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MURILO TRETTEL

Deferred grazing and supplementation with ammonium nitrate as a strategy for greenhouse gas emissions mitigation

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Corrected Version

Dissertation presented to Faculdade de Zootecnia e Engenharia de Alimentos of Universidade de São Paulo, as part of requirements for obtaining Master's Degree in Animal Science.

Area: Animal Quality and Production

Advisor: PhD. Paulo Henrique Mazza Rodrigues

Co-advisor: PhD. Patrícia Perondi Anchão Oliveira

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RESUMO

TRETTEL, MURILO. **Pastejo diferido e suplementação com nitrato de amônio como estratégia de mitigação de gases de efeito estufa**. 2022. 62f. Dissertação (Mestrado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

Este experimento avaliou os efeitos de práticas apropriadas de manejo de pastagens e suplementação proteica como estratégias para reduzir a emissão de gases de efeito estufa. Foram utilizadas 24 novilhas Nelore (372 kg de PV e ± 15 meses de idade), sendo 6 animais por tratamento. Em cada tratamento, quatro novilhas foram utilizadas para a medição da produção de metano (técnica do gás traçado SF_6) e todas para avaliação do desempenho. Os animais foram distribuídos aleatoriamente em 8 piquetes em delineamento de blocos ao acaso (localização espacial), durante o ano (total de três repetições). Os tratamentos foram compostos por dois sistemas de pastejo e dois suplementos nutricionais (fatorial 2x2): 1) pastagem diferida e suplementação com ureia (DfG + UR), 2) pastagem diferida e suplementação com nitrato de amônio (DfG + AN), 3) pastagem não diferida e suplementação com ureia (NDG + UR) e 4) pastagem não diferida e suplementação com nitrato de amônio (NDG + AN). Os suplementos foram isoproteicos e deixados à vontade para os animais. Foram realizadas análises de desempenho, ingestão de suplemento e emissão de GEE dos animais. Os animais apresentaram diferença de emissão metano/animal/dia entre as estações, devido ao crescimento dos mesmos. Com relação à emissão de metano/ganho de peso diário houve uma menor emissão para animais no sistema diferido no inverno. A emissão de metano/Energia Bruta ingerida e a emissão de metano/CMS apresentaram interação tripla, sendo que em ambos os casos a maior emissão foi para animais no rotacionado suplementados com ureia durante a primavera, enquanto a quantidade de energia bruta dispendida em emissão de metano (Ym%) foi maior em animais durante a primavera. Esse resultado mostra a viabilidade da intensificação dos sistemas de produção com redução das emissões de metano por carne produzida.

Palavras-chave: Bovino de corte. Manejo de Pastagem. Metano. Proteinado.

ABSTRACT

TRETTEL, MURILO. **Deferred grazing and supplementation with ammonium nitrate as strategy for greenhouse gas emissions mitigation**. 2022. 62 p. M.Sc. Dissertation – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2022.

This experiment evaluated the effects of appropriate pasture management practices and proteic supplementation as strategies to reduce the greenhouse gases emission. 24 Nellore heifers (372 kg in BW and ± 15 months old), 6 animals per treatment, were used. In each treatment, four heifers were used for methane production measurement (SF_6 tracer gas technique), and all of them for performance evaluation. The animals were randomly allotted to 8 paddocks in randomized block (terrain location) design during a year (total of three replicates). Treatments were composed by two grazing systems and two nutritional supplement (2x2 factorial): 1) deferred grazing and urea supplementation (DfG+UR), 2) deferred grazing and ammonium nitrate supplementation (DfG+AN), 3) non-deferred grazing and urea supplementation (NDG+UR), and 4) non-deferred grazing and ammonium nitrate supplementation (NDG+AN). The supplements were isoproteic and left at will to the animals. The analyzes were carried on animals performance, supplement intake and GHG emission. Animals showed a difference in methane/animal.day emission between seasons, due to their growth. Regarding the emission of methane/daily weight gain, there was a lower emission for animals in the deferred system in winter. The animals presented methane emission/Gross Energy intake and methane emission/DMI with triple interaction, and in both cases the highest emission was for animals in rotated supplemented with urea during spring, while the gross energy amount spent in methane emissions (Ym%) was higher in animals during spring. This results shows feasibility in intensification of production systems as a reduction in methane emissions per meat produced.

Keywords: Beef cattle. Methane. Pasture management. Proteic.

SUMMARY

FIGURES LIST

1. INTRODUCTION

Brazil had about 264.1 million cattle. It is the 2nd largest herd and the 2nd largest producer of beef in the world, reaching the mark of the largest beef exporter in the world (USDA, 2022).

With the growing demand for food, due to population increase projected by the United Nations (UN, 2017), Brazil for being one of the largest producers is considered as one of the countries that will be able to supply this need. Demand for food increase will also apply to animal origin products and consequently a possible GHG production increase by this source, bringing the need for strategies so that this production is more effective and productive.

The present average stocking rate in Brazil, of less than 1.0 animal per ha, represents a potential loss of more than US$ 10 billion in beef value, considering a potential stoking rate of 2.0 animals per ha, which could be easily obtained with improvements in pasture management, assuming that the national or international market could absorb such large quantities of beef at present prices (OLIVEIRA, 2015).

One important characteristic of brazilian livestock production systems is that grasslands constitute the main food resource used for ruminant production (BEZABIH et al., 2014). The variable growth of the tropical grasses throughout the year widely fluctuate in their quality and quantity of forage mass produced. During the rainy season, these plants grow rapidly; in the dry season, they grow more slowly.

Despite being an alternative to guarantee the availability of forage during the winter, the low nutritional value of deferred pastures creates the need for supplementation to achieve acceptable levels of animal production. According to Moore and Moser (1995), the advancement of plant’s age is to be the main determinant for the nutritional value of the produced forage. Supplementation is a common practice during the use of deferred grazing.

In addition to correcting degraded pastures, other strategies are evaluated as reducing greenhouse gas emissions. Nitrate´s inclusion in ruminant diet has been shown an effective methane mitigation strategy (HRISTOV, 2013) and its use has been reviewed (MARTIN et al., 2010). The extent to which nitrates can be included in the diet has been widely evaluated, because its use is limited by adverse factors such as nitrate and nitrite toxicity, but little attention is given to their effect as a methane mitigator (RICHARDSON et al., 2019).

Agriculture is the sector that emits the most GHG, with 33% of the total, and the methane enteric fermentation subsector, released by ruminants (cattle, buffalo, goats and sheep), represented 19% of the total (MCTI, 2022). According to Berchielli et al. (2012), the Brazilian bovine herd is responsible for approximately 3.3% of the methane produced worldwide by human activities, 11.3% of the enteric methane produced in the world.

However, not all aspects of environmental issues in the Brazilian cattle industry are negative. Comparing the performance of the world's 10 largest beef exporters between 1988 and 2007, Brazil had the greatest growth rates in annual methane emission estimates (2.12%/year); however, the Brazilian beef cattle industry also had the greatest growth rate of beef production (4.01%/year), which resulted in the largest negative value (−1.82%/year) for the net increase in the rate of methane emissions per unit of product (kilograms of methane/kilogram of beef) when compared to other countries.

Methane emissions are expressed in terms of either production per animal unit or production per gross energy consumed by the animal. Decreasing methane per unit of product, like meat or milk, is part of a strategy to reduce the negative impact of livestock production on global warming (KNAPP et al., 2014; OLIVEIRA et al., 2020). In fact, when GHG emissions are expressed per unit of animal product, this enhances data accuracy.

Considering all these points, the recuperation and intensification of pastures through adequate management, fertilization and adequate animal supplementation may positively impact the sustainability of livestock production systems. Since there could be an improvement in animal performance and also an increase in gains per area, in addition to the decrease in the greenhouse gases emission.

2. HYPOTHESIS and OBJECTIVES

The *hypothesis* was that differed grazing and ammonium nitrate supplementation can stimulate animal performance, decreasing GHG emissions when expressed per unit of animal product.

The *objective* of this experiment was to investigate the effects of adequate practices of grazing management and proteic supplementation on animal (performance and GHG emission).

3. LITERATURE REVIEW

3.1 Methane Emission

According to IPCC (2019), total global GHG emission by anthropic action, calculated between 2007-2016, was 52 ± 4.5 Gt CO_2-eq/year, with GHG emission coming from fossil fuels burning, industrial processes and other non-agricultural sources responsible for approximately 77% of total emitted, while agriculture, livestock, forestry, and other land uses account for 23% of GHG emission by anthropic action.

These values take into account carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), already using global warming potential (GWP) calculation, having CO_2 equivalent (CO_2 eq) as a unit.

In the global agricultural sector, the most impacting GHGs are CH_4 and N_2O, with enteric fermentation by ruminants being the largest emitter (57.5%), followed by agriculture (35%) and other activities (7.5%) (MCTI, 2016).

Methane production in rumen occurs through anaerobic fermentation, where a CO_2 compound works as an electron acceptor and hydrogen drain (KOZLOSKI, 2011). This process of removing hydrogen in the rumen occurs mainly in the absence of nitrates, sulfates, oxidized metals and, especially, oxygen (PERNA Jr, 2018). In a meta-analysis study, Ungerfeld (2015) observed that H_2 accumulation in rumen slows down rumen microbiota growth and, consequently, plant materials degradation, hindering the rumen fermentation process.

Use of different methane mitigation strategies has been studied by several groups around the world. Among these strategies are: adequate pastures management, use of grains and concentrated feed, fodder processing and conservation, legumes, tannins and saponins use, saturated and unsaturated fats and oils, ionophores, nitrate, yeasts, malate and fumarate, essential oils and plant extracts (BERNDT, 2010).

Improve the digestibility of provided diet for animals on pasture, either by using proteic-energetic supplementation or providing better quality pastures, are alternatives that mitigate CH_4. In this way, there is an increase in microbial growth, increasing efficiency of rumen fermentation process with a decrease in methanogenesis per unit of degraded carbohydrate (ZOTTI and PAULINO, 2009).

The higher dry matter intake leads to higher methane emissions (KURIHARA, et al. 1999). O´Hara (2003) showed that as cows increase consumption, milk production also increases, making methane emission by milk produced lower in more productive animals. Likewise, when milk production is equalized, animals that are subjected to a better quality diet, eat less and thus also emit less methane per unit of milk produced.

3.2 Greenhouse gases in soil

Animal production, including ruminants and non-ruminants, accounts for more than 65% of total nitrous oxide emissions (FAO, 2015). Nitrogen deposition in the soil promotes an increase in N_2O emission (PIVA et al., 2014), which, in cattle production, comes mainly from animal waste addition in the soil, but it can also come from nitrogen fertilizers, soil leaching, burning of agricultural residues and organic matter mineralization in soil (BRUNES and COUTO, 2017).

Agricultural systems with low zootechnical indexes and productivity, generally caused by degraded pastures, result in higher greenhouse gas emissions per unit of production, considering meat or milk (IPCC, 2006). On the other hand, in grazing ruminants production, with well-managed grasses, animals develop a better productive efficiency. In this case, system function as a GHG mitigation agent instead a polluter (WANG, 2015).

Cardoso (2012) affirms that there is carbon sequestration in pastures kept in good conditions, capturing and storing CO_2 from the atmosphere, which as a consequence contains and reverses the warming resulting from the greenhouse effect.

Thus, when evaluating the Brazilian production chain, it is possible to conclude that potential for methane mitigation and nitrous oxide, using recovery strategies for degraded pasture areas and intensive and correct management of forages, can be much greater (PBMC, 2012).

3.3 Deferred grazing

Forage conservation is a process that aims save food for future use (ALLEN, 2011), which can be done through ensiling, hay and haylage making as well as deferred grazing. The authors define deferred grazing as a process to provide forage for later use. Similarly, deferred grazing has often been defined as the discontinuation of the use of a pasture at the end of the growing season for a specific period to allow accumulation of forage that can be used during periods of shortage (EUCLIDES, 2007).

In comparison with other forms of conservation, defered grazing is very important due to the fact that it is one of the most easily adopted and least-cost strategies (SANTOS et al., 2009).

During dry seasons of the year, grasses may have crude protein contents below 7%, considered as the minimum required for fibrolytic activity of ruminal microbiota, resulting in decreased voluntary consumption and diet digestibility. These factors reduce animal performance and contribute to increasing enteric methane emissions (ARCHIMÈDE et al., 2011). In this way, deferred grazing is an interesting strategy to circumvent the Brazilian climate seasonality and thus improve the beef cattle productivity, reinforcing that, in fact, the Brazilian herd low productivity is a forage scarcity reflection resulting from the management lack (NUSSIO and SHIMDT, 2010).

Forage nutritional quality, during dry period, is lessened due to the plant's increase in structural carbohydrates, and there is a reduction in their crude protein content. In addition to reducing the animal's performance, these factors also contribute to increased enteric methane emission, either expressed in its relation to the consumed forage or in relation to the final product generated (ARCHIMÈDE et al., 2011).

In deferred pastures, gains ranging from 490 to 725 grams were reported by Silva et al. (2016), while Garcia et al. (2014) found 840 grams daily gains. In both cases performance was avaliated by 90 days. All of these gains were with supplementation use. Counter point, Silva et al. (2009), in a literature review, recorded cattle weight losses of up to 300 grams daily in not deferred pastures during the dry period.

3.4 Ammonium Nitrate

In Brazil, ammonium nitrate is commomly commercialized as fertilizer. Besides to being a useful non-protein nitrogen (NPN) source for ruminants, as urea, nitrate supplementation has a capacity to mitigate CH_4 in ruminants (VAN ZIJDERVELD et al., 2011; LEE et al., 2014; FENG et al., 2020), wich have been increase the specialist attention. Nitrate (NO3-) work as an electron receptor (H+) in the rumen (LENG and PRESTON, 2010) reducing methane production as it competes with these microorganisms for hydrogen. Some microbes can reduce nitrate to nitrite and further to ammonia (LEWIS, 1951; IWAMOTO et al., 2002), confirming enteric methane mitigation by nitrate salts in rumen (NOLAN et al., 2010; VAN ZIJDERVELD et al., 2011; HULSHOF et al., 2012; LI et al., 2012; VILLAR et al. 2020).

Newbold et. al. (2014) and Velazco et. al. (2014) affirm that this occurs because, when transformed, the nitrate competes with the CH_4 producing route, consuming H_2 electrons and reducing methanogenic microorganisms, due to electrons availability lower than nitrite toxicity level for these microorganisms.

Villar et al. (2020) observed that NO_3^- was an effective additive to reduce CH_4 emissions, and a source of additional nitrogen (N) for microbial protein synthesis via N-recycling into saliva and the gut.

El Zaiat et al. (2014) found a 50% reduction in methane emissions in lambs submitted to an encapsulated nitrate diet against a control using urea, without changing dry matter consumption or daily weight gain.

According to Lee and Beauchemin (2014), there are many studies with nitrates addition in ruminants diet, but very few with animals on pasture. The possibility of reducing methane emissions add to the fact that ammonium nitrate, like urea, is also an NNP source for ruminants. This is a great importance point since the base of beef cattle breeding in Brazil is carried out in a grazing system, which is susceptible to seasonality.

Despite the beneficial effects, nitrate has a potential risk of toxicity to animals when the reduction of nitrite exceeds the conversion of nitrite to ammonia. Thus, nitrite is absorbed by the rumen wall and reaches the bloodstream, which may lead to the oxidation of hemoglobin to methemoglobin preventing oxygen transport leading the animal to death (MCALLISTER et al., 1996; LEE and BEAUCHEMIN, 2014).

Therefore, a gradual adaptation period is fundamental to reducing the risk of toxicity (LIN et al., 2013). Besides that, most of the studies are using encapsulated nitrate as a slow-release form of NO_3^- to minimize potential toxicity (ALEMU et al., 2019). In Brazil, there is no commercial form for nitrate animal feed, nitrate is commercialized as fertilizer.

Methane reduction can increase the energy available for production because this gas production is a source of energy loss for the ruminant (FERNANDES, 2015). Furthermore, it is speculated that nitrate may also directly inhibit some methane producing microorganisms growth (GRANJA-SALCEDO et al., 2019; ZHAO et al., 2015).

The main polluting gas emitted during the fermentation processes of ruminants, methane, is released by microorganisms of the Archea group through the use of CO_2 and H^+.

Nitrate ingested in large quantities by non-adapted animals can generate a rumen nitrite accumulation, that when absorbed oxidizes hemoglobin to the ferric form, hindering its oxygen transport capacity (COCKBURN et al., 2013). However, an animal adapted through the gradual nitrate inclusion, increase its activities of reducing rumen nitrate and nitrite, minimizing the possibility of intoxication (LIN et al., 2013).

4. MATERIALS and METHODS

The experiment was carried out at College of Veterinary Medicine and Animal Science (FMVZ/USP), Pirassununga, Sao Paulo State, Brazil (Figure 1), for one consecutive year. In order to cover all seasons of the year, it began in June 2019 (winter) and ended in April 2020 (summer). The experimental animals were handled and managed according to the Ethics Committee on Animal Use on Research (FZEA/USP) under protocol number 1488090919. A total of 24 Nellore heifers, of approximately 372 kg BW and 15-16 months old, used as experimental animals. It was used six animals in each treatment, four of them were used for methane production measurement (SF_6 tracer technique), and all of them for performance evaluation.

Figure 1. Experimental area

Source: FZEA/USP

4.1 Experimental design, pasture system, and treatments

The 24 experimental animals were randomly allotted to 8 modules and allotted to a randomized block design (blocks were formed as a function of terrain location) during one year (total of four replicates). Treatments were composed by two grazing systems (Figure 2) and two nutritional supplements (2x2 factorial): 1) deferred grazing and urea supplementation (DfG+UR), 2) deferred grazing and ammonium nitrate supplementation (DfG+AN), 3) non-deferred grazing and urea supplementation (NDG+UR), and 4) non-deferred grazing and ammonium nitrate supplementation (NDG+AN). Urea supplementation was a convencional mineral protein supplement with urea as a non-protein nitrogen source, while the other treatment had ammonium nitrate as a non-protein nitrogen source.

Figure 2. Rotated grazing system

Source: own autorship

The different groups of animals were left to continuously graze and had free access to clean and freshwater in each experimental unit. The nutritional supplementation (urea or ammonium nitrate) was available ad libitum to all animals. The conventional supplement was composed of corn grain, soybean meal, urea, common salt and mineral premix, while the nitrate supplement was composed of corn grain, soybean meal, ammonium nitrate, common salt and mineral premix (Table 1). The supplements were isoproteic and were left at will to the animals. Before starting treatment, an adaptation of 5 days was made. Both supplements were formulated for about 0.1% of BW intake.

Table 1. Formulations and Nutritional composition

Ingredients		Adaptation		Winter		Summer/Spring/Autumn	
		Urea	Nitrate	Urea	Nitrate	Urea	Nitrate
		(%)					
Ground Corn		55	55	48	45	72	69
Urea		10	-	22	-	13	-
Sodium Chloride		20	15	15	10	7	5
Mineral Mix[1]		15	15	15	15	8	8
Ammonium nitrate		-	15	-	30	-	18
Nutritional composition (DM)							
CP	%	33.14	33.49	66.34	61.13	43.01	43.34
TDN	%	48.22	48.22	42.02	39.46	63.13	60.50
CF	%	1.27	1.27	1.10	1.04	1.66	1.59
EE	%	1.60	1.60	1.39	1.31	2.09	2.00
NDF	%	4.35	4.35	3.79	3.56	5.69	5.45
ADF	%	1.43	1.43	1.25	1.17	1.87	1.79
Ca	%	2.70	2.70	2.69	2.69	1.45	1.45
P	%	2.54	2.54	2.52	2.52	1.47	1.46
Na	%	7.81	5.86	5.86	3.91	2.74	1.96

CP: Crude Protein; TDN: Total Digestive Nutrients; NPN: Non-Protein Nitrogen; CF: Crude Fiber; EE: Ether Extract; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber;
[1]Mineral mixture, quantity per kg of product: 200 g of calcium, 160 g of phosphorus, 60 g of sulfur, 185 g of sodium, 200 mg of cobalt, 2.5 g of copper, 1.6 g of fluorine, 125 mg of iodine, 2.25 g of manganese, 50 mg of selenium, 7.5 g of zinc.
Source: own autorship

The experimental area had 14.4 ha divided into eight experimental units as well as management corridors, four of these areas was subdivided into six paddocks with 0.3 ha each one (non-deferred areas) and other four modules (deferred grazing areas) was with 1.8 ha each one. The area was established in 1999 with *Urochloa brizantha* cv. Marandu. Additionally, 13.1 ha was being used for the allocation of extra animals to be used to adjust the stocking rate, while necessary.

4.2 Characterization of pasture productive components

Pasture height was evaluated in March (beginning of the dry season) and were limed with 1900 kg dolomitic limestone per hectare and fertilized with 57 kg per hectare with sulfur, using sulfate ammonium. Pasture height in all paddocks, except for the deferred treatments, was systematically decreased using natural grazing with animals. Pasture height was decreased to 20 cm in non-deferred treatments. Soon after mowing, paddocks (except for deferred pastures) was fertilized with 250 kg of NPK 20-0-20 per hectare, which is equivalent to 50 kg of nitrogen per hectare, this amount was used twice a year. Fertilization was performed using a centrifugal distributor with disc, and gravimetric dosing coupled to the tractor (Figure 3).

Figure 3. Fertilization

Source: own autorship

The deffered grazing treatment consisted of four similar modules with 1.8 ha area (area replication) and was managed under continuous grazing.The non-deferred grazing treatment consisted of four similar modules of 1.8 ha area (area replication), with 6 paddocks of 0.3 ha each, intermittently grazed, with 7 day of occupation and 35 days of resting.

All pastures were submitted to stocking rate adjustments using the "put and take" technique (Mott & Lucas, 1952) and evaluation of forage availability. The “put and take” technique consists in adjust the stocking rate periodically to compensate for changes in the forage supply, and the aim is to keep the grazing pressure as close to the carrying capacity as possible throughout the trial.

For forage mass production determination in each paddock, forage samples were collected from each experimental unit weekly. A metal frame of 0.25 m^2 was randomly thrown and all the forage contained in its interior was harvested at ground level. In order to compose a sample, this procedure was repeated 4 times in each paddock, totaling 1 m^2. All harvested forage was weighed in a precision scale and conditioned in paper bag, weighted and dried in forced air circulation at 65°C during 72 hours for determination of dry matter content and subsequent determination of forage mass at the paddock.

4.3 Animal performance

The individual performance (daily live weight gain) of animals was obtained by dividing the difference in weight between two successive weighings by the interval of days between measurements. Animals were weighed at the beginning of the experiment and subsequently at regular intervals of 28 days. An initial fasting weighing was performed and another occurred at the end of experimental period. However, due to structural limitations, it was decided to perform intermediate weighing (every 28 days) without fasting.

4.4 Dry matter intake

An indirect method with markers was used for consumption estimates. Titanium dioxide (TiO_2) was used as an external marker and iNDF as an internal marker. Titanium capsules were supplied orally to animals within the containment corral but without the need for an applicator (Figure 4). The external marker was administered for 9 days in the amount of 15 g per animal per day. During the last 5 days of the administration, feces samples were collected in containment corral. The material was frozen at -20ºC in plastic bags properly identified for further analysis of titanium and iNDF content using a technique described by Myers et al. (2004).

Figure 4. Titanium dioxide administration

Source: own autorship

To determine forage dry matter intake, a known external marker amount was administered (15 g/day) and then, through fecal collection, the marker concentration in feces was determined, calculating the fecal excretion of the following form:

Fecal excretion = TiO_2 diet (kg/day)/ TiO_2 feces(kg)

TiO_2 diet: Titanium oxide administered; TiO_2 feces: Titanium oxide present in feces (kg)

Having the fecal excretion data (Figure 5), forage dry matter intake was calculed by the iNDF from pastures and feces as internal marker (%), was used the following equation:

Forage DMI (kg.day) = [(Fecal excretion)*(% iNDF on feces)]/(% iNDF on forage).

To the determination of supplement dry matter intake, Chromium Oxide was used as an external marker, and it was used the following equation to attain supplement DMI (kg.day):

Supplement DMI (kg.day) = (Fecal excretion*Cr_2O_3 on feces)/Cr2O3 on supplement

The apparent digestibility coefficients (ADC) were calculated based on the TiO_2 content of the diet and feces using the following equations:

$$ADC_{DM} = 100 - (100 \times \frac{\mathrm{TiO2\ (\%)\ in\ diet}}{\mathrm{TiO2\ (\%) in\ feces}})$$

$$ADC_{N} = 100 - 100 \times \frac{(\%\mathrm{TiO2d})}{(\%\mathrm{TiO2f})} \times \frac{(\%\ \mathrm{Nf})}{(\%\mathrm{Nd})}$$

Where: ADC_{DM} = DM apparent digestibility coefficient; ADCN = Nutrient apparent digestibility coefficient; % TiO_2d = Titanium dioxide content in diet; % TiO_2f = Titanium dioxide content in feces; % Nd = Nutrient content in the diet; % Nf = Nutrient content in feces.

Figure 5. Feces collection

Source: Own autorship

Feed and feces dry matter content were determined by drying in a forced air oven at 65ºC for 72 hours according to AOAC (1995). After drying, samples were milled in willy-type knives mill of 1 mm sieves and stored. All analyzes were corrected for analytical dry matter content determined at 105°C overnight. Mineral material was obtained by calcination in a muffle furnace at 550ºC for 4 hours, and the organic matter (OM) calculated as the difference between 100 and mineral material (AOAC, 1990). Crude protein was determined by the total N content (N x 6.25) using the micro-Kjeldahl technique (method 920.87; AOAC, 1990). Ether extract was determined with the ANKOM XT15 Extractor® equipment (method Am 5- 04; AOCS, 2005). NDF and ADF were determined by the method described by Van Soest et al. (1991). Non-fibrous carbohydrate (NFC) content was obtained by subtracting the amounts expressed in percentage of DM of CP, EE, MM and NDF from 100.

Supplement intake was also measured in two different ways. During the collection period, a supplement was provided with chromium oxide addition, which through feces analysis it was obtained the supplement individual consumption. In addition, the average batch consumption was measured by the difference between provided and leftovers.

4.5 Enteric methane measurements

Methane was collected once a season, totaling 4 times a year. Sulfur hexafluoride (SF_6) tracer gas was used as the method for measuring eructated CH_4 (JOHNSON et al., 1994; adapted to Brazil by PRIMAVESI et al., 2004, improved by BERNDT et al., 2014 and JOKER et al, 2020). During five consecutive days each animal was sampled daily. Animals were fitted with gas collection halters 14 d before methane sampling to allow animal acclimation in order to facilitate sampling. A small brass permeation tube, with a known SF_6 permeation rate, was placed in the reticulum 72 h before sample collection to allow the tracer gas to equilibrate in the rumen. Eructed gas samples were continuously obtained through a capillary tube connected to a collection canister placed on the neck of the animal (Figure 6). A halter with 0.127-mm stainless-steel capillary tubing and an in-line 15-μm filter was placed on the animal’s head and connected to an evacuated sampling canister. Before the experiment, collection PVC pipe canisters was attached to a vacuum pump in the laboratory to create a negative pressure (around the −13.15 psi).

The negative pressure, generated by the vacuum in the sampling vessel, constantly and slowly draws the air sample around the animal's mouth and nose.

Figure 6. Cappilary Tube

Source: Own autorship

Additional canisters were placed near the experimental pastures to monitor daily background concentration of CH_4 and SF_6 during each sampling period. Sampling started daily at 07:30 h when animals were removed from the paddocks and moved to working corral to facility for sampling. After sample collection and pressuring the canisters with nitrogen, CH_4 and SF_6 in the canisters was measured, by using gas chromatography (Agilent HP-6890, Delaware, USA; and Shimadzu GC-2014, Columbia, MD, USA). The CH_4 flux was calculated according to Westberg et al. (1998), using the following equation:

$$QCH_4 = QSF_6[(CH_4)y–(CH_4)b]/[(SF_6)y-(SF_6)b]$$

Where: QCH_4 = CH_4 emission rate by animal; QSF_6=known SF_6 emission rate from the capsule in the rumen; $(CH_4)y$ = CH_4 concentrations in the collection apparatus; $(CH_4)b$ = basal CH_4 concentration; $(SF_6)y$ = SF_6 concentration in the collection apparatus and $(SF_6)b$ = basal SF_6 concentration in the surrounding air.

Methane emission (g per animal per day and kg per animal per year), grams of methane per kilogram of DM intake, and gram of methane per BW, or per kg of weight gain, was calculated. The gross energy percentage intake spent in methane emission (Ym%) was calculated by dividing the daily methane output of each animal by gross energy daily intake during the methane sampling.

4.6 Soil greenhouse gas flux measurements

For soil N_2O flux determination, samples was collected in the pasture systems and in the nearest natural vegetation areas weekly. It was used PVC chambers installed in the experimental plots, according to the chamber technique described by Davidson & Schimel (1995) and Allen et al. (2010). The chambers for gas sampling followed the design described by Varner et al. (2002). They consist of a round PVC base (30 cm diameter, 20 cm height, 0.014 m^3), with a 10 cm deep lid containing a small valve to prevent overheating and subsequent increase in the chamber’s internal pressure. The chambers were installed by inserting the base into the soil to a depth of 3 cm. The lid of the chamber made also with PVC was fitted with a three-way valve for gas sampling, with a headspace height from 18 to 25 cm on average. Gas samples was taken using a 20 mL BD plastic syringe (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and transferred to evacuated 12 mL LABCO vials. The first sample was collected soon after the chamber is closed and the remaining samples after 20 and 40 minutes.

Gas samples was analyzed at Embrapa Environment, Jaguariúna, SP, Brazil, using a Shimadzu GC-2014 gas chromatograph (Shimadzu Co., Columbia, MD, USA). The chromatograph was equipped with a packed Porapak Q column, an electron capture detector to analyze N_2O and a flame ionization detector to quantify CO_2 and CH_4. Prior to detection, CO_2 was reduced to CH_4 using a methanizer. Soil N_2O fluxes was calculated according to Jantalia et al. (2008):

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{V_m}$$

Where: $\Delta C/\Delta t$ is the change in GHG concentration inside the chamber during the period (Δt) that the chamber is closed; V and A are, respectively, the volume of the chamber and the area of soil covered by the chamber; m is the molecular weight of each GHG (CO_2, CH_4, and N_2O) and Vm is the molar volume. The emission rate was computed using a linear regression based on the curve generated from the gas values measured along the 30 minutes intervals. Annual cumulative estimates of GHG was calculated by linear interpolation between sampling events (ALLEN et al., 2010).

4.7 Statistical analyses

The grazing units (some with a different number of paddocks) were considered the experimental units for data obtained per area and the heifers were the experimental units for data obtained per animal. Data was statistically analyzed using the SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Before the actual analysis, the data was analyzed for the presence of disparate information ("outliers") and the normality of residuals (Shapiro-Wilk). When the normality assumption was not accepted, the logarithmic or the square root transformation was tested. Data was analyzed according to the mixed procedure (PROC MIXED), and the season was considered as a repeated variable (split-plot in time). Among the 15 different covariance structures tested, the chosen one was based on the lower value of the corrected Akaike Information Criterion (AICC) (WANG and GOONEWARDENE, 2004).

The model included the effects of treatment (grazing systems and supplementation), season and the interaction between treatments and season as fixed factors. The block effects (replicate area) was considered as random factors. In the presence of treatment*season interaction, effects of one factor inside the other was evaluated using the SLICE command of Mixed Procedure. All means was presented as least-squares means and the treatment effects was separated by LDS test, using the PDIFF option of SAS. It was considered as statistically significant when $P \leq 0.05$.

5. RESULTS

5.1 Animal performance

Influence of grazing systems, nitrogen sources and seasons in animal performance results are presented in Table 2.

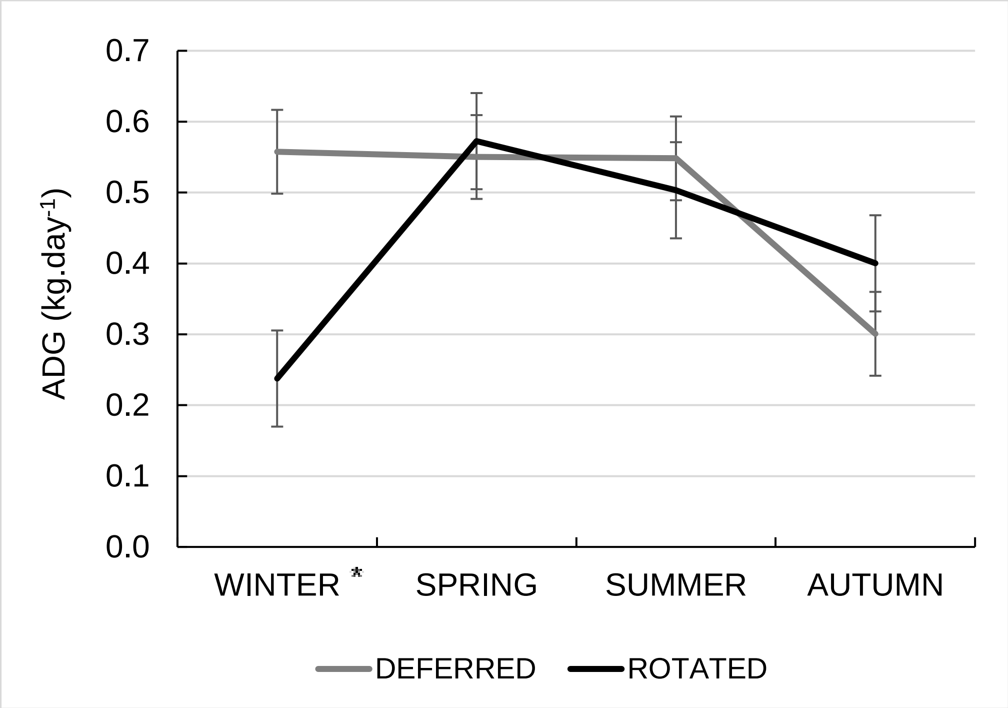
Table 2. Effects of grazing systems and nitrogen source in animals performance data during the year: average daily gain (ADG), initial body weight (IBW) and final body weight (FBW).

Fixed effects			***Item****		
			Mainly Effects		
Grazing	**N_source**	**Season**	**ADG (kg)**	**IBW**	**FBW**
Deferred			0.4773	368.5000	536.3800
Rotated			0.4520	375.6200	527.6300
	Nitrate		0.4314	372.0000	523.6300
	Urea		0.4979	372.1200	540.3800
		Winter	0.4034		
		Spring	0.5672		
		Summer	0.5316		
		Autumn	0.3563		
Average Data					
Average			0.4646	372.0600	532.0000
SEM			0.0218	7.5000	9.2450
Statistics Probabilities					
Grazing			0.6492	0.6753	0.6684
N_source			0.3086	0.9942	0.4173
Season			<.0001		
Grazing*N_source			0.1950	0.7291	0.8444
Grazing*Season			0.0282		
N_source*Season			0.2765		
Grazing*Season*N_source			0.6802		

IBW = initial body weight; FBW= Final body weight; ADG= Average daily gain

Heifers initial body weight (IBW), as expected, did not show (P > 0.05) any significant difference (Table 2). Animals final body weight (FBW) also did not show (P > 0.05) any significant difference.The average daily gain (ADG) was affected by season*grazing system interation (P=0.0282). Difference between rotated and deferred only occurred in winter, where ADG was better in deferred grazing system. Deferred grazing had a lower ADG in autumn, while in rotated grazing, winter had the lowest ADG and spring had the highest ADG (Figure 7).

Figure 7. Heifers average daily gain in differents production systems ($kg.day^{-1}$) by season. Seasions marked with an asterisk had statistical difference ($P < 0.05$).



Source: Own autorship

5.2 Animal Intake

Effects of treatments on forage and supplement dry matter intake results are presented in Table 3.

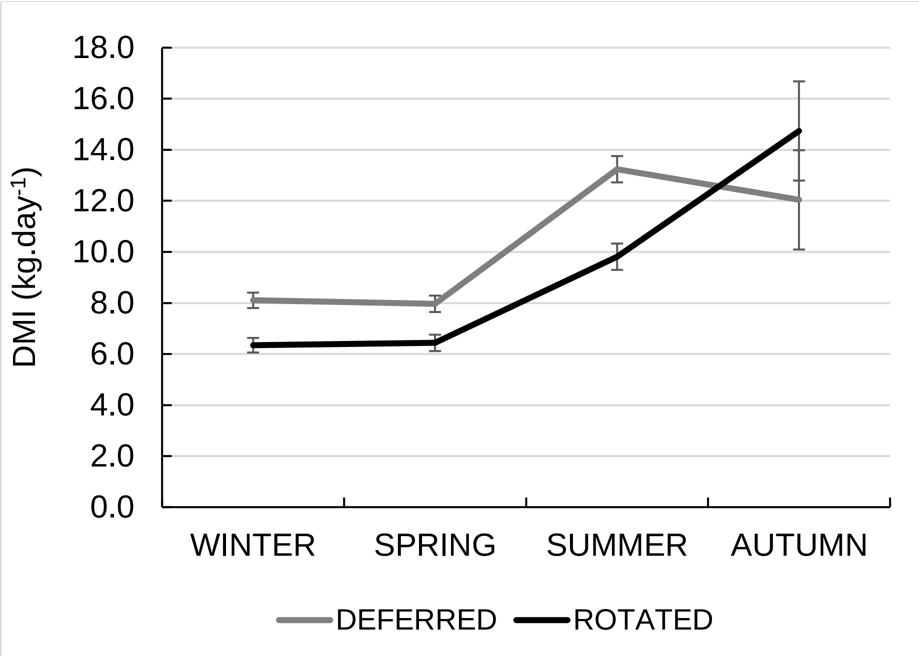
Table 3. Effects of grazing systems and nitrogen source in animals intake during the year: Total dry matter intake in kilograms per day, body weight perentage and g/kg Metabolic Weight , forage intake (kg) and supplement intake (kg).

Fixed effects			***Item****				
			Mainly Effects				
Grazing	**N_source**	**Season**	**Supplement Intake (kg)**	**Forage Intake (kg)**	**Total DMI kg**	**Total DMI %BW**	**Total DMI g/kg MW**
Deferred			0.8410	9.4582	10.2992	2.2401	103.5031
Rotated			0.4716	8.5094	8.9430	1.9418	89.7402
	Nitrate		0.5536	8.8408	9.3460	2.0607	94.8849
	Urea		0.7693	9.1269	9.8962	2.1213	98.3584
		Winter	0.2826	6.6509	6.6367	1.8771	82.2480
		Spring	0.6508	6.1068	6.6367	1.5785	71.4267
		Summer	0.8365	10.6890	11.5255	2.3910	111.9781
		Autumn	0.9001	12.4886	13.3888	2.5173	120.8331
Average Data							
Average			0.67	8.98	9.6211	2.09	96.62
SEM			0.07	0.49	0.5197	0.09	4.28
Statistics Probabilities							
Grazing			<.0001	0.6499	0.2397	0.0154	0.0287
N_source			0.0002	0.7302	0.4522	0.4919	0.4859
Season			<.0001	<.0001	<.0001	<.0001	<.0001
Grazing*N_source			0.4023	0.4659	0.6453	0.8732	0.8385
Grazing*Season			0.0005	0.0316	0.0020	0.0009	0.0012
N_source*Season			<.0001	<.0001	0.3451	0.4027	0.4358
Grazing*Season*N_source			0.0034	<.0001	0.1345	0.0162	0.0147

DMI = dry matter intake; %BW = percent of body weight; MW = metabolic weight

Season*grazing system interation affected dry matter intake (DMI) in kilograms too, showing that DMI was higher in deferred grazing animals than in rotated grazing animals during winter, spring and summer (P=0.0020). The only exception was in autumn, where there was no statistical difference (Figure 8).

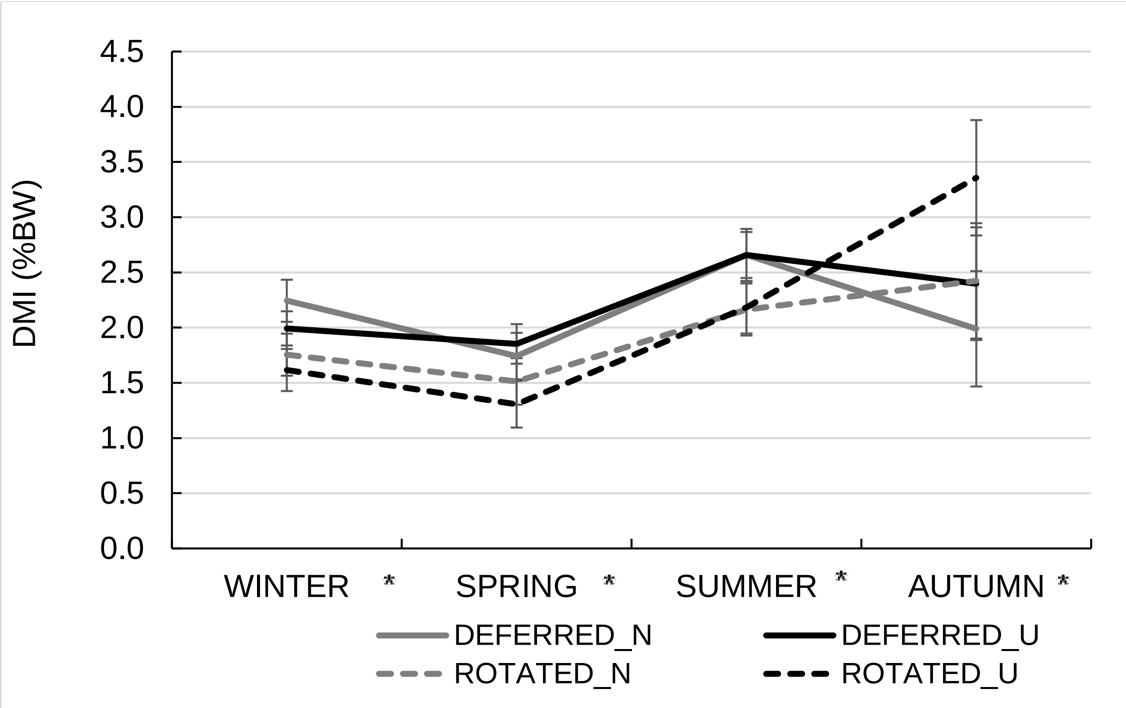
Figure 8. Heifers dry matter intake (kg.day^{-1}) by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Dry matter intake in body weight percentage was also affected by triple interation of seasion*grazing systems*Nitrogen source (P<0.0162), when consumption was higher in animals in deferred system and supplemented with ammonium nitrate in winter, spring and summer (Figure 9 and Table 4).

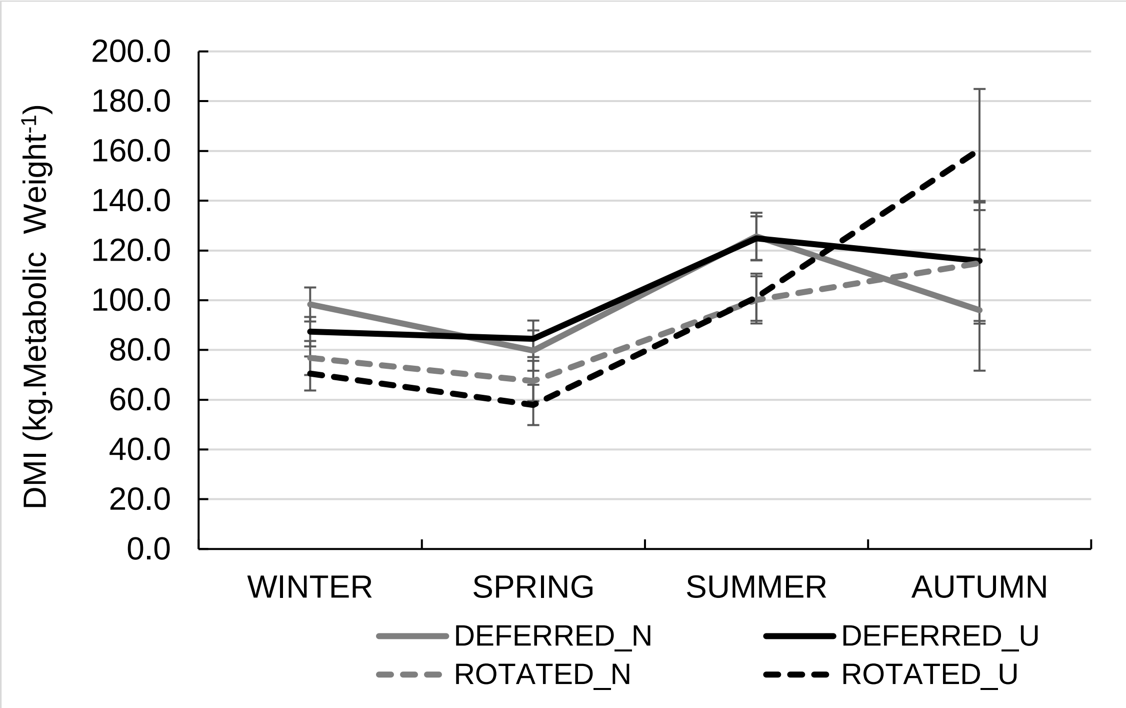
Figure 9. Heifers dry matter intake in body weight percentage (kg.day^{-1}) by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

As it is proportionally equal, even with different values, dry matter intake per Metabolic Weight follows the same statistical pattern as dry matter intake per %BW. In this way, there was also a triple interaction of seasion*grazing systems*Nitrogen source (P < 0.0147), with consumption was lower in animals in rotated system and supplemented with urea than animals in both deferred grazing system in winter, spring and summer (Figure 10 and Table 4).

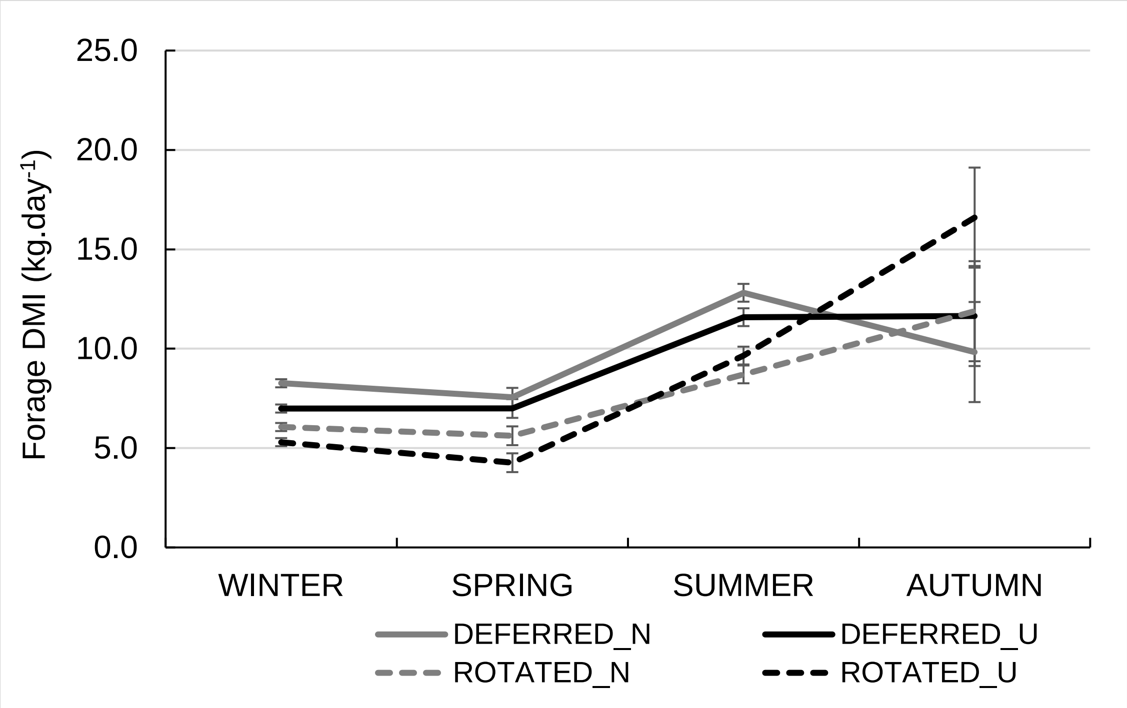
Figure 10. Heifers dry matter intake (kg.PW^{-1}) by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Forage dry matter intake also was affected by triple interation of seasion*grazing systems*Nitrogen source (P < 0.0001), demonstrating that animals in defered grazing and supplemented with ammonium nitrate had a higher forage dry matter intake during the winter and summer than the other treatments. During the winter and spring animals in rotated grazing supplemented with urea had the lowest intake (Figure 11 and Table 4).

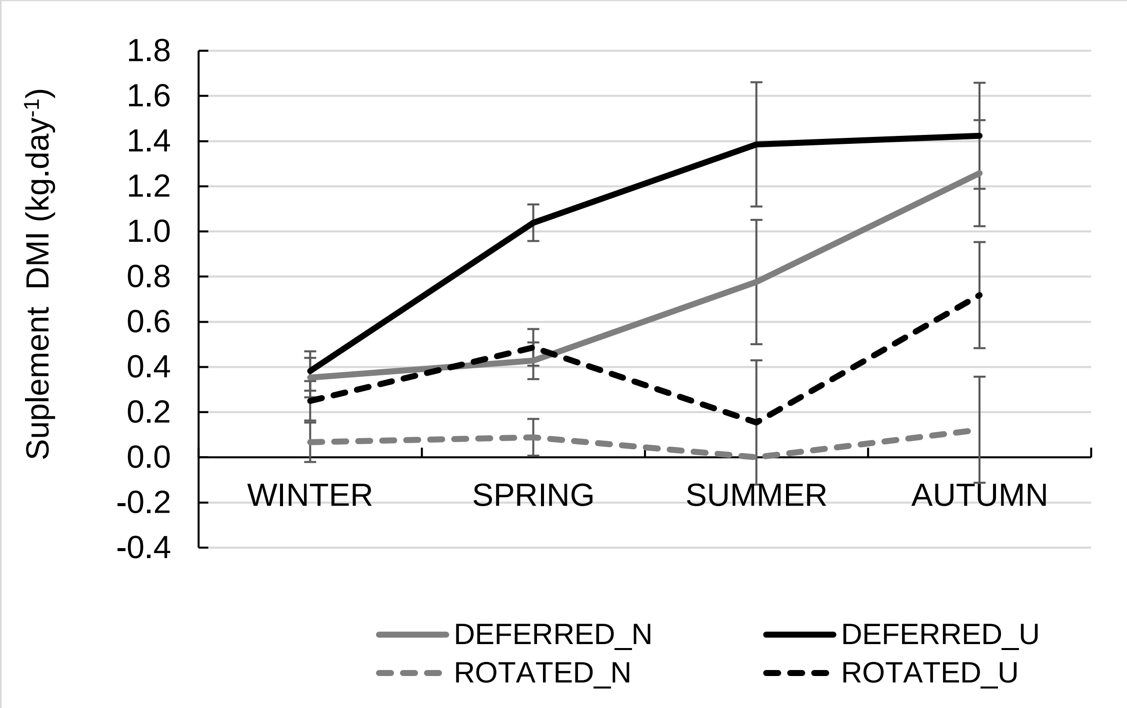
Figure 11. Forage dry matter intake of heifers in differents production systems (kg.day-1) by season.



Source: Own autorship

Supplement dry matter intake also was affected by triple interation of seasion*grazing systems*Nitrogen source (P = 0.0034), demonstrating that during the spring and summer animals in defered grazing and supplemented with urea had a highest supplement intake. During the winter, spring and autumn animals in rotated grazing supplemented with nitrate ammonium had the lowest intake (Figure 12 and Table 4).

Figure 12. Supplement dry matter intake ($kg.day^{-1}$) by season.



Source: Own autorship

Table 4. Means of variables animals consumption that showed triple interaction: Supplement Intake, Forage Intake, DMI %BW, DMI g/kg MW.

	Winter	Spring	Summer	Autumn
		Supplement Intake		
Deferred_N	0.3529a	0.4274b	0.7759b	1.2579a
Deferred_U	0.382a	1.0385a	1.3855a	1.4235a
Rotated_N	0.06656a	0.08856c		0.1221c
Rotated_U	0.2501b	0.4866d	0.1541c	0.7181b
		Forage Intake		
Deferred_N	8.26a	7.555a	12.812a	9.8315b
Deferred_U	6.9885b	6.9905a	11.584b	11.644ab
Rotated_N	6.0575c	5.6185b	8.7065d	11.885ab
Rotated_U	5.2975d	4.263c	9.6535c	16.594a
		DMI %BW		
Deferred_N	2.2428a	1.7416a	2.6581a	1.9891b
Deferred_U	1.9925ab	1.8522a	2.6575a	2.3975ab
Rotated_N	1.7547b	1.5122ab	2.1637b	2.4232ab
Rotated_U	1.6159b	1.3054b	2.1822b	3.3567a
		DMI g/kg MW		
Deferred_N	98.289a	79.7557ab	125.66a	96.0222b
Deferred_U	87.3468ab	84.486a	124.86a	115.78ab
Rotated_N	76.7665bc	67.5155bc	100.14b	114.93ab
Rotated_U	70.5485c	57.9048c	101.2b	160.56a

Means followed by different letters differ from each other.

5.3 Animal efficiency

Effects of treatments in feed conversion and ADG/N of supplement intake are presented in Table 5.

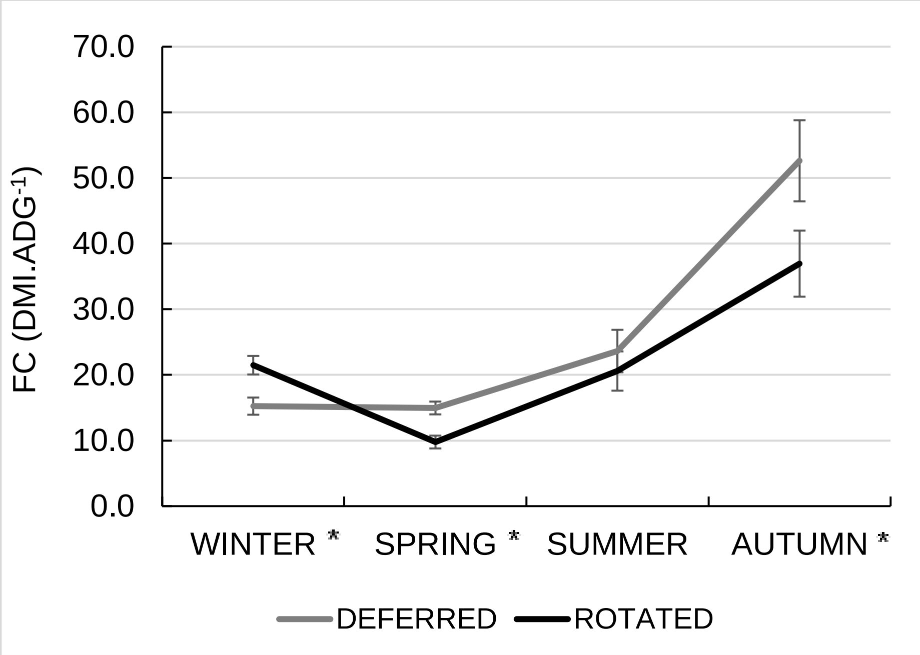
Table 5. Effects of grazing systems and nitrogen source in animal efficiency during the year: Feed conversion and ADG/N os supplement inake.

Fixed effects			***Item****	
			Mainly Effects	
Grazing	**N_source**	**Season**	**FC**	**ADG_N**
Deferred			24.3475	2.9135
Rotated			22.1221	4.2101
	Nitrate		24.0047	4.9145
	Urea		22.3907	2.2531
		Winter	17.9508	4.9008
		Spring	12.3638	3.8925
		Summer	22.0708	3.3092
		Autumn	42.4084	1.9858
Average Data				
Average			23.20	3.64
SEM			1.87	0.48
Statistics Probabilities				
Grazing			0.2776	0.1061
N_source			0.5826	<.0001
Season			<.0001	0.0005
Grazing*N_source			0.0517	0.6572
Grazing*Season			0.0062	0.0636
N_source*Season			0.2307	0.0315
Grazing*Season*N_source			0.6042	0.0100

FC = feed conversion; ADG/N intake = average daily gain/nitrogen intake

Feed conversion was affected by interation of seasion*grazing systems (P=0.0062), showing that animals in rotated system was the worst (highest value) feed conversion during the winter, but during the spring and autumn animals in deferred system had the the highest conversion (Figure 13).

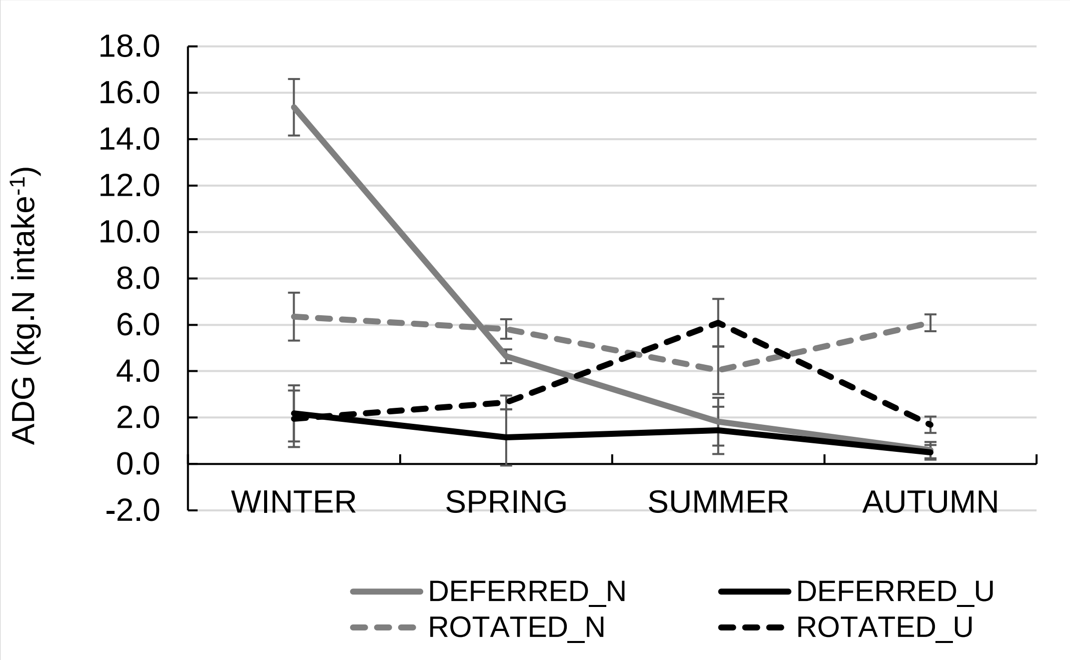
Figure 13.Heifers feed conversion in differents production systems by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Average daily gain in relation to nitrogen intake was affected by triple interation of seasion*grazing systems*Nitrogen source (P<0.0100), showing that animals in deferred system and supplemented whit ammonium nitrate has the highest ADG per N intake during the winter, followed by animals in rotated system supplemented with nitrate ammonium. In spring and autumn animals in rotated system supplemented with nitrate ammonium has higher ADG per N intake (Figure 14 and Table 6).

Figure 14. Heifers average daily gain for Nitrogen intake by season.



Source: Own autorship

Table 6. Means of average daily gain for nitrogen intake.

	Winter	Spring	Summer	Autumn
		ADG_N (kg ADG/ kg N intake)		
Deferred_N	15.376a	4.6424b	1.823b	0.5966c
Deferred_U	2.1798c	1.1425d	1.4481b	0.5008c
Rotated_N	6.3511b	5.819a	4.0403a	6.0851a
Rotated_U	1.9487c	2.6535c	6.0856a	1.6905b

Means followed by different letters differ from each other.

5.4 Methane emission

Methane emission results presented take into account animal averages in each batch and are presented in table 5. Methane emission presented significative difference for season (Table 5). Animals during the winter had the lowest methane emission, folowed by animals in spring. Animals during the summer and autumn presented the higher methane emission per head.

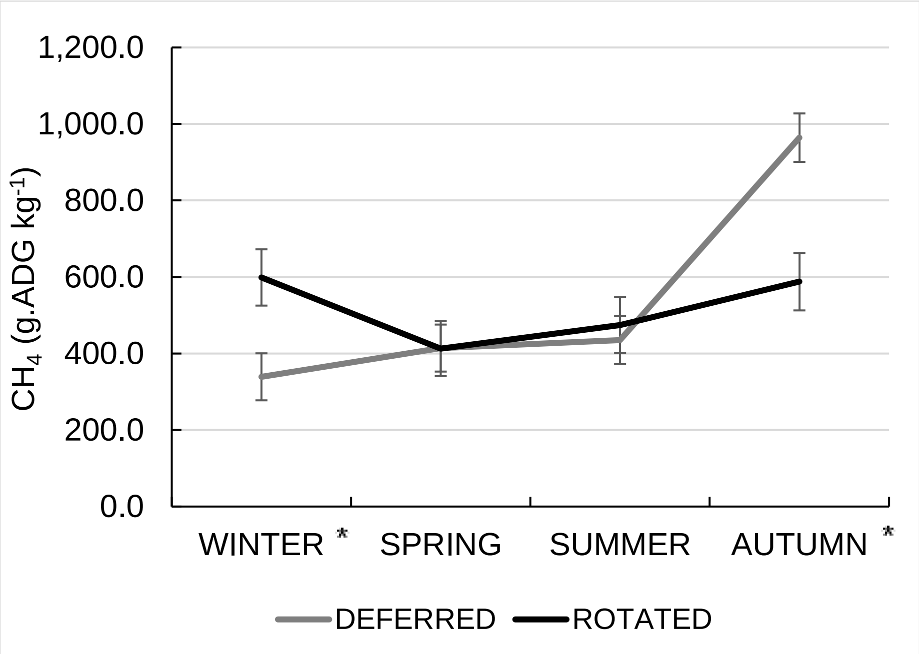
Table 7. Effects of grazing systems and nitrogen source in methane emission during the year: daily methane emission, methane emission per ADG, methane emission per DMI, Ym, methane emission per gross energy intake and methane emission per nitrogen intake.

Fixed effects			***Item****					
			Mainly Effects					
Grazing	**N_source**	**Season**	**CH_4 (g/day)**	**CH_4 (g/kg ADG)**	**CH_4 (g/kg DMI)**	**Ym**	**CH_4 (g/kg GEi)**	**CH_4 (g/kg N)**
Deferred			231.0896	527.7941	23.8093	8.0979	6.0881	1171.7000
Rotated			213.1679	473.4705	28.0043	9.4290	7.0887	2217.8500
	Nitrate		225.8943	540.0795	25.7054	8.6959	6.5377	2377.8100
	Urea		218.5308	463.4820	26.0470	8.8117	6.6247	983.1473
		Winter	172.0191a	450.8298	25.1494	8.6838	6.5286	1905.3200
		Spring	219.4854b	404.5223	34.6826	11.6613	8.7671	1766.0300
		Summer	245.2325c	441.1781	21.9954	7.4956	5.6351	1499.2500
		Autumn	254.3520c	762.6587	21.3862	7.0654	5.3119	1526.2000
			Average Data					
Average			222.2710	501.1088	25.8735	8.7529	6.5805	1669.0500
SEM			5.3056	26.8830	1.0720	0.3590	0.2699	172.0171
			Statistics Probabilities					
Grazing			0.2782	0.1472	0.5004	0.6902	0.6911	0.7753
N_source			0.5045	0.5694	0.9757	0.7910	0.7915	<.0001
Season			0.0059	<.0001	<.0001	<.0001	<.0001	0.5677
Grazing*N_source			0.8725	0.0908	0.9099	0.8216	0.8220	0.0205
Grazing*Season			0.2068	<.0001	0.0216	0.0012	0.0012	0.6723
N_source*Season			0.0534	0.7770	0.0232	0.1183	0.1183	0.0035
Grazing*Season*N_source			0.4252	0.9426	0.1209	0.1963	0.1963	0.0468

CH_4 = methane; ADG = average daily gain; DMI = dry matter intake; Ym = gross energy spent in methane emission; GEi = gross energy intake; N = nitrogen
Means followed by different letters differ from each other.

Methane emission per average daily gain (ADG) was affected by interation of seasion*grazing systems, when in winter the animals in rotated grazing emitted more methane per ADG than the animals in deffered grazing (Figure 15). While in autumn animals in deferred grazing system emitted more methane per ADG than the animals in rotated grazing.

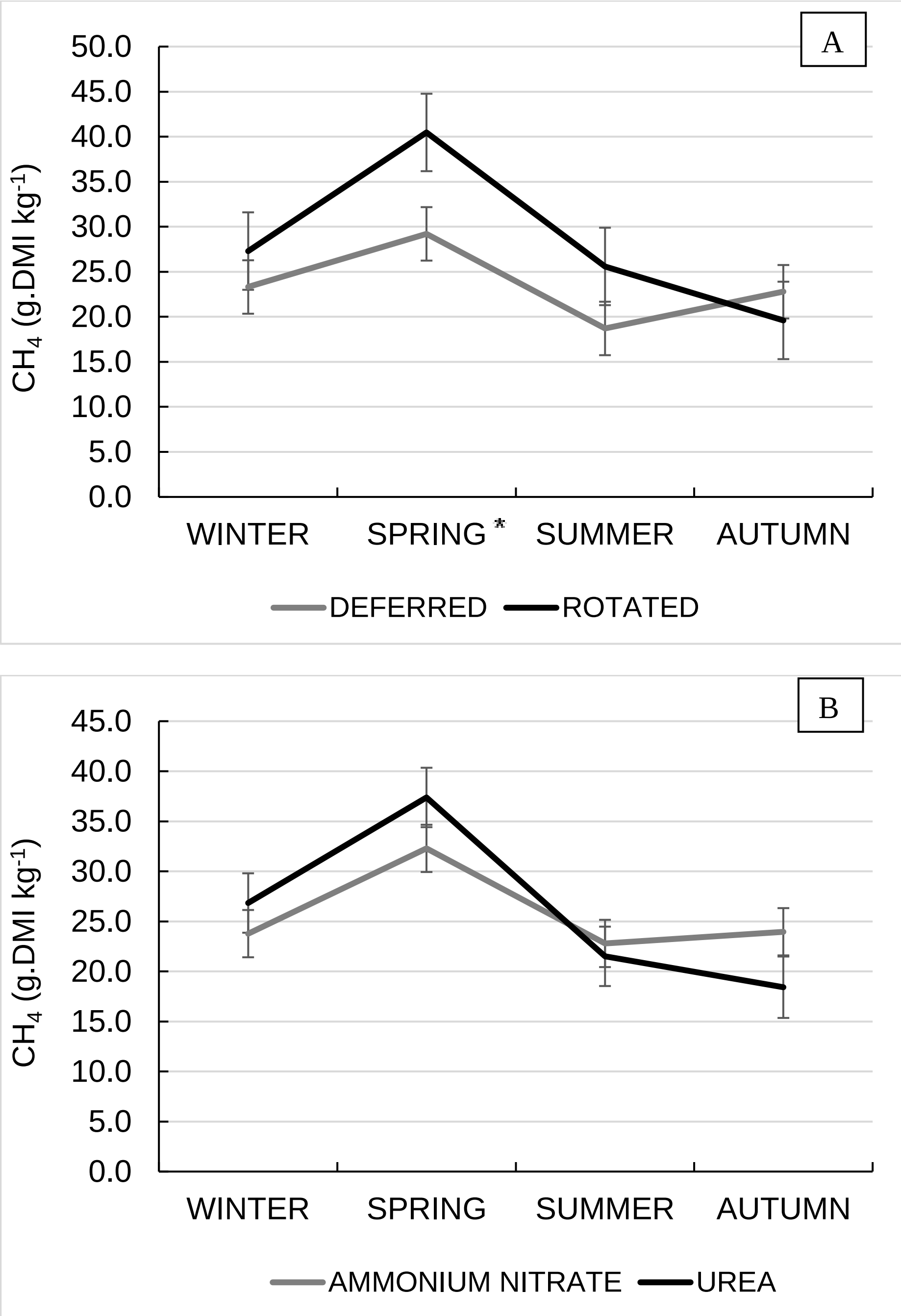
Figure 15. Methane emission per daily gain ($g.kg^{-1}$) of heifers in differents production systems by season. Seasions marked with an asterisk had statistical difference ($P < 0.05$).



Source: Own autorship

Methane emission per dry matter intake (DMI) was affected by interation of seasion*grazing systems (P=0.0216), showing that during spring animals in rotated grazing had a higher methane emission than the others treatments (Figure 16). Methane emission per dry matter intake was also affected by interation of seasion*nitrogen source (P=0.0232). This interation showed that animals supplemented with ammonium nitrate had a higher emission in autumn (Figure 16).

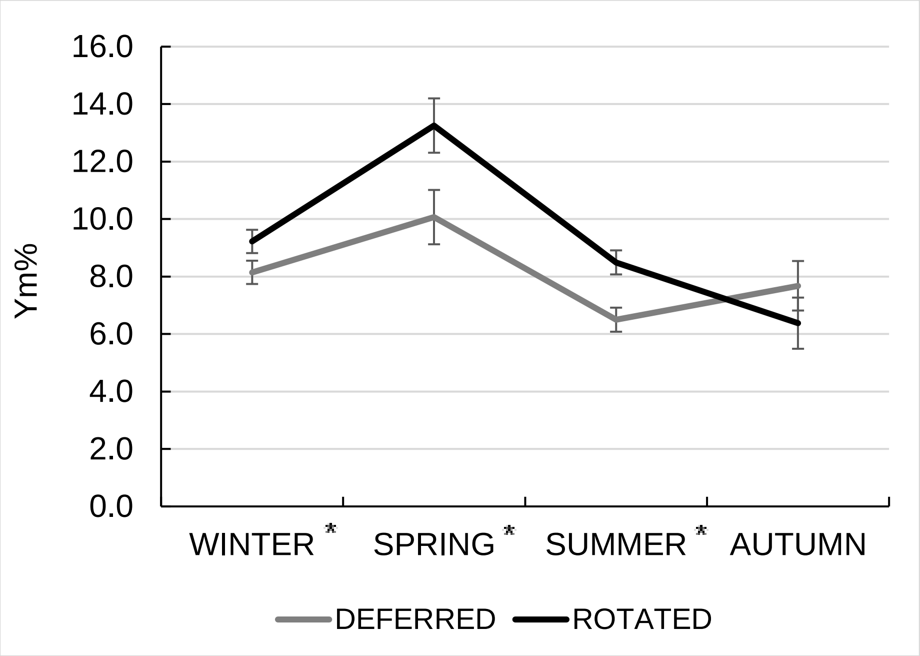
Figure 16. Methane emission per dry matter intake (g.kg^{-1}) of heifers in differents production systems by season (A) and with differents supplementation by season (B). Seasons marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Ym variable, that is gross energy percentage ingested lost in the methane emissions form, was affected by interation of seasion*grazing (P=0.0012). Thus animals had a higher Ym in the rotated grazing system during winter, spring and summer (Figure 17).

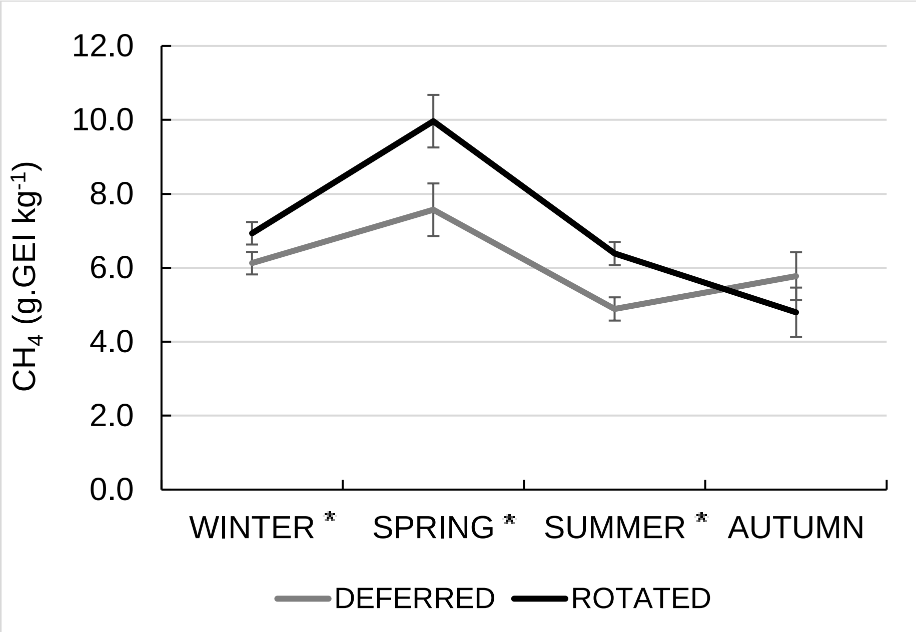
Figure 17. Ym% of heifers in differents production systems by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Methane emission per gross energy intake (GE) also was affected by interation of seasion*grazing systems (P=0.012), demonstrating that during the winter, spring and summer animals in rotated grazing had a higher methane emission than the other treatment (Figure 18).

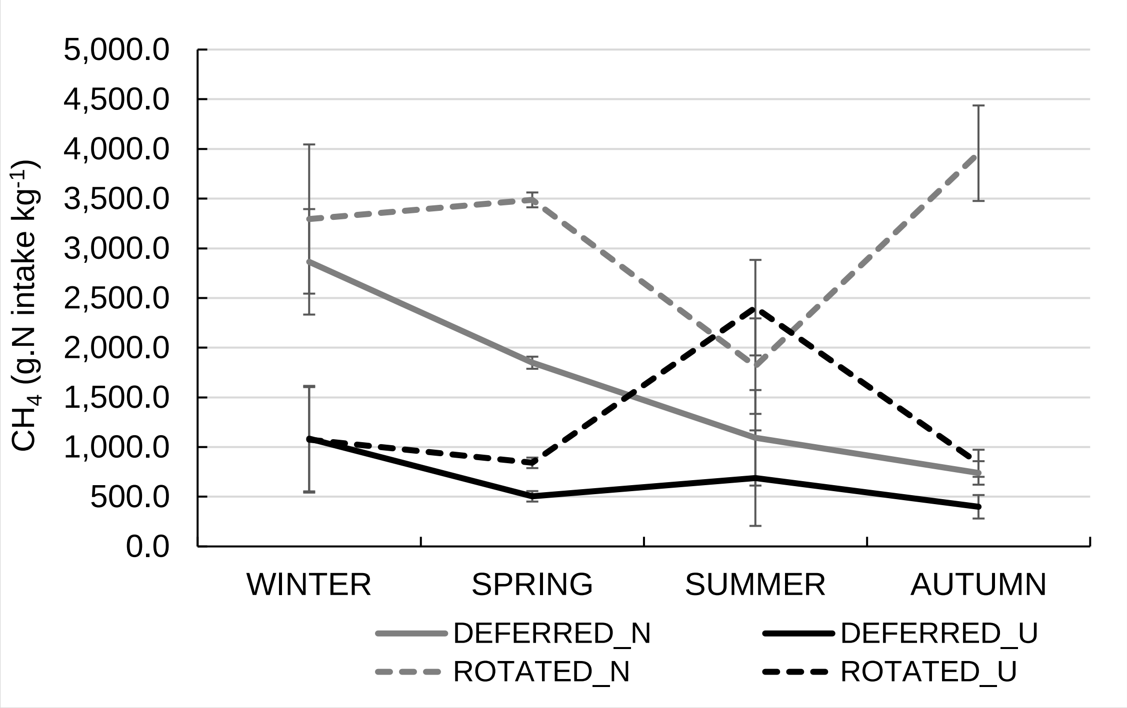
Figure 18. Methane emission per gross energy intake (g.kg^{-1}) of heifers in differents production systems by season. Seasions marked with an asterisk had statistical difference (P < 0.05).



Source: Own autorship

Methane emission per nitrogen intake (GE) was affected by triple interation of season*grazing system*Nitrogen source showing animals in rotated grazing system and supplemented with ammonium nitrate had a higher emission per nitrogen intake during spring and autumn (Figure 19 and Table 8).

Figure 19. Methane emission per Nitrogen intake (g.kg-1) of heifers in differents production systems and supplementation by season.



Source: Own autorship

Table 8. Means of average daily gain for nitrogen intake.

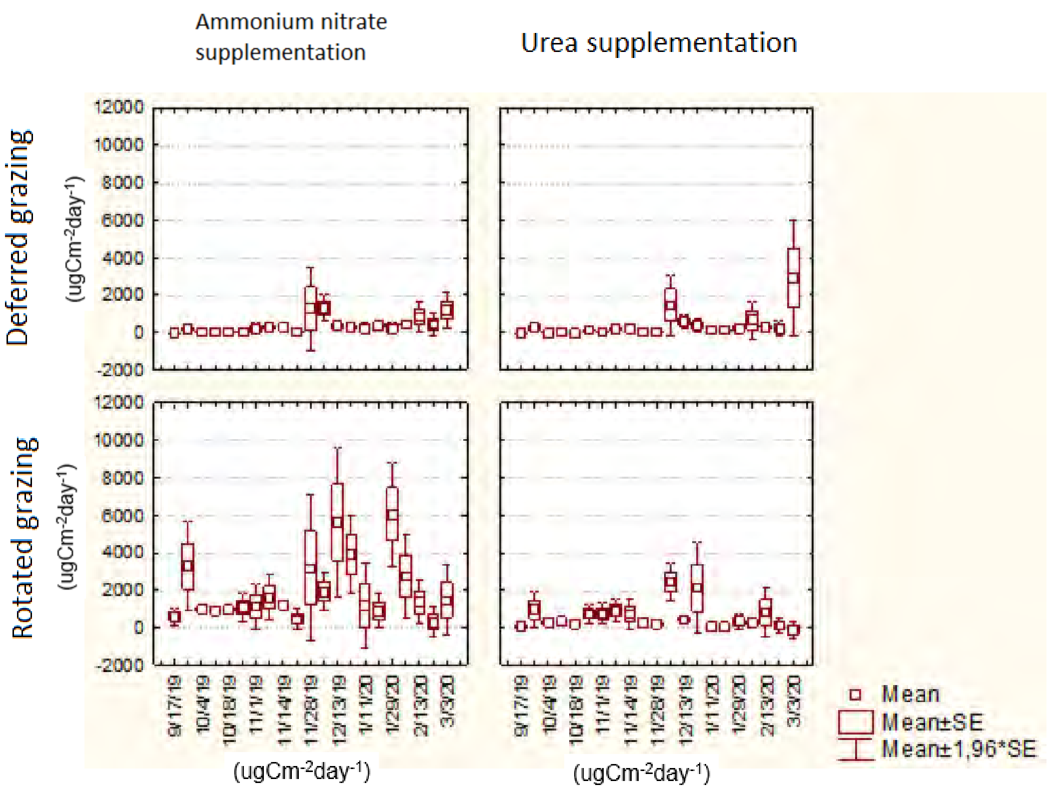
	Winter	**Spring**	**Summer**	**Autumn**
		CH4 (g/kg N)		
Deferred_N	2863.89a	1848.66b	1093.04bc	739.65b
Deferred_U	1084.77b	504.35d	687.14c	399.15c
Rotated_N	3294.56a	3487.16a	1814.22ab	3956.83a
Rotated_U	1072.69b	841.1c	2402.58a	836.82b

Means followed by different letters differ from each other.

5.5 Soil greenhouse gas emission

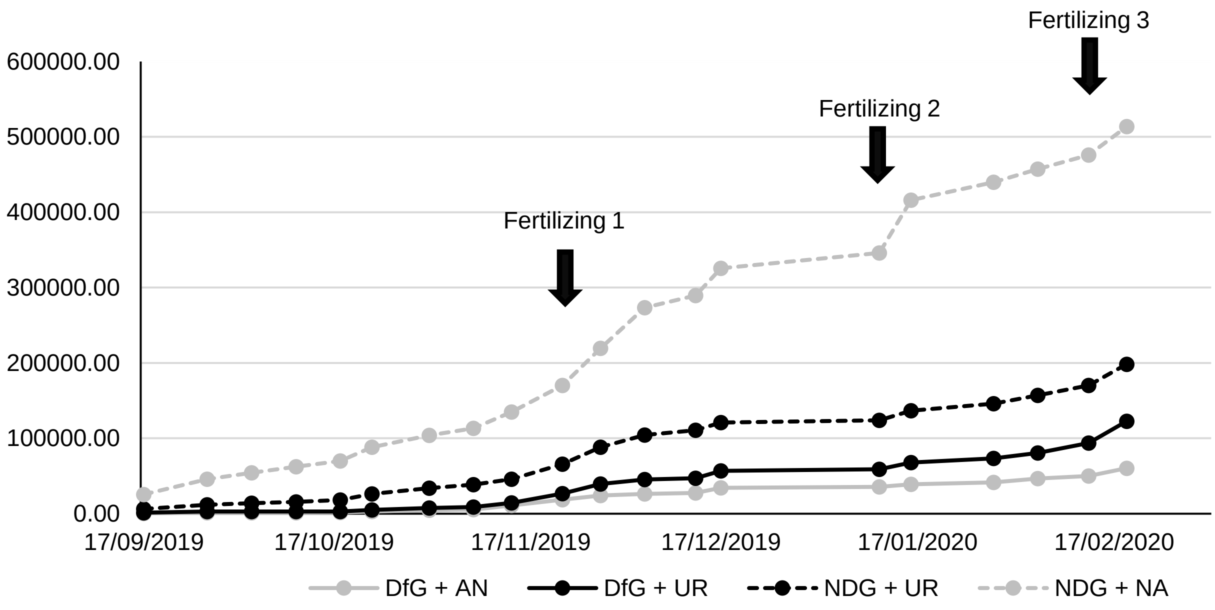
Nitrous oxide (N_2O) soil emission was analysed by treatments, trough accumulation over the time (Figure 20 and Figure 21).

Figure 20. N_2O emission accumulated over time, divided by grazing systems and nitrogen supplementation.



Source: Own autorship

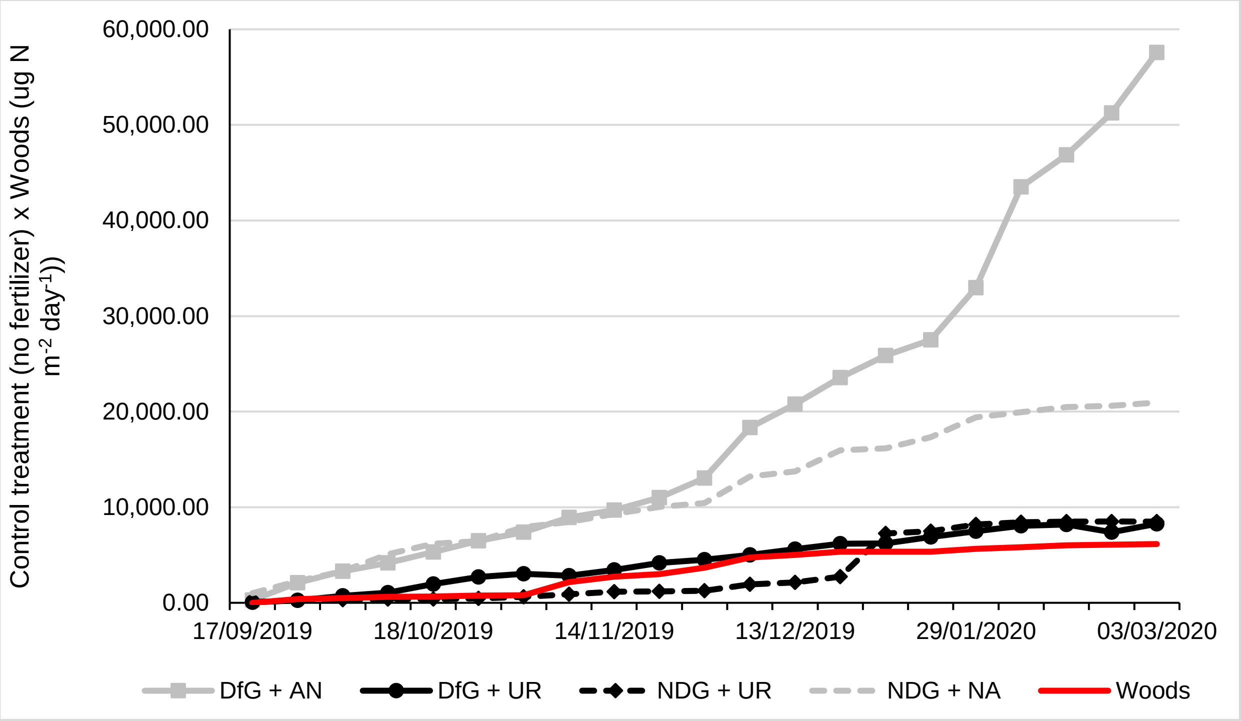
Figure 21. N_2O emission accumulated over time(ug N m^{-2} day^{-1}), divided by trataments.



Source: Own autorship

Non-fertilizing chamber that was allocated in each treatment, was analysed with the woods chambers, in order to compare the N_2O emission of each treatment, excluding fertilization (Figure 22).

Figure 22. Non-fertilizing chambers N_2O emission accumulated over time, divided by trataments and woods.



Source: Own autorship

6. Discussion

The average methane emission value was 222.17 g.day^{-1}, a value above that found by the IPCC in feedlot beef cattle (EGGLESTON et al., 2006), which is 156.0 (g/day). A significant result was found for methane emission in relation to seasons, due to animals live weight increase. Pinares-Patiño et al. (2016) also conclude that there is a positive correlation between CH_4 emission and the animal's live weight, as well as Méo-Filho (2020), who shows that animals that yield higher final live weight had higher methane emission.

Methane emission/weight gain showed an interaction between season and grazing system, since grazing system was determinant for the animals' weight gain. Similar results was obtained by Méo-Filho (2020), when it shows that less intensified systems and with less availability had a higher emission/gain.

This reinforces the idea that higher the animal's performance, lower the methane emission/weight gain, showing a feasibility in reducing environmental impact through the methane emission as it is possible to intensify the animals' performance.Sakamoto (2018) also observed that there was a higher emission per kg/ADG in treatments than lower ADG.

Demarchi et al. (2016) also found, in *Urochloa brizantha* cv. Marandu pasture, differences in methane emissions between seasons, indicating that pasture quality can interfere in the emission. This result is similar to that found in this experiment, as well as Moraes et. al. (2014) observed that methane emission can be affected by difference in diet nutritional composition in pasture and feed behavior cattle grass production systems.

Weight gain was greater in seasons and systems in which the dry matter consumption was higher, consequently, methane emission/dry matter intake, methane emission/gross energy ingestion and Ym% repeated the same pattern. Similarly, Buddle et al. (2011) states that the dry matter intake is the main factor that can modify how methane is produced in ruminants, being able to increase or decrease methane emission per unit of DMI depending on consumption level. Méo-Filho (2020) also found differences in methane emission/DMI and in Ym%, due differences observed by dry matter intake, because there were no differences in methane emission/animal/day between treatments, that consists in differents integration systems of farming-livestock-florest.

Sakamoto (2018) showed that, ideal grazing strategy for each time of the year, must be adjusted, to ensure an increase in animal productivity, increasing the product per area without affecting the sustainable development of the activity.

Feng (2020), in a meta-analysis, found that a higher nitrate dose supplemented enhances the nitrate mitigating effect on CH4 production and yield, whereas an increased DMI reduces the mitigating effect of nitrate on CH4 production. This study did not showed that nitrogen source was a factor to reduces or increase methane emission, independent of the variable was utilized. Regarding dry matter consumption, it is possible to assess that the higher total dry matter consumption, is greater the methane emission, because there is a relationship with animal live weight. On the other hand, when we evaluated methane emission by consumption, we noticed a higher methane emission for animals in the rotated system during spring, the season that presented numerically the lowest DMI.

7. Conclusion

Methane emission in grams per day does not suffer a difference due to treatment to the grazing system or the nitrogen source supplemented in any season of the year, and it is possible to observe a difference only as the live body weight changes.

Observing the rotated grazing, in an isolated way, it is possible to observe that as animals leave dry season and enter in rainy season, heifers had an increase in consumption and, consequent, increase in ADG, this made methane emission/ADG also decrease. This behavior was similar in deferred system during the dry season, when this treatment is the one with the greatest pasture availability.

Regarding supplementation with different nitrogen sources, no significant differences were observed.

Therefore, animal performance is altered according to the systems, being better in systems where there is a greater pasture availability, making methane emission/ADG lower in systems where animals performance was better, showing feasibility in intensification of production systems as a reduction in methane emissions per meat produced.

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