1994). The SF_6 permeation tubes used had an average permeation rate of 2.854 mg/day (2014) and 1.521 mg/day

(2015). The tubes were deployed in the reticulum of the animals (*per os* dosing). The same three test animals were used for OM intake and CH_4 measurements.

Air sampling devices consisted of stainless steel cylinders (0.5 L volume) with the sample flow regulated by a brass ball-bearing (Gere and Gratton, 2010). The cylinders were cleaned with high purity nitrogen gas (N_2) and pre-evacuated prior to each sample collection. The flow regulators were calibrated to allow a remaining vacuum in the canister of about 500 mb at the end of the sample collection period (five consecutive days).

After each OM intake measurement, CH_4 measurement was performed. In 2014, in the treatments RN and RT, four and three CH_4 measurements were performed, respectively. In 2015, three CH_4 measurements were performed for both treatments (RN and RT).

Samples were analysed for concentrations of CH_4 (ppm by volume) and SF_6 (ppt by volume) using gas chromatography (Shimadzu 2010, Japan). Four standards of CH_4 and SF_6 mixtures were used to calibrate the gas. The daily CH_4 emissions were calculated by the specific permeation rate of SF_6 and the CH_4:SF_6 ratio (volume/volume) in breath samples, after correction for atmospheric gas concentrations (see Savian et al., 2014).

The CH_4 emission was calculated as g CH_4/OM intake, g CH_4/digestible OM intake, g CH_4/ha/day and g CH_4/kg ADG (average daily gain). To calculate the CH_4 emissions per ha and per ADG, animal production data were used (CHAPTER III).

5.2.3.3. Rumen fluid samples and analyses

The rumen fluid samples were collected from one test animal per experimental unit. After each measurement period of CH_4 emission was taken of a rumen fluid sample. The measurements were performed approximately three hours after the strip-grazing allocation and the next morning at 9 am h. An esophageal probe was used to collect 20 mL of rumen fluid per animal. After measurement, the sample was then strained through cheesecloth and a pH reading was taken. The sample was alkalinized with NaOH (10 *N*) for measurement of short-chain fatty acid concentration. All samples were then frozen. Before the analyses, the samples were centrifuged. Short-chain fatty acid concentration in the rumen fluid was performed by high performance liquid chromatographer (Shimadzu®, 14-B model).

5.2.4. Data analyses

Data were analyzed with analysis of variance at 5% level of significance for interpretation of statistical analyses. The analyses were performed using the R software for statistical computing version 2.12.0 (R Development Core Team, 2010). Linear models and mixed linear models (lme) were tested and the best model was selected by likelihood ratio test e Akaike's Information Criterion (AIC). The treatments were considered as fixed effect and the measurements (during the stocking period), blocks and years were considered as random effects. The measurements during the stocking period were considered to be repeated measures. Data were transformed to improve the normality and homogeneity of the residuals if necessary.

5.3. Results

5.3.1. Sward characteristics

Italian ryegrass characteristics are shown in Table 1. Sward height values were near the targets of the experiment. Pre-grazing leaf mass was greater (P<0.05) for the RT than the RN treatment, however in the post-grazing, the leaf mass was greater (P<0.05) for the RN than the RT treatment. Therefore, the stem mass was greater (P<0.05) for the RT than the RN treatment (pre- and post-grazing). Leaf/stem ratio was greater (P<0.05) for the RN than the RT treatment (pre- and post-grazing).

Table 2 shows the chemical composition of Italian ryegrass pastures. Acid detergent lignin did not differ between treatments (P>0.05). Neutral detergent fiber, acid detergent fiber and organic matter content were greater (P<0.05) for the RT than the RN treatment, however ash and crude protein content were greater (P<0.05) for the RN than the RT treatment.

5.3.2. Herbage intake and methane emissions

The herbage digestibility and intake of OM, digestible OM and metabolisable energy by sheep grazing Italian ryegrass were greater for the RN than the RT treatment (Table 3). The OM intake (% LW) in the RN treatment was 12% greater (P<0.05) than the RT treatment (Table 3).

CH_4 emission per animal (g/day) was greater (P<0.05) for the RN than the RT treatment, however, the CH_4 emission per kg of metabolic live weight did not differ (P>0.05) between both grazing managements, with average of 1.71 ± 0.01 g CH_4/kg $LW^{0.75}$ (Table 4). The CH_4 emission per kg of digestible OM and OM intake, per hectare per day and per kg of ADG by grazing sheep were lower ($P<0.05$) for the RN than the RT treatment (Table 4).

5.3.3. Rumen parameters

The rumen pH of sheep grazing Italian ryegrass did not differ ($P>0.05$) between both grazing managements, with average of 6.92 ± 0.22 (Table 5). The concentration of short-chain volatile fatty acids in the rumen of sheep grazing Italian ryegrass did not differ ($P>0.05$) between the two grazing managements, with average values of 58.8 ± 0.01, 30.0 ± 0.47 and 11.2 ± 0.48% molar for acetic, propionic and butyric acid, respectively (Table 5).

5.4. Discussion

This study showed that the grazing management modified the sward structure and consequently influenced the herbage intake and CH_4 emission by sheep grazing Italian ryegrass pastures. Therefore, “rotatinuous stocking” (RN) based on animal behaviour resulted in lower environmental impact, with lower CH_4 intensity and production by sheep than traditional rotational stocking method (RT).

5.4.1. Implications of herbage intake at methane emission

The CH_4 emission per animal per day was 12% lower for the RT than the RN treatment (Table 4). This is explained mainly by the grazing management proposed for the RN treatment that resulted on herbage intake by sheep 23% greater than the RT treatment (Table 3). This is a classic response described by the literature, in other words, when the forage intake increased, the CH_4 emission also increased (Kurihara et al., 1999; Hammond et al., 2013; Savian et al., 2014; Moorby et al., 2015; Amaral et al., 2016; Charmley et al., 2016; Zhao et al., 2016). Another important factor that may aid in explaining the CH_4 emission by ruminants is the diet quality (Johnson and Johnson, 1995). In this experiment diet quality may have helped to reduce CH_4 emission for the RN treatment and minimize the difference between treatments, even with mean values (g CH_4/animal/day) greater than the RT treatment (Table 4). This may be concluded by the greater leaf mass and lower stem mass (greater leaf/stem) (Table 1), mainly in the post-grazing (RN treatment), showing that the animals had the opportunity to eat a high leaf proportion until the moment of change for a new strip-grazing, with better herbage chemical composition (Table 2) than RT treatment (e.g. more crude protein and lower neutral detergent fiber, respectively). Therefore, the digestible OM and metabolisable energy intake by animals in RN treatment also were 26 and 15% greater than RT treatment, respectively (Table 3).

Savian et al. (2014) showed similar CH_4 emission for male growing sheep grazing Italian ryegrass pastures. However, these authors did not find a difference between grazing management (stocking methods and grazing intensities), with an average of 22.7 g CH_4/animal/day. The difference in individual daily CH_4 emission is not common for animals with similar LW eating the same forage species (Wims et al., 2010; Boland et al., 2013; Nascimento et al., 2015; Muñoz et al., 2016) and often when eating different forage species

(Dini et al., 2012; Williams et al., 2016) or pure versus mixed pastures (Enriquez-Hidalgo et al., 2014a), except when the grazing management is very contrasting, as in the present study. This is confirmed by Hammond et al. (2013), who reported that when the feeding level increases from 0.8 to 2.5 x MEm (requirements for maintenance), the CH_4 emission by sheep fed Perennial ryegrass also increases (from 13.1 to 31.9 g CH_4/animal/day, respectively).

Nonetheless, the CH_4 emission expressed per animal per day is not an ideal parameter to compare different treatments and/or livestock production systems. Thus, the CH_4 per kg of OM intake and per kg of digestible OM intake were 14 and 16% lower for the RN than the RT treatment (Table 4). These results show that the RN treatment was more efficient in reducing the CH_4 production as a consequence of greater herbage intake by animals in this treatment. Hammond et al. (2013) showed that when the feeding level increases from 0.8 to 2.5 x MEm, the herbage intake increases from 490 to 1510 g DM/animal/day and the CH_4 yield decreases from 27 to 23.9 g CH_4/kg DM intake by sheep fed Perennial ryegrass.

The values found in this study were similar or lower than values described by Dall-Orsoletta et al. (2016) assessing CH_4 yield by dairy cows grazing Italian ryegrass, with results ranging between 31.2 and 37.4 g CH_4/kg DM intake and Kurihara et al. (1999) when evaluating cattle fed Rhodes grass and Angleton grass, with average of 64.6 and 75.4 g CH_4/kg digestible OM intake, respectively. Hammond et al. (2014) also showed that young dairy heifers fed with Perennial ryegrass hay had CH_4 emissions of 31.6 g CH_4/kg OM intake and 43.1 g CH_4/kg digestible OM intake.

5.4.2. Implications of rumen parameters for methane emission

This study showed that the pH and concentration of short-chain fatty acids in the rumen fluid of sheep grazing Italian ryegrass was not affected by grazing management strategies. Despite diet quality being better in the RN than the RT treatment (Table 2), this did not result in different rumen parameters. The change in short-chain fatty acids was found with specific diets, such as use of monensin for example (McGinn et al., 2004). Aguerre et al. (2011) studied the CH_4 emission by dairy cows fed with variable forage-to-concentrate ratio (from 47 to 68% forage), and concluded that CH_4 emission could not be predicted from ruminal short-chain volatile fatty acid patterns alone. Enriquez-Hidalgo et al. (2014b) also showed that the acetate and propionate acids in the rumen of dairy cows grazing pure Perennial ryegrass or mixed Perennial ryegrass and white clover pastures were not altered. Even diets with nitrate did not interfere with the acetic and propionic acid concentrations of beef cattle (Hulshof et al., 2012). Different growth stages of the *Urochloa brizantha* forages harvested for hay had no effect on rumen pH, acetic and propionic acid concentrations in the rumen of steers, even with differences in hay chemical quality, which varied between treatments from 4.2% and 77% to 10.7% and 70% for crude protein and neutral detergent fiber, respectively (Nascimento et al., 2016).

Hünerberg et al. (2015) also found that the rumen pH by itself is not a strategy for CH_4 mitigation and not the main determining factor for the CH_4 reduction.

5.4.3. Implications of animal production for methane emission

The most effective way to express CH_4 production in animals is in relation to unit of animal product (Berndt and Tomkins, 2013). Therefore, the CH_4 intensity (g/kg ADG) is a consequence of animal production and the RN treatment resulted in 170% lower CH_4 intensity than the RT treatment but associated with a higher performance (Table 3). These results were supported firstly by the greater herbage intake (801 and 653 g OM/animal/day) and/or energy intake and consequently by greater ADG (119 and 47 g/animal) by sheep in the RN than the RT treatment, respectively (CHAPTER III). According to Boval and Dixon (2012) a management priority to increase output of animal products from grasslands should be based in the objective to achieves the highest possible intakes of metabolizable energy; as observed in the RN treatment. Savian et al. (2014) showed that growing lambs grazing Italian ryegrass in continuous stocking had lower CH_4 intensity (g/kg ADG) than rotational stocking (171 and 263 g CH_4/kg ADG, respectively). This was a consequence of greater herbage intake (1345 and 1075 g DM/animal/day) and greater ADG (150 and 90 g/animal) in the continuous than the rotational stocking, respectively, with both grazing managements offering the same herbage allowance (13.4 kg DM/100 kg LW). DeRamus et al. (2003) also reported that when proper grazing management practices were implemented, heifers grazing ryegrass pastures increased animal productivity (from 780 to 1260 g ADG/animal) and decreased CH_4 intensity (from 214 to 125 g CH_4/kg ADG). According to Muñoz et al. (2016), grazing management strategies that favor better herbage chemical composition (e.g. increasing crude protein from 17% to 22% and reducing neutral detergent fiber from 49% to 43%), resulted in increases of daily forage intake (from 12.1 to 13.7 kg DM/cow), in increases of daily milk production (from 21.6 to 24.4 kg/cow) and reduction of CH_4 intensity (from 15.3 to 13.6 g/kg of milk) by lactating dairy cows grazing *Lolium perene* pastures.

We simulated a production system where the sheep start grazing at 22 kg of LW and are slaughtered at 35 kg LW which is a live weight that lambs attain a fat class of 2 to 3 (on a scale of 0 to 5) (Prache et al., 2011). Thus, from the results this experiment, the sheep needed 109 and 276 days in grazing to achieve this LW (RN and RT treatment, respectively). The total CH_4 emission per animal during the whole stocking period was 2.7 kg for the RN treatment and 6.1 kg for the RT treatment. The RN grazing management strategy resulted in lower CH_4 emission and intensity, and animals had reduced turn-off time, therefore demonstrating that only by manipulating sward management will significant mitigation in CH_4 emission can be achieved. According to Herrero et al. (2016) the reduction of age at slaughter by increase of individual ADG, results in decreased greenhouse gas emissions per unit of product.

In addition to the CH_4 emission per herbage intake or unit of animal product, the topic that must also be considered is the total emission per area. This variable also was 64% lower for the RN than the RT treatment (Table 4), which confirms again the greater efficiency of the “rotatinuous stocking” (RN) as a grazing management strategy with high potential to mitigate the total CH_4 emissions by grazing ruminants.

The reduction of gas emissions per area is a global goal. This involves many important and necessary strategies to reduce greenhouse gas by livestock, such as use of tannin-rich plants (Archimède et al., 2015), legumes (Archimède et al., 2011), concentrate diets (Doreau et al., 2011), additives, as nitrate (Hulshof et al., 2012; Lee et al., 2015), saponin and ropadiar (Wang et al., 2009), sunflower oil and monensin (McGinn et al., 2004). However, there are few grazing management strategies that have been studied for this purpose. Grazing management is the main goal before any other strategies, because the pasture (grazing systems) is the base of ruminant production in the world. An example is Brazil that has 90% of beef cattle production on grazing systems (ANUALPEC, 2015).

First, the basics must be covered and then the system can be made more complex, diversifying the system with inclusion of other species, such as legumes and using concentrate levels and additives in the concentrate when for example the feed availability and quality are low. However, some ingredients as oil and monensin are expected to increase the cost of feeding (McGinn et al., 2004), which may be a hindrance to farmers using this technology.

The results of the “rotatinuous stocking” (RN) strategy confirmed that CH_4 emission per area is also a consequence of animal performance, because to maintain the low grazing intensity with high grazing frequency, a consequence is greater animal production (individual and per area) with automatic reduction of stocking rate (from 1235 to 850 kg LW/ha, RT and RN treatment, respectively) (CHAPTER III). Thus, good grazing management is the first step to increase animal productivity and reduce environmental impact.

Improved animal management, including increases in reproductions rates, feed availability and reduction of herd size, are alternatives to reducing greenhouse gas emissions (Herrero et al., 2016).

Smart livestock (FAO, 2013) management should be based on a process that reduces the trade-off between forage production and animal production. CH_4 emission is just a consequence of this process, in others words, smart livestock management should not be synonymous with high intensification. Soussana and Lemaire (2014) point out that grassland intensification needs to overcome trade-offs between production and environmental goals. These authors emphasized also that grassland soil organic carbon increases with the net primary production and the high stocking density impairs soil carbon sequestration. For Henderson et al. (2015), the impacts of animals would fully offset all of the carbon sequestration gains from improved grazing management.

5.5. Conclusion

The ideal sward structure and greater diet quality of Italian ryegrass pastures found in the grazing management based on animal behaviour (“rotatinuous stocking”) resulted in greater herbage digestibility and intake of organic matter and metabolisable energy by grazing sheep than traditional rotational stocking method.

Our study concluded that the grazing management is the key strategy to reduce the environmental impact through lower CH_4 emissions in livestock grazing systems. “Rotatinuous stocking” was the most efficient grazing management strategy for mitigation of CH_4 emissions and intensity by grazing sheep, with 64% less CH_4 production per area and 170% less CH_4 emission per unit of animal product than the traditional rotational stocking method.

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Table 1. Variables of Italian ryegrass pastures grazed by sheep under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
Sward height (cm)				
Pre-grazing	17.9	27.1	0.02	<0.001
Post-grazing	11.1	7.8	0.01	<0.001
Leaf mass (kg DM/ha)				
Pre-grazing	855	1309	55.7	<0.001
Post-grazing	475	379	31.2	<0.001
Stem mass (kg DM/ha)				
Pre-grazing	419	942	64.7	<0.001
Post-grazing	396	635	41.1	<0.001
Leaf/stem ratio				
Pre-grazing	3.40	2.12	0.20	<0.001
Post-grazing	1.65	0.88	0.10	<0.001

P = probability; MSE = mean standard error; DM = dray matter.

Table 2. Chemical composition of Italian ryegrass pastures grazed by sheep under different grazing management strategies (RN and RT).

Variables (g/kg DM)	RN	RT	MSE	P
Organic matter	875	884	1.22	<0.001
Ash	124	115	0.12	<0.001
Crude protein	215	159	0.48	<0.001
Acid detergent lignin	26.8	24.5	0.04	0.067
Acid detergent fiber	227	257	0.31	<0.001
Neutral detergent fiber	436	466	0.35	<0.001

P = probability; MSE = mean standard error.

Table 3. Herbage digestibility and intake by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
OM intake (g/animal/day)	801	653	12.09	<0.001
OM intake (% LW)	2.52	2.25	0.04	<0.001
Digestible OM intake (g/animal/day)	638	507	9.71	<0.001
ME intake (kj/kg LW/day)	291	252	0.001	0.005
OM digestibility (g/kg OM)	798	779	3.13	0.009

P = probability; MSE = mean standard error; OM = organic matter; LW = live weight; ME = metabolisable energy.

Table 4. Methane emission by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
g/animal/day	24.8	22.2	1.29	0.035
g/kg $LW^{0.75}$	1.72	1.70	0.01	0.296
g/kg OM intake	31.4	35.8	2.04	0.013
g/kg digestible OM intake	39.5	45.7	2.52	0.010
g/kg ADG	217	586	84.9	<0.001
g/hectare/day	645	1056	91.8	<0.001

P = probability; MSE = mean standard error; OM = organic matter; ADG = average daily gain; LW = live weight.

Table 5. Rumen parameters of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	MSE	P
pH	6.94	6.90	0.02	0.772
Short-chain fatty acids (% molar)				
Acetic	58.8	58.8	0.01	0.959
Propionic	30.5	29.6	0.47	0.213
Butyric	10.7	11.7	0.48	0.163
Acetic:propionic ratio	1.97	2.05	0.04	0.318

P = probability; MSE = mean standard error.

6. CHAPTER VI[5]

Faecal methane, nitrous oxide emissions and nitrogen excretion by grazing sheep: implications of grazing management strategies

[5]Prepared in accordance with the standards of Nutrient Cycling in Agroecosystems.

Faecal methane, nitrous oxide emissions and nitrogen excretion by grazing sheep: implications of grazing management strategies

Abstract: The objective of this study was to evaluate the effect of different grazing management strategies on the amount of faeces' production, nitrogen excretion and faecal greenhouse gas emissions (CH_4 and N_2O) from male sheep grazing Italian ryegrass pastures under a rotational stocking method. The experiment was carried out in 2014 and 2015, in the south of Brazil. The experimental design was a randomized complete block with four replicates and two grazing management strategies; traditional rotational stocking method (RT) with pre- and post-grazing sward heights of 25 and 5 cm, respectively and, “rotatinuous stocking” (RN) with pre- and post-grazing sward heights of 18 and 11 cm, respectively. RN treatment presented a greater daily DM faecal excretion and nitrogen excretion per animal compared with RT treatment. However, daily DM faeces excretion and nitrogen excretion per hectare were lower for the RN than the RT treatment. In the RN treatment, faecal CH_4 and N_2O emissions were higher, not only per animal and hectare but also, for the measurement period (29 days). However, the potential gas emission (CO_2eq per kg of herbage crude protein accumulation) did not differ between treatments. In conclusion, “rotatinuous stocking” (RN) had a faecal chemical quality greater than the traditional rotational stocking method (RT), resulting in a greater daily nitrogen excretion per animal and greater faecal CH_4 and N_2O emissions.

Keywords: faecal emission factor, greenhouse gases, Italian ryegrass, nutrient excretion, rotational stocking, sward management.

6.1. Introduction

It is known that the diet quality is the key factor which regulates the nitrogen excretion by ruminants. The higher the nitrogen intake, the higher is the faecal and urinary nitrogen excretion by animals (Dijkstra et al. 2011; Bannink et al. 2013; Gardiner et al. 2016), with a consequent increase mainly in N_2O production and fluxes (Luo et al. 2013). Therefore, in grazing systems, the key point is to develop strategies to improve a grazing management which favours a balanced nutrient intake by animals for their maximum production (Boval and Dixon 2012). Thus, a trade-off between nitrogen excretion and animal production may be expected, as well as a trade-off between nitrogen excretion and CH_4 emission by animals (Dijkstra et al. 2011; Bannink et al. 2013). For example, in dairy systems, when nitrogen fertilization was reduced, the nitrogen excretion was also reduced from 15.1 to 11.3 g/kg of fat and protein corrected milk, but the CH_4 emission increased from 14.9 to 16.1 g/kg of fat and protein corrected milk (Dijkstra et al., 2011).

According to Hünerberg et al. (2014), higher dietary (corn dried distillers' grains) levels increase nitrogen excretion, as well as, the greenhouse gas emissions from beef cattle production, mainly due to a higher emission of N_2O from manure. In steers grazing winter oats pastures of a high quality chemical composition, not only the faecal chemical composition (e.g. a higher crude protein) but also, the faecal CH_4 emission, were greater compared with a winter oats pastures of lower quality (Priano et al., 2014).

Based on the fact that the grazing management (sward structure) is the main factor that regulates the herbage intake and consequently the performance by grazing animals, a new grazing management target denominated “rotatinuous stocking” (RN) (Carvalho 2013), based on animal behaviour, was developed with the objective and necessity of identifying the ideal pre- and post-grazing sward heights that maximize and maintain the herbage intake per unit of grazing time in grazing ruminants. Considering that the traditional grazing management in rotational stocking method is based mainly on the greater forage accumulation and the maximum herbage harvested by the animals.

In this context, this study focused on faecal nitrogen excretion and faecal greenhouse gas emissions by sheep grazing Italian ryegrass pastures in a rotational stocking. Therefore, it was hypothesized that the optimal sward height (pre- and post-grazing) in the “rotatinuous stocking” (RN) which present a greater herbage quality, herbage intake (CHAPTER V), animal performance (CHAPTER III) and, a lower CH_4 intensity by the enteric fermentation (CHAPTER V) results in a greater amount of faeces and faecal nitrogen excretion and consequently, a greater faecal greenhouse gas emissions of grazing sheep, compared to the traditional rotational stocking method (RT). Thus, the objective of this study was to evaluate the effect of different grazing management strategies in rotational stocking on the amount of faeces production, nitrogen excretion and greenhouse gas emissions (CH_4 and N_2O) by faeces from sheep grazing Italian ryegrass pastures in the south of Brazil.

6.2. Material and Methods

6.2.1. Experiment 1 – Faecal production

6.2.1.1. Site, design and treatments

The experiment was carried out at the experimental field of the Federal University of Rio Grande do Sul, Rio Grande do Sul State, Brazil (30°05´S, 51°39´W). The data were collected in 2014 and 2015, between May and October. The mean air temperature and rainfall were 18.8°C and 1455 mm, respectively. The climate in this region is subtropical humid and the soil at the experimental site was classified as a Typic Paleudult (USDA 1999) with 17.5% clay, 20% silt and 62.5% sand.

The experimental design was a randomized complete block with two grazing management strategies and four replicates (paddocks). The grazing managements studied were: RT) traditional rotational stocking method, with pre- and post-grazing target heights of 25 and 5 cm, respectively and RN - "rotatinuous stocking" (Carvalho, 2013) with pre- and post-grazing target heights of 18 and 11 cm, respectively (Amaral et al., 2013). In the RT treatment, the moment to start grazing was defined by maximum herbage accumulation and by high grazing pressure to harvest all herbage. The new sward management strategy (RN) was defined by optimal sward height (pre-grazing), when the animal herbage intake per unit of grazing time is maximized (Amaral et al., 2013) and reduction of pre-grazing sward height to 40% of the initial height (Fonseca et al., 2012; Mezzalira et al., 2014).

6.2.1.2. Sward management

The experimental area had eight experimental units (paddocks), of 0.22 hectares each. Pure Italian ryegrass (*Lolium multiflorum*) was sown in April in both years (2014 and 2015) and received one application of 300 kg ha^{-1} of fertilizer (NPK, 5-30-15) together with the sowing and 140 kg ha^{-1} of nitrogen fertilization (urea source) before the start of the stocking period (experimental period).

The grazing management was based on a 1-day strip-grazing regime. Every day, the animals started a new strip-grazing between 2 pm and 3 pm h. The sward management was performed according to the initial sward heights proposed by both treatments. The number of strips-grazing per experimental unit depended on the herbage growth, wherein the animals started a new stocking cycle when the first strip had the ideal height proposed by the treatments. The stocking period was 146 and 140 days (RN and RT, respectively) in 2014 and, 156 and 150 days (RN and RT, respectively) in 2015. The sward height was measured using a sward stick (Barthram 1985) and 100 measurements were performed in the strip-grazing (pre- and post-grazing) every two days, in all the experimental units. The real sward average heights were of 17.9 cm and 11.1 cm for the RN treatment (pre- and post-grazing, respectively) and, 27.1 cm and 7.8 cm for RT treatment (pre- and post-grazing, respectively).

6.2.1.3. Faeces collection

The experiment was carried out with growing male sheep with an average live weight (LW) of 26.2±0.9 kg in 2014 and 22.1±1.8 kg in 2015, at the beginning of the stocking period. The number of animals per experimental unit was three (tester animal) and, in addition, a variable number of animals (using the put-and-take technique, Mott and Lucas (1952)). The faeces collections (measurements) were performed three and four times during the stocking period in 2014 (RN and RT, respectively) and during 2015, three faeces measurements were performed in both treatments. For measuring the total faeces production, harnesses and faeces collection bags were used in all tester-animals during five consecutive days. Bags were emptied once per day. The faeces were weighed, homogenized and subsequently a sub sample (20% of the total) was withdrawn in each case. These samples were dried at 55°C for 72 hours, pooled per animal (sub samples of five days), grounded and, the analyses of DM (Easley et al. 1965), OM and total nitrogen (AOAC 1975) were performed. Based on these results, the faeces production and the faecal nitrogen excretion were calculated per animal and per hectare.

6.2.2. Experiment 2 – Gases fluxes measurements

6.2.2.1. Experimental conditions

This experiment was conducted during July, in 2015, in a small isolated area of the Experiment 1; this area was cultivated with Italian ryegrass and remained intact (non-grazing) for a period of 60 days circa. In the middle of stocking period (Experiment 1 - 2015) and during the second faeces collection measurement (02/07/2015), faeces samples from the three tester-animals of each experimental unit were mixed to form a composite sample of 500 grams. Each sample was collected and immediately deposited on the soil, surrounded by a PVC cylindrical base with 25 cm of diameter previously driven into the soil (5 cm deep). The composite sample formed a homogeneous layer that covered the entire PVC base in each case. Besides, in this experiment there were two treatments as in Experiment 1 (RT and RN) and three replicates (PVC base). Also, three PVC bases without faeces were used to measure gases fluxes at the soil-atmosphere interface and were considered as the control chambers. The soil from these chambers was fertilized just with 15 kg of nitrogen per hectare together with the sowing as was previously indicated. Furthermore, another PVC cylindrical base with the same amount of faeces was used to monitor the temperature of the faeces during each measurement. During the measurement period, the average of air temperature ranged between 14.5 °C (minimum) and 29.6 °C (maximum) and the average of soil temperature ranged between 13.5 °C (minimum) and 19.1 °C (maximum).

6.2.2.2. Gas flux measurements

During July, CH_4 and N_2O emission from nine PVC cylinders chambers were measured with the static chamber method. Cylindrical bases were closed only during measurements with the purpose of accumulating the gases emitted in the head-space of the chamber. The lid had a fan attached inside to ensure air mixing and, a valve at the top for extracting air´s samples. The PVC cylindrical bases were closed with a lid (PVC cylinder of 15 cm height and 25 cm diameter) during the measurement periods. Nine measurements were carried on until the emission rate was close to zero. The first measurements were taken on the 2nd of July, 2015 between 9 am and 1 pm h (Jantalia et al. 2008), considering a daily average value (Priano et al. 2014).

Gas samples were drawn at regular intervals (Priano et al. 2014), using 20 ml syringes with a three-way stopcock and, were immediately transferred to 12 ml glass vials with vacuum (Labco Exetainer®). Gas concentration was analysed with a gas chromatographer (Shimadzu Corp. 2014) equipped with flame ionization and electron capture detectors.

Air temperature from the head-space was measured with a digital thermometer (Zanatta et al. 2010) during the flux measurements and it was used to estimate the mass concentration of CH_4 and N_2O.

6.2.2.3. Calculations

Gas fluxes (CH_4 and N_2O) at the faeces–atmosphere interface were calculated from the linear regression of the varying gas concentration versus the time spent in the chambers. Mean daily fluxes at soil-atmosphere interface (control chambers) were subtracted from average daily fluxes at faeces–atmosphere interface for each treatment.

The N_2O-N emission factor for nitrogen applied as faeces was calculated using the equation proposed by De Klein et al. (2003) and adapted by Tomazi et al. (2015). The N_2O-N (g N_2O/kg DM) was multiplied by the factor 28/44 that consider the molecular weight of 2 nitrogen and N_2O, respectively, and divided by nitrogen excretion (g of nitrogen per kg DM).

To estimate the faecal CH_4 and N_2O emissions per animal and per area, data of the two years (Experiment 1) were used and, it was assumed that the CH_4 and N_2O emissions (Experiment 2) were equal in both years. Thus, gas emission per animal was calculated using the average daily faeces excretion (g

DM day^{-1}) and, the CH_4 and N_2O emissions per kg of DM. Therefore, the gas emissions per hectare were estimated using the daily CH_4 and N_2O emissions per animal and the stocking rate in both treatments. Subsequently, to estimate the total faecal emissions by grazing sheep, the faecal CH_4 and N_2O emissions were transformed to carbon dioxide equivalent (CO_2eq), using global warming potentials of 25 and 298, respectively (Forster et al. 2007). CO_2eq per kg of herbage crude protein accumulation was estimated using CO_2eq emission per area per day and daily herbage accumulate rate (CHAPTER III) and herbage chemical quality (CHAPTER V).

6.2.3. Statistical analyses

Data were analysed using the ANOVA procedure. The analyses were performed using the R software for statistical computing version 2.12.0 (R Development Core Team, 2010).

For faecal chemical composition, faecal dry matter and nitrogen excretion, linear models and mixed linear models (lme) were tested and the best model was selected by likelihood ratio test and Akaike's Information Criterion (AIC). The treatments were considered as fixed effects and, the faecal collection measurements, blocks and years were considered as random effects. The faecal collection measurements during the stocking period were considered as repeated measures. Data were transformed to improve the normality and homogeneity of the residuals if necessary. For faecal gas emission (per animal and hectare) the same procedure, as it was mentioned before, was performed, but the treatments were considered as fixed effect and the paddocks were considered as random effects.

In order to determine if there were statistical differences in CH_4 and N_2O emission between treatments, an ANOVA test was used. The treatments were considered as fixed effects and the chambers were considered as random effects.

6.3. Results

6.3.1. Experiment 1 - Faecal and nitrogen excretions

The faecal chemical quality and faecal excretion are shown in Tables 1 and 2. Faecal organic matter and ash did not differ ($P>0.05$) between treatments, with an average of 774.8 and 225.1 g kg DM^{-1}, respectively. However, the faecal crude protein was greater ($P<0.05$) for the RN than the RT treatment (Table 1).

The daily DM faecal excretion and nitrogen excretion per animal were greater ($P<0.05$) for the RN than the RT treatment. However, daily DM faeces excretion per hectare and nitrogen excretion were lower ($P<0.05$) for the RN than the RT treatment (Table 2).

6.3.2. Experiment 2 - Faecal gas fluxes

Faecal CH_4 emission for RN treatment was three times greater ($P<0.05$) than RT treatment for the experimental period (29 days), with an average of 0.233 g CH_4/kg of fresh faeces and 0.079 g CH_4/kg of fresh faeces, respectively, and maximum emission values during the first days for both treatments (Fig. 1a). According to Table 2, mean faecal CH_4 emission were 1.065 g CH_4/kg DM and 0.359 g CH_4/kg DM for RN and RT treatment, respectively.

In the RN treatment, faecal N_2O emission (Fig. 1b) showed values of 9.97 mg N_2O/kg of fresh faeces (or 45.56 mg N_2O/kg DM), which were four times greater compared with the RT treatment, with an average of 2.29 mg N_2O/kg of fresh faeces (or 10.36 mg N_2O/kg DM). Contrary to the CH_4 emission, the maximum N_2O emission values were observed on the 10^{th} and 24^{th} day after the beginning of the measurement (RT and RN treatments, respectively).

Furthermore, the mean CH_4 soil flux (control chambers) was -2.17 g/hectare/day and the mean N_2O soil flux was 1.32 g/hectare/day.

As shown in Table 3, faecal gas emissions (CH_4 and N_2O) per animal and per hectare were greater ($P<0.05$) for the RN than the RT treatment. Even when the values were transformed for CO_2eq, the same response was found. However, the potential gas emission (CO_2eq per kg of herbage crude protein accumulation) did not differ between treatments ($P>0.05$) (Table 3).

6.4. Discussion

6.4.1. Faecal and nitrogen excretion

The greatest faecal and nitrogen excretion per animal for the RN treatment could be the result of the best sward structure and consequently, the better diet quality for this grazing management. The optimum pre-grazing sward height associated with a 40% of herbage depletion, resulted in a greater crude protein and a lower NDF for the RN (21 and 44%, respectively) compared with the RT treatment (16 and 47%, respectively) (CHAPTER V). However, due to small difference of NDF content between both treatments, daily herbage intake, which is modified by sward structure, could interfere on faecal excretion per animal. This is supported mainly because diets with low quality had longer retention time in the rumen (Panjaitan et al. 2010) than diets with high quality, due to their slower passage rate (Poppi et al. 1981).

Due to the herbage quality and daily herbage intake were greater for the RN than the RT treatment (CHAPTER V), we can assume that nitrogen intake was also greater, which resulted in greater faecal nitrogen excretion by animals (Dijkstra et al. 2011; Bannink et al. 2013; Gardiner et al. 2016). The same response was found in many studies with different forage and animal species. Azevedo et al. (2014) found that faecal crude protein concentration was greater for sheep grazing Italian ryegrass with high chemical quality. Luo et al. (2015) also reported that a higher nitrogen concentration in forage rape compared with perennial ryegrass, resulted in a daily nitrogen intake 23% higher in sheep. Hünerberg et al. (2013) showed that the greater the nitrogen intake by growing beef cattle, the greater was the nitrogen excretion in the faeces and urine. Lastly, Oenema et al. (2005) pointed out that nitrogen excretion by dairy cows increases almost linearly with milk production and protein content of the animal feed. Therefore, it is important to consider from the results of this study that, despite having more nitrogen excreted in the RN treatment, sheep had a greater performance compared with the RT treatment (CHAPTER III).

As a result of the high values of nitrogen excretion, the nitrogen excretion by sheep could have an important role for the soil system. In an integrated crop-livestock system, Da Silva et al. (2014) found that faeces from grazing beef cattle in the pasture phase (livestock) increased a 23% of the soybean grain yield (crop phase) in relation to the absence of faeces. The decoupling of carbon and nitrogen of the plants by animals results in a little part which accumulates in the animal itself, and a highest part which returns to the system (faeces and urine), therefore, a high grazing intensity (e.g. RT treatment) affects the dynamics of the cycling system by the highest carbon and nitrogen decoupling (Soussana and Lemaire 2014). Assmann et al. (2015) found that carbon and nitrogen release rates from pasture and faeces were greater in soybean-beef cattle integration under moderate grazing compared with the low and high grazing intensity and, in non-grazed areas. These authors also pointed out that despite the nitrogen cycling did not supply sufficient nitrogen amounts for the integrated systems, it maintained the soil biological activity, enabling a greenhouse gas sequestration and decreasing the fertilizer demand under long-term conditions. Therefore, strategies to improve the spatial distribution of dung are necessary in order to reduce its high concentration in areas which are regularly used by cattle and, in consequence, it could lead to a reduction in the greenhouse gas emissions. Eckard et al. (2010) pointed out that if animal urine in grazing systems was spread more evenly across the paddock, the effective nitrogen application rate would be reduced, potentially reducing N_2O emissions.

As the diet quality was greater for the RN treatment (CHAPTER V), the herbage passage rate may have been also greater (Poppi et al. 1981), which may result in a greater frequency of defecation events by the sheep and consequently, a more uniform spread of faeces in the area. This is an advantage of the rotational stocking method where grazing systems are more uniform and may result in an uniform excreta distribution compared with the continuous stocking method. According to Ledgard et al. (2007), the use of salt supplementation for increasing the water intake by ruminants is one strategy to implement which reduces the nitrogen concentration in urine and increases the urination events and consequently, the urinary nitrogen will be more uniformly distributed under grazing areas.

In this study, sheep showed lower excretion of faeces uniformly distributed in the same place, compared with large herbivores. This is the result of smaller amounts of excretion per sheep (198 g DM day^{-1}, in average) (Table 2) and a high number of sheep per area (35 sheep per hectare, in average) compared with beef cattle, with an average of 1.5 kg DM (faeces) $steer^{-1}$ day^{-1} (adapted from Sánchez Chopa et al. (2016)) with an average of 4 steers per hectare (adapted from Kunrath et al. (2014)). Therefore, there is a higher N_2O emission of excreta in dairy cattle systems under grazing conditions compared with grazing sheep, as it was observed by Saggar et al. (2007).

The use of conserved forages with low nitrogen concentrations which dilute the nitrogen from forages with a high chemical quality, (e.g. Italian ryegrass) is necessary (Gregorini et al. 2016) to improve the nitrogen utilization and to reduce the nitrogen excretions by ruminants in grazing systems.

Furthermore, in livestock under grazing systems with more complex plant communities, it will be an advantage to improve the equilibrium between nitrogen intake, nitrogen excretion, animal performance and greenhouse gas emissions.

6.4.2. Faecal CH_4 and N_2O emissions

In general, this study showed that the faecal greenhouse gas emissions (CH_4 and N_2O) were greater for the grazing management strategy based on the animal behaviour ("rotatinuous stocking", RN), with a greater herbage and animal production (CHAPTER III) and, lower CH_4 emissions per area and per unit of animal production (CHAPTER V) compared with the traditional rotational stocking method (RT). Therefore, the CO_2eq emission was 3.5 times (per animal) and 2 times (per hectare) greater for the RN than the RT treatment. This was the result of the greater faecal nitrogen content, with a higher value (18%) of nitrogen in the RN treatment than in the RT treatment (Table 1).

This study shows values of CH_4 emissions from sheep faeces of 80 g animal^{-1} year^{-1} for the RN treatment and, 25 g CH_4 animal^{-1} year^{-1} for the RT treatment. The highest emission correlates with the greatest faecal chemical quality. These values were lower compared with the first national report from Brazil to the IPCC (2002), with CH_4 emission factor from sheep of 160 g CH_4 head^{-1} year^{-1} for temperate climates. Priano et al. (2014) reported the same response for steers grazing oat pastures under a strip grazing management (morning vs. afternoon treatments). They found that the total CH_4 emission was greater for the morning treatment, which was associated with a greater faecal chemical quality (0.067 kg CH_4 animal^{-1} year^{-1}) compared with the afternoon treatment, which was associated with a lower faecal chemical quality (0.012 kg CH_4 head^{-1} year^{-1}).

It will be significant to consider the incorporation of these emission factors in the Brazilian greenhouse gas inventory, considering that the national inventory is based on default emission factors (mainly N_2O-N), proposed by the IPCC Guidelines (Tier 1) (Brasil 2010), which represent a 1% for sheep excreta.

This study reported faecal emission factors (N_2O-N) lower than the Tier 1 factor, with averages values of 0.85 and 0.22% for RN and RT treatments, respectively. Faecal emissions factors were found in the range of 0.06% and 0.09%, in Brazil (Tomazi et al. 2015), in New Zealand (Luo et al. 2013) and in Germany (Hoeft et al. 2012) in sheep. On the other hand, in cattle, faecal emissions factors were found in the range of 0.05% and 2.03%, in temperate climates (Hoeft et al. 2012) and subtropical climates (Sordi et al. 2014; Costa Junior et al. 2015).

As reported in this work, Luo et al. (2015) also reported that faeces from sheep fed forage rape, with a greater chemical quality resulted in an greater emission factor (N_2O-N: 0.08%), compared with a forage ryegrass diet, with a lower chemical quality (N_2O-N: 0.03%).

It is known that inputs from animal excreta in grazing systems, such as faeces and urine, result in an increase of NH_4^+ and NO_3^- content in the soil and consequently, an increase in N_2O production (Luo et al. 2013). However, it is necessary to understand the effect of grazing management strategies and their implications and trade-offs regarding nitrogen excretions and greenhouse gas emissions, in pastoral ecosystems. The trade-off between N_2O emissions for manure and enteric CH_4 emissions from beef cattle was reported by Hünerberg et al. (2014). These authors showed that high-fat distillers' grains in the diet of feedlot cattle may decrease enteric CH_4 emissions. However, at high dietary levels they produce an increase in nitrogen excretion resulting in a net increase in greenhouse gas emissions, mainly due to a higher N_2O emission from manure. On the other hand, nutritional measures to reduce nitrogen excretion, may not necessarily reduce greenhouse gas emissions (Bannink et al. 2013; Gardiner et al. 2016). Dijkstra et al. (2011) pointed out that dietary measures which reduce total nitrogen excretion by cattle, are expected to lead to an increase in CH_4 production. According to Gregorini et al. (2016), there is not an optimum diet which optimizes all the aims simultaneously in a production system.

We must understand the processes which take place in the grazing systems and seek the equilibrium between animal production (e.g. meat) and greenhouse gas emissions. Although urinary excretion and emission were not evaluated in this study, it is known that the faecal emissions, mainly of N_2O, are lower compared with the urinary emissions (Hoeft et al. 2012), because 70-80% of the total nitrogen excreted by the animal is in the urine (Soussana and Lemaire 2014). In general, faecal CO_2eq emissions represent less than 1% of enteric CH_4 emission. This pattern represents a small part of the total emissions of the system. Beauchemin et al. (2010) evaluated the life cycle of greenhouse gas emissions from beef production in Canada and concluded that the greater part (63%) of total emissions (CO_2eq) was derived from enteric CH_4 emission.

Moreover, it was hypothesized in this study that the total greenhouse gas emissions may be mainly compensated by the soil carbon sequestration in the “rotatinuous stocking” (RN), which presented herbage production of 28% higher compared with the traditional rotational stocking method (RT) (CHAPTER III). When the emission potential was evaluated, faecal CO_2eq emission per kilogram of herbage crude protein accumulation, both grazing managements had the same result. This outcome indicates that even though the “rotatinuous stocking” (RN) had a greater faecal CO_2eq emission, it may be compensated by a greater herbage production. For Soussana and Lemaire (2014), overgrazing (e.g. RT treatment, 45 sheep per hectare) is often the most limiting factor for the net primary productivity and soil carbon sequestration and, therefore, moderate grazing intensities (e.g. RN treatment, 26 sheep per hectare) would restore forage productivity and soil carbon sequestration stocks, while reducing nitrogen and greenhouse gas losses.

6.5. Conclusion

“Rotatinuous stocking” (RN) showed a faecal chemical quality greater than the traditional rotational stocking method (RT), resulting in a greater daily nitrogen excretion per animal. However, total faecal and nitrogen excretions per hectare were lower for the RN than the RT treatment. Therefore, the faecal CH_4 and N_2O emissions from sheep were also greater for the RN than the RT.

This study showed the importance of understanding the trade-offs in grazing systems. High levels of herbage and animal production (CHAPTER III) and, lower levels of CH_4 production from grazing sheep (RN treatment, CHAPTER V) had, as a consequence, a greater faecal CH_4 and N_2O emissions than RT treatment. However, the faecal CO_2eq emission per kg of herbage crude protein accumulation was equal for both treatments.

In general, greenhouse gas emissions (in CO_2eq) from sheep faeces represent a small part of the total gas emissions in the pastoral ecosystems, less than a 1% of the CH_4 emission (enteric fermentation transformed to CO_2eq). Therefore, we must take concern to find directions for the mitigation of enteric CH_4 production from sheep, in pastoral ecosystems.

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Table 1. Chemical composition (g kg DM^{-1}) of faeces excreted by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
Organic matter	785.2	764.5	0.512	7.55
Ash	214.8	235.4	0.291	7.55
Crude protein	186.3	157.7	<0.001	2.61

DM = dry matter; P = probability; MSE = mean standard error.

Table 2. Faecal dry matter and nitrogen excretions by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
g DM animal^{-1} day^{-1}	205.7	191.3	<0.001	3.91
kg DM ha^{-1} day^{-1}	5.28	8.51	<0.001	0.57
g N animal^{-1} day^{-1}	6.02	4.72	<0.001	0.11
g N ha^{-1} day^{-1}	154.6	207.3	<0.001	9.19

N = nitrogen; DM = dry matter; P = probability; MSE = mean standard error.

Table 3. Faecal gas emissions by sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT).

Variables	RN	RT	P	MSE
CH_4				
g animal^{-1} day^{-1}	0.21	0.07	<0.001	0.03
g ha^{-1} day^{-1}	5.61	3.05	<0.001	0.50
N_2O				
mg animal^{-1} day^{-1}	9.57	2.16	<0.001	1.41
g ha^{-1} day^{-1}	0.25	0.09	<0.001	0.03
Total (CO_2eq)				
g animal^{-1} day^{-1}	8.33	2.36	<0.001	1.13
g ha^{-1} day^{-1}	213.6	105.2	<0.001	20.8
g CO_2eq per kg HCPA	19.05	19.95	0.525	0.64

DM = dry matter; HCPA = herbage crude protein accumulation; P = probability; MSE = mean standard error.

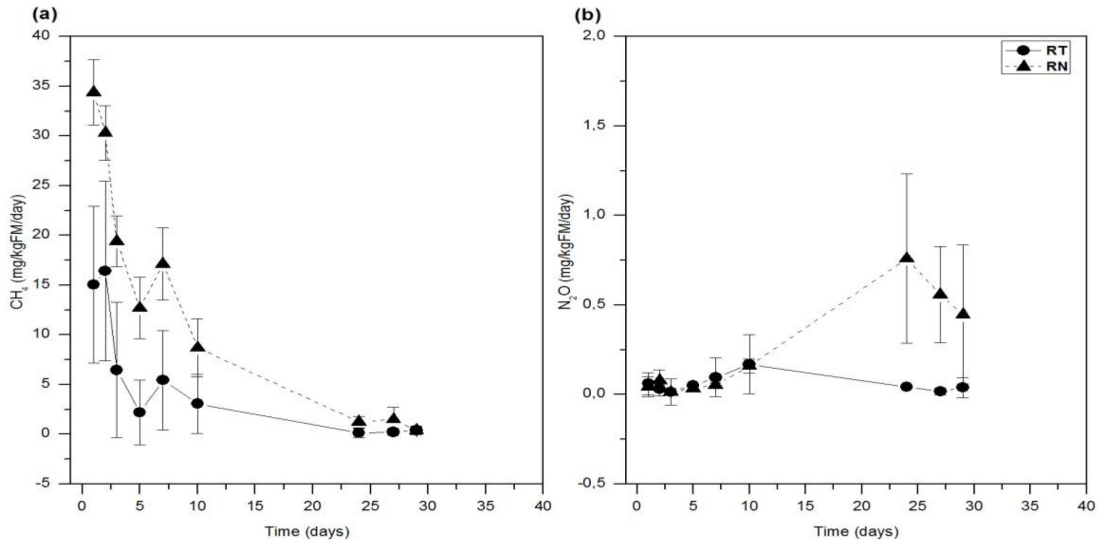

Figure 1. CH_4 (a) and N_2O (b) emissions from fresh faeces of sheep grazing Italian ryegrass pastures under different grazing management strategies (RN and RT). Standard errors are shown as vertical bars.

7. CHAPTER VII

7.1. Final considerations

According to Food and Agriculture Organization (2015), demand for ruminant meat is projected to increase globally at almost the same rate as milk demand (+90%). Consequently, it is necessary to produce large amounts of food with quality, and with low environmental impact. Therefore, it is important to know the processes involved in food production systems, and grazing systems in particular, which are the basis of ruminant production in the world. Thus, grazing management strategies have an essential role in this rationale in order to foster climate smart livestock/agriculture.

Smart livestock should be based on processes that reduce trade-offs between forage and animal production, because the balance of C and N coupling and decoupling is a consequence of that and directly linked to GHG emissions.

Thus, sound grazing management is the first step to increase animal productivity and reduce environmental impact. According to HERRERO et al. (2016), improving animal management, including increases in reproduction rates, feed availability and reduction of herd size, are alternatives to reducing greenhouse gas emissions.

Based on the necessity for the development of more adequate production systems, this thesis investigated a concept in grazing management based on animal behaviour (“rotatinuous stocking”). The “rotatinuous stocking” was the best grazing management strategy in practically all parameters assessed by this thesis. “Rotatinuous stocking” resulted in better herbage chemical composition, greater leaf/stem ratio, greater grazing time and bite rate, and consequently greater daily herbage intake by the sheep. A greater daily intake was a consequence, since the original concept of rotatinuous was based on greater herbage intake rate (FONSECA et al., 2012; MEZZALIRA et al., 2013; AMARAL et al., 2013; MEZZALIRA et al., 2014). In addition, the greater nutrient intake by the sheep on “rotatinuous stocking” resulted in greater individual live weight gain and per hectare. Moreover, the consequence of well managed swards (mainly by the high post-grazing residue in each grazing event) was the greater herbage production. Results prove that it is possible to have good scores of animal production and herbage production in the same stocking season and, the traditional trade-off between these variables may be overcome just with adjusted grazing management targets.

As the live weight gain was greater for the “rotatinuos stocking”, the final live weight, carcass and commercial cut weights from grazing sheep also were greater compared to the traditional rotational stocking method. This proves that the new grazing management was able to finish the animals before, which indicates a reduced turn-off time of animals on the farm.

Finally, the “rotatinuous stocking” had a greater faecal chemical composition, resulting in a greater daily nitrogen excretion per animal and greater faecal CH_4 and N_2O emissions. However, the “rotatinuous stocking” was the better management system for mitigation of CH_4 emissions, with 64% less CH_4 production per area and 170% less CH_4 per unit of animal product than the traditional rotational stocking method. Therefore, the more important point in pastoral ecosystems, with regard to finding directions for the mitigation of greenhouse gas, is to achieve strategic reductions of enteric CH_4 production from animals, as was proven by the “rotatinuous stocking”. This is because, in general, greenhouse gas emissions (in CO_2eq) from sheep faeces represent a small part of the total gas emissions in the pastoral ecosystems, less than a 1% of the CH_4 emission (enteric fermentation transformed to CO_2eq).

In summary, this thesis demonstrates that grazing management is the main strategy to improve the animal production and mitigation of the CH_4 emissions by ruminants in pastoral ecosystems, and grazing management based on animal behaviour, the so called “rotatinuous stocking”, may be used in different production systems and regions of the world since the rule “*take the best and leave the rest*” proposed by CARVALHO (2013) and the optimum pre-grazing sward heights targets are adopted.

In the future, there is a need to investigate the effect of this new grazing strategy in medium/long term and its implications for soil organic carbon, soil carbon sequestration and net balance of carbon fluxes of the system. We hypothesize that well managed swards, such as “rotatinuous stocking”, present greater soil organic carbon stocks than traditional rotational stocking method, mainly due to the higher herbage production and post-grazing residue after each grazing event.

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9. AUTHOR BIOGRAPHY

Jean Víctor Savian, son of Valdomiro Fermino Savian and Antoninha Angelina Ponte Savian was born in May 25th, 1988, in Santa Maria city, Rio Grande do Sul State, Brazil.

He started his Undergraduate Degree in Animal Science in 2006 at the Federal University of Santa Maria (UFSM, Palmeira das Missões Campus) and finished in 2010.

In March 2011, he started his Master of Science Degree in Animal Science at the Grazing Ecology Research Group (GPEP) - Federal University of Rio Grande do Sul (UFRGS), under supervision of Dr. Paulo César de Faccio Carvalho. In 2012, had sandwich period at the "*Universidad Nacional del Centro de la Provincia de Buenos Aires"* (UNICEN), Tandil city, Argentina, under supervision of Dr. Horacio Gonda. In March 2013, he completed his Master of Science Degree.

In April 2013, he started his PhD in Animal Science at the GPEP-UFRGS, under supervision of Dr. Paulo César de Faccio Carvalho. In 2016, had sandwich period at The University of Queensland, Gatton Campus, Australia, under supervision of Dr. Dennis Poppi and Dr. Marcelo Benvenutti. In February 2017, he was submitted to the PhD thesis defence jury at Federal University of Rio Grande do Sul, Porto Alegre city, Brazil.