Universidade Federal de Minas Gerais

Desempenho e emissões de gases de efeito estufa de bovinos zebuínos e cruzados em sistema intensivo e integrado de produção

Isabella Cristina de Faria Maciel

Belo Horizonte

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“Our greatest weakness lies in giving up. The most certain way to succeed is always to try just one more time.”

Thomas A. Edison

SUMÁRIO

LISTA DE TABELAS

LISTA DE FIGURAS

LISTA DE SIGLAS E ABREVIATURAS

AG – Ácidos graxos

AGV – Ácidos graxos voláteis

C - Carbono

CH_4 – Metano

CMS – Consumo de matéria seca

CO_2 – Dióxido de carbono

EM – Energia metabolizável

GEE – Gases de efeito estufa

H_2 - Hidrogênio

ha – hectares

ILP – Integração lavoura pecuária

MS – Matéria seca

N_2 – Nitrogênio

N_2O – Óxido nitroso

NH_3 – Amônia

NH_4^+ – Amônio

NO_3^- – Nitrato

NRC – National Research Council

O_2 – Oxigênio

PC – Peso corporal

QCH_4 – Taxa de emissão do CH_4

QSF_6 – Taxa de emissão do SF_6

R - Repetibilidade

SF_6 – Hexafluoreto de enxofre

t - toneladas

TP – Taxa de permeação do SF_6

UA – Unidade animal (equivale a 450 kg de peso corporal)

ACRONYMS AND ABBREVIATIONS LIST

ADF – Acid detergent fiber

ADG – Average daily gain

ADGc – Average daily gain of carcass

AHM – Available herbage mass

AN – Angus x Nellore crossbred

BW – Body weight

$BW^{0,75}$ – Metabolic body weight

C – Carbon

Ca – Calcium

CC – Cell content

Cel - Cellulose

CH_4 – Methane

Co – Cobalt

CO_2 – Carbon dioxide

CP – Crude protein

Cu – Copper

CY – Carcass yield

DAA – Days after application

DM – Dry matter

DMI – Dry matter intake

EE – Ethereal extract

EF – Emission factor

FBW – Final body weight

Fe – Iron

FL - Feedlot

FP – Fecal production

GHG – Greenhouse gases

ha – hectares

Hem - Hemicellulose

I – Iodine

IBW – Initial body weight

ICL – Integrated crop-livestock

iNDF – Indigestible NDF

Mg – Magnesium

Mn – Manganese

$MWCH_4$ – CH_4 molecular weight

$MWSF_6$ – SF_6 molecular weight

N – Nitrogen

N_2O – Nitrous oxide

Na – Sodium

NDF – Neutral detergente fiber

Nel - Nellore

NH_4^+ – Ammonium

NO_3^- – Nitrate

OM – Organic matter

P – Phosphorous

PVC – Polyvinyl chloride

RCH_4 – CH_4 emission rate

RSF_6 – SF_6 emission rate

S – Sulfur

SD – Standard deviation

Se - Selenium

SF_6 – Sulfur hexafluoride

t – toneladas

TDN – Total digestible nutrients

TiO_2 – Titanium dioxide

WHC – Weight of hot carcass

Zn - Zinc

RESUMO

Objetivou-se avaliar o desempenho animal e a produção de metano (CH_4) entérico de dois grupos genéticos de bovinos de corte em um sistema intensivo de produção, com recria à pasto em sistema de integraçãolavoura-pecuária (ILC) e terminação em confinamento, além de determinar as emissões de óxido nitroso (N_2O) e CH_4 e o fator de emissão (FE) do N_2O das fezes e urina de bovinos de corte depositados em confinamento. No ensaio I, 70 animais de dois grupos genéticos, Nelore (Nel) e cruzados Angus x Nelore (AN), foram comparados quanto ao desempenho e às emissões de CH_4 em um sistema de produção intensivo. No início do experimento, novilhos de 10 meses de idade pastejaram *Megathyrsusmaximus* 'Mombaça' na fase de recria (taxa de lotação de 5,5 UA/ha, produção de forragem de 4884 kg MS/ha, oferta de forragem de 5,9 kg MS/100kg PC) e depois foram terminados em confinamento (dieta 35:65% silagem de milho:concentrado). Novilhos (n=8) de cada grupo genético foram selecionados aleatoriamente em cada fase para medir a produção de CH_4 usando a técnica dohexafluoreto de enxofre e o consumo de matéria seca (CMS) utilizando dióxido de titânio. Comparado com Nel, AN tiveram ganho total e GMD superior no período de pastejo. Além disso, AN apresentou maior GMD no confinamento, apesar do período menor de terminação, resultando em maior rendimento de carcaça e GMD de carcaça. A produção de metano (kg/período) foi 19% menor em Neldo que AN em pastejo ($P<0,01$), e não houve diferença no confinamento. Animais Nel tiveram maior intensidade de CH_4 (g CH_4/GMD) em comparação com AN em confinamento. O grupo genético não influenciou o rendimento de CH_4 (g CH_4/CMS) em pastejo e em confinamento, apesar da diferença deCMS (kg/dia) noconfinamento. Os animais cruzados tem potencial para reduzir a intensidade de CH_4 em climas tropicais, resultando em menoremissão de metano por kg de carne produzida. No ensaio II, para investigar os efeitos do tipo de excreta depositado em solos confinados nas emissões de N_2O e CH_4, foi obtido um pool de amostra de cada excreta, fezes e urina, de 25 novilhos em confinamento (PC médio = 393 kg). Urina (1,3 l) e fezes (1,3 kg) foram aplicados uma vez no início do experimento e os gases foram monitorados durante 92 dias após a aplicação das excretas, utilizando a técnica de câmaras estáticas. Os resultados mostraram que os fluxos de N_2O tiveram dois picos paraurina, o primeiro no 1° dia após a aplicação (DAA) das excretas e o segundo após os eventos de precipitação (70 DAA). Para as fezes, foi observado um pico de N_2O aos 70 DAA. Os fluxosde CH_4foram instáveis e apresentaram vários pulsos ao longo do período de mensuração,alterando entre valores positivos e negativos. As emissões médias de CH_4 do solo permaneceram próximas de zero (-

8,4, -3,2 e -14,8 µgC/m/h para fezes, urina e controle, respectivamente. A presença de excretas aumentou a umidade do solo em 44,5 e 55,4% para fezes e urina, respectivamente, em comparação ao controle. A alta concentração de N mineral na urina resultou em altos valores e diferença significativa de amônio (NH_4^+) e nitrato (NO_3^-) em relação às fezes e ao controle. As concentrações de NH_4^+ e NO_3^- nos solos tratados com urina atingiram o pico aos 13 DAA, enquanto asfezes atingiram o pico aos 42 DAA. O FE para o N2O (FE; porcentagem de nitrogênio das excretas perdido como N_2O) da urina foi significativamente (P <0,0001) maior do que das fezes (2,83 versus 0,32%, respectivamente), resultando em um FE combinado de 1,83%, que é 8,5% menor do que o FE padrão recomendado pelo IPCC.
Palavras-chave: bovinos de corte, gases de efeito estufa, ruminantes, sistemas integrados, sustentabilidade

ABSTRACT

This study aimed to evaluate animal performance and enteric methane (CH_4) production from two breed compositions in a Brazilian beef cattle production system– rearing in integrated crop-livestock (ICL) system and finishing in feedlot (FL), besides to determine nitrous oxide (N_2O) and CH_4 emissions and the associated emission factor (EF; percentage of urine and dung-N lost as N_2O-N) for beef cattle excreta deposited onto a FL land. In trial I, to assess how breed composition affects performance and methane emissions, 70 animals of two breed compositions, Angus x Nellore crossbred (AN) and Nellore (Nel), were compared in an intensive production system. At trial onset, 10 mo old steers grazed *Megathyrsus maximus* 'Mombaça' in the rearing phase (stocking rate 5.5 AU/ha, herbage mass 4,884 kg DM/ha, forage allowance 5.9 kg DM/100kg BW) and then were finished in FL (35:65% corn silage:concentrate diet).Steers (n = 8) from each breed composition were randomly selected in each phase to measure CH_4 production using a sulfur hexafluoride technique and DMI using titanium dioxide. Compared with Nel, AN had both superior total gain and ADG in the grazing period. Also, the AN presented greater ADG in FL with a shorter finishing period, and resulted in greater carcass yield and carcass ADG. Methane production (kg/period) was lower in Nel (19% less) than AN in grazing (P<0.01), and no difference in FL was observed. Nel had greater CH_4 intensity (g CH_4 per unit of ADG) compared to AN in FL. Breed composition did not influence the CH_4 yield (g CH_4 per unit of DMI) in grazing or FL, despite the difference in DMI (kg/day) in FL. In our study the introduction of Angus into Nellore has potential to reduce CH_4 intensity in tropical climates, resulting in less methane emission per kg beef produced. In trial II, to investigate the effects of excreta type deposited in feedlot soils on N_2O andCH_4emissions, sample' pool of each excreta were obtained from 25 steers in feedlot (Average BW = 393 kg). Urine (1.3 l) and dung (1.3 kg) were applied once and gases fluxes were monitored lasted 92 days, by using static chambers technique. The results showed that N_2O fluxes had two peaks for the urine treatment, the first at 1stday after application (DAA) of excreta and the 2ndafter the rainfall events (70 DAA). Also, the N_2O fluxes for the dung had a peak at 70 DAA. The CH_4fluxes were unstable and presented several pulses throughout the measurement period and was altered between positive and negative flow values. Soil CH_4 emissions remained near zero and the treatments showed low levels up CH_4 uptake (-8.4, -3.2 and -14.8 μgC m^{-2} h^{-1} for dung, urine and control, respectively). The excreta presence increased soil moisture by 44.5 and 55.4% for dung and urine, respectively, compared to control. The high mineral N concentration in the urine caused that high values

and significant difference of ammonium (NH_4^+) and nitrate (NO_3^-) compared to dung and control. The NH_4^+ and NO_3^- soil concentrations in the cattle urine treated soils peaked at 13 DAA, while for dung treated soils peaked at 42 DAA. The N_2O EF from urine was significantly ($P<0.0001$) higher than the EF from feces (2.83 vs. 0.32%, respectively), resulting in a combined excretal EF of 1.83%, which is <8.5% of the IPCC default EF for excretal returns.

Key-words: beef cattle, greenhouse gases emissions, integrated systems, ruminants, sustentability

CAPÍTULO I – REVISÃO DE LITERATURA

1.1.Introdução geral

Os desafios dos sistemas de produção animal serão produzir alimentos em qualidade e quantidade suficientes para suprir o aumento da população e, ao mesmo tempo, reduzir os impactos ambientais (Duthie et al., 2017). A expectativa é que a população global aumentará para mais de 9,5 bilhões de habitantes até o ano de 2050 (FAO, 2009). Concomitante com esse aumento populacional, e com mudanças no padrão de consumo, observa-se aumento na demanda poralimentos de alta densidade nutricional, como carne, leite e ovos.

Apecuária global é responsável por 14,5% do total de gases de efeito estufa (GEE) emitidos para a atmosfera, dos quais 6,4% correspondem às emissões de metano (CH_4) e 4,2% às emissões de óxido nitroso (N_2O)(Gerber et al., 2013).Embora essa valor não seja tão expressivo, o sistema de produção de carne tem sido apontado como fonte significativa de emissões de GEE.

Aprincipal fonte de emissão deCH_4na pecuária advém da fermentação entérica (Moraes et al., 2014), a qual ocorre pela fermentação microbiana do alimento no rúmen do animal, resultando na formação do CH_4 (Desjardins et al., 2012).As emissões de N_2O por sua vez, são provenientes principalmente da deposição de excretas dos animais no solo e uso de fertilizantes nitrogenados.

Atualmente a produção de carnebovina brasileira ocupa uma posição de destaque no cenário mundial, a qual responde por 15,5% da produção mundial (FAO, 2015). Assim, a busca pelo equilíbrio entre o aumento na produção de alimentos e a redução dos impactos ambientais, mediante a identificação e análise de cenários de produção animal mais sustentáveis, se faz necessário. Além disso, a utilização de estratégias com potencial de mitigação neste setor é extremamente importante no cumprimento das metas de redução de emissões de GEE (IPCC, 2014).

Diante disso, o Brasil propôs inúmeras estratégias para mitigação de GEE apresentadas no Plano Brasileiro de Mitigação e Adaptação às Mudanças Climáticas(Plano…, 2012). O foco principal para redução de GEE é acontenção do desmatamento, o que será viável mediante a recuperação de 15 milhões de hectares de pastagens degradadas até 2020, além da adoção de sistemas integrados de produção. Essas medidas visam reduzir as emissões diretamente pela melhoria da eficiência produtiva, resultando em menor emissão de GEE por

kg de carne produzida, além do aumento dos estoques de carbono orgânico no solo(Silva et al., 2018).

Os dados nacionais de inventário de emissões de GEE (Brasil, 2014) mostram que o impacto do desmatamento nas emissões de CO_2 diminuiu de 57 para 15% em 2005 e 2012, respectivamente, o que é parcialmente explicado pela eficiente política de controle do desmatamento (Arimaetal, 2014; Lapolaetal., 2014).A área de pastagem tem diminuído ao longo das duas últimas décadas, enquanto o número de bovinos tem aumentado (FAO, 2015). Correspondentemente, a produção de carne aumentou, o que comprovaos ganhosna eficiência dos sistemas de produção de bovinos de corte.

A melhoria da eficiência produtiva conferida pelas práticas modernas de gestão e pela utilização das tecnologias pode favorecer a produção de carne bovina de forma sustentável (Capper e Hayes, 2012). Assim, projeta-se a adoção de sistemas multifuncionais, como os sistemas integrados de produção, planejados para explorar o sinergismos e interações existentes entre solo-planta-animal-atmosfera (Carvalho e Moraes, 2011).

Para que as práticas de manejo adotadas em sistemas de produção tropicaispossam serapontadas como sustentáveis, é necessário que as mensurações de GEE sejam realizadas de forma acurada para que diferenças entre tratamentos sejam identificadas. Esses dadospossibilitarão a avaliação de estratégias de mitigação de GEE da agropecuária, além de reduzir as incertezas dos inventários de GEE, já que geralmente são baseados em estudos de países de clima temperados(Prajapati e Santos, 2017).

Com este trabalho, objetivou-se: *i)*avaliar o desempenho e a emissão de metano de bovinos zebuínos e cruzados em sistema intensivo e integrado de produção e, *ii)*mensurar as emissões de gases de efeito estufa provenientes da deposição de excretas de bovinos em confinamento.

1.2.Sistema de produção de bovinos de corte no Brasil

A produção de bovinos de corte tem grande importância para a economia do Brasil, que detém o maior rebanho bovino comercial do mundo (218 milhões), e lidera as exportações mundiais de carne, embora ainda apresente taxas produtivas abaixo de suas reais potencialidades, como taxa de lotação menor que 1 unidade animal/hectare (UA/ha) e produtividade menor que 120 kg de peso vivo ou 4 arrobas/ha/ano (IBGE, 2017). De acordo com a ABIEC (2019), a contribuição da pecuária é de 8,7% do produto interno bruto (PIB) total brasileiro.

A pecuária de corte brasileira se desenvolve principalmente em sistemas a pasto, ocupando aproximadamente 171 milhões de hectares de pastagens (IBGE, 2017). Em decorrência disso, a maioria dos bovinos de corte são criados e terminados em pastagens no Brasil (Silva et al., 2016).

A preocupação atual com a redução dos recursos naturais, a mudança climática e a aceitabilidade social das práticas de produção de carne bovina tem provocado questionamentos sobre a intensificação dos sistemas de produção (Xue et al., 2010). A expansão das novas áreas de pastagens, chamada de intensificação horizontal, em detrimento de matas e florestas, são inadmissíveis nos dias atuais, devido ao grande impacto causado ao ambiente pelo desmatamento. Estudo recente mostrou que apenas 10% dos aumentos de produção ocorrem devido à expansão de pastagens; os restantes 90% resultam de ganhos em produtividade (Silva et al., 2016).

No período de 1990 a 2014, o aumento da produtividade da bovinocultura superou o aumento das emissões de GEE. Além disso, a produção de carne por unidade de emissões de gases teve um salto produtivo da ordem de 10 toneladas em 1990 para cerca de 19 toneladas de carne produzidas por unidade de emissão em 2014, o que demonstra menor emissão de GEE por animal abatido (Vieira Filho, 2017).

A atual pressão para extinguir o desmatamento, concorrentemente com a mitigação de GEE sinalizam para uma intensificação vertical, em que é preconizado maior produção de carne em menor área mediante utilização de estratégias que permitam aumentar a taxa de lotação, a fertilidade do rebanho, o ganho médio diário, o peso da carcaça, dentre outros. Essa intensificação resulta em uma pecuária de ciclo curto, com redução no tempo de abate, área de pastagem e emissões de GEE por kg de produto (Berndt e Tomkins, 2013).

Dentre as alternativas, manejo de pastagem, suplementação proteica-energetica-mineral para animais a pasto, integração lavoura-pecuária, confinamento, utilização de cruzamentos com raças mais precoces (*Bostaurus x Bosindicus*) podem contribuir para maior produtividade do sistema, uma vez que permitem redução no tempo de abate e aumento no peso de carcaça dos animais.

A intensificação da pecuária pode impactar naquantidade de carne produzida por área (kg/ha/ano) e nas emissões de GEE. Os sistemas pecuários modernos e intensivos, como sistemas de terminação de animais com grãos, exigem menor área para produção e reduzem as emissões de GEE por quilograma de carne comparado aos sistemas tradicionais e extensivos (Swain et al, 2018).

Embora somente 13% dos animais abatidos tenham sido terminados em confinamento em 2015, observa-se um aumento anual do número de bovinos confinados, além de uma redução na idade ao abate e aumento do peso da carcaça (ABIEC, 2019).

A redução nas emissões de GEE por ruminantes também tem sido estabelecida em estudos utilizando sistemas de pastagens melhoradas (Dick et al., 2015; Wang et al., 2015). Nesse aspecto, sistemas de produção consorciados como a integração lavoura pecuária (ILP) apresentam grande potencial, proporcionando ganhos produtivos, econômicos e ambientais. Esses sistemas são identificados como uma estratégia eficiente de uso da terra para restaurar áreas degradadas, aumentando a produção das culturas e da carne bovina e fornecendo potencial técnico de armazenamento de carbono (C) no solo como opção para compensar as emissões de CH_4 e óxido nitroso (N_2O) da pecuária bovina (Figueiredo et al., 2017).

Estudos mostram que a melhoria da produtividade das pastagens resulta em aumento do estoque de carbono no solo (Braz et al., 2013; Stanley et al., 2018), com remoções de CO_2 atmosféricas líquidas de aproximadamente 1MgC $ha^{-1}ano^{-1}$ ao comparar pastagens degradadas e melhoradas (IPCC, 2006).

O aumento da produtividade e o menor ciclo de produção também podem ser alcançados mediante melhoramento genético animal. Independente do grau de intensidade dos sistemas, os rebanhos apresentam uma predominância dos genótipos zebuínos, em especial da raça Nelore, mas nas últimas décadas animais taurinos tem sido utilizado em cruzamentos, destacando-se as raças Aberdeen Angus, Hereford, Simental e Charolês.

Resultados apresentados por Silva et al. (2016) suportam a afirmativa de que para a produção de carne ambientalmente sustentável é necessário a intensificação dos sistemas de produção, que demanda menor área para produção de maior volume de carne bovina. Além disso, as emissões de GEE podem ser reduzidas em até 10% em um cenário sem desmatamento e com aumento de 30% no consumo de carne atual.

1.3.Emissão de gases de efeito estufa pela pecuária

1.3.1.Metano

O gás metano (CH_4), juntamente com o dióxido de carbono (CO_2) e o óxido nitroso (N_2O) constituem as fontes primárias de gases de efeito estufa (GEE) (Knapp et al., 2014). As emissões desses gases ocorrem mediante processos naturais ou antropogênicos, que incluem atividades agrícolas, fermentação ruminal, entre outras atividades (Herrero et al., 2013).

O aumento da concentração de GEE é visto como um dos principais propulsores da mudança climática. Enquanto as emissões de CO_2 são decorrentes principalmente do uso de combustível fóssil, as emissões de CH_4 e N_2O surgem principalmente da agricultura (Smith et al., 2007). Embora a quantidade de CH_4na atmosfera seja menor do que o CO_2, o potencial de aquecimento do CH_4é significativamente maior, pois capta 25 vezes mais calor quando comparado ao CO_2. Já o N_2O possui potencial de aquecimento global aproximadamente 300 vezes maior do que o CO_2 (IPCC, 2007) e sua concentração na atmosfera aumenta a uma taxa de 0,73 ppb/ano (Ciais et al., 2013).

O CH_4 entérico é um dos produtos finais do processo de digestão microbiana, sendo produzido em condições anaeróbias no rúmen pelas *Archaea*metanogênicas, que utilizam o CO_2 e o hidrogênio (H_2) presentes no ambiente ruminal. A formação de metano no rúmen depende tanto do suprimento de H_2 da fermentação da dieta por bactérias e protozoários, quanto pela subsequente conversão do H_2 e do CO_2 em CH_4. Assim, o processo de formação do CH_4 possibilita que o H_2 oriundo do metabolismo microbiano seja eliminado (McAllister e Newbold, 2008).

As emissões de CH_4 entérico resultam em diminuição da eficiência alimentar, representando uma perda de energia bruta para o animal (estimada entre quatro e 10%), dependendo do tipo, qualidade e quantidade de alimento consumido (Lassey, 2007). Essa energia perdida poderia ser utilizada pelo animal para produção, como por exemplo, para a produção de carne (Cottle et al., 2011; Gerber et al., 2013).

1.3.2.Óxido nitroso

As emissões de N_2O representam aproximadamente de 6% das emissões globais de GEE, sendo 90% dessas emissões derivadas de práticas agrícolas (Forster et al., 2007; Smith et al., 2007). O N_2O nos solos é produzido em grande parte pelo processo microbiano de desnitrificação e, em menor grau, pela nitrificação. A nitrificação é um processo aeróbio que oxida amônio (NH_4^+) em nitrato (NO_3^-), com N_2O como subproduto, enquanto a desnitrificação é um processo anaeróbio que reduz NO_3^- a N_2, com formação do N_2O como um intermediário obrigatório. As altas taxas de emissão de N_2O geralmente coincidem com as condições dos solos favoráveis à desnitrificação (anaerobiose, boa oferta de NO_3^-) (De Klein e Eckard, 2008).

Devido ao efetivo bovino brasileiro, 40% da emissão nacional estimada de N_2O são derivadas de urina e fezes excretadas em áreas de pastagens (Brasil, 2010). Aproximadamente 80 a 95% do nitrogênio (N) que é lançado ao solo como urina ou fezes de bovinos advêm do

N que é ingerido (Bolan et al., 2004). Assim, as excretas são consideradas fontes importantes de N_2O, capazes de impactar na quantidade global desse gás (Mosier et al., 1998).

Acredita-se na tendência de aumento das concentrações de N_2O nas próximas décadas devido à intensificação da pecuária brasileira que avança, concomitante, com o aumento no volume de excretas e na utilização de fertilizantes nitrogenados, o que contribui para elevar as emissões desse gás(Smith et al., 2007).

1.4.Ação dos microorganismos ruminais na produção de metano

Os ruminantes desempenham um papel crucial na segurança alimentar, sendo capazes de converter forragens e alimentos não comestíveis por humanos em produtos (carne e leite) para consumo humano por meio da fermentação entérica de carboidratos celulósicos (Duthie et al., 2017).

Os alimentos ingeridos pelos ruminantes, após serem transformados em partículas menores pela mastigação inicial e pela remastigação durante a ruminação, serão decompostos pela ação microbiana. Os microrganismos ruminais, bactérias, protozoários e fungos, degradam a maioria dos componentes poliméricos da alimentação e depois fermentam os monómeros e oligômeros resultantes (Janssen, 2010).

Os produtos da fermentação são principalmente os ácidos graxos (AG) voláteis acetato, propionato e butirato, embora também sejam formados compostos tais como o formato, o etanol, o lactato, o succinato e os AG de cadeia ramificada. Além disso, a amônia, CO_2 e o H_2 são produzidos. Os principais AG voláteis (AGV), acetato, propionato e butirato,são fundamentais para os requisitos de energia e carbono dos ruminantes e são amplamente absorvidos pela parede do rúmen (Janssen, 2010).

A formação do acetato e do butirato, principalmente como resultado da fermentação de carboidratos estruturais (embora quantidades razoáveis de butirato sejam produzidas a partir de carboidratos solúveis), resultam na produção de H_2, que é um substrato usado pelas *Archaeas*metanogênicas para reduzir o CO_2. O resultado final dessa reação é a produção de CH_4. O propionato, por outro lado, produzido em grande parte pela fermentação de carboidratos não-estruturais, serve como uma via competitiva para a utilização do H_2 no rúmen e é acompanhado por uma diminuição na produção de CH_4 (Hegarty, 1999).

A dieta tem grande efeito sobre a população microbiana ruminal, o padrão de fermentação e as proporções dos AGV; essas variáveis diferem principalmente em função das proporções volumoso:concentrado das dietas (Fernando et al., 2010; McCann et al., 2014). Animais alimentados com dietas forrageiras produzem maior proporção de AGV,

principalmente acetato, e, portanto, maior quantidade de H_2 está disponível para a metanogênese, enquanto que os animais alimentados com dietas com alto teor de grãos produzem maior proporção de propionato e, portanto, menos H_2 está disponível para a produção de CH_4 (Janssen, 2010).

Enquanto o tipo de carboidrato presente na dieta parece determinar a população microbiana presente no rúmen e, portanto o perfil de AGV, outros mecanismos como pH ruminal e taxa de passagem também influenciam na produção total de CH_4, diretamente em organismos metanogênicos ou indiretamente através de mudanças na taxa de digestão (Ellis et al., 2008)

Além da interferência da dieta nas emissões de metano por bovinos, Roehe et al. (2016) relataram que os microrganismos ruminais foram influenciadas pelo genótipo do animal. Esses autores sugeriram que a abundância de *Archeas* na digesta ruminal está sob controle genético podendo ser usada para selecionar geneticamente animais que produzem menor quantidade de metano.

Além disso, Wallace et al. (2015) demonstraram a influência dos microrganismos presentes no rúmen nas emissões de CH_4. Os autores encontraram relação positiva entre a abundância relativa de *Archaeas* em amostras de rúmen coletadas no abate e o CH_4 produzido e emitido pelos animais (Wallace et al. 2014).

1.5. Mensurações de metano pela técnica SF_6

A mensuração exata e/ou precisa da emissão de CH_4 dos animais, além de necessária para estabelecer inventários nacionais, também auxilia na determinação das emissões decorrentes de práticas de manejo, avaliação de estratégias de mitigação e desenvolvimento de protocolos de quantificação (Machado et al., 2011). Existem diversas técnicas sendo usadas em todo o mundo para quantificar a emissão de CH_4 entérico, as quais diferem em sua aplicação, custo, acurácia e precisão (Hammond et al., 2016).

A utilização de câmaras respirométricasé considerada como "padrão-ouro" por apresentar resultados mais precisos para medições de CH_4(Blaxter e Clapperton, 1965; Grainger et al., 2007). No entanto, essa metodologia apresenta elevado custo, exige mão de obra demasiada, além de não poder ser utilizada no ambiente de produção do animal. Os animais necessitam serem treinados para evitar alteração do comportamento e possíveis reduções do consumo de matéria seca (CMS)(Johnson e Johnson, 1995;Arthur et al., 2017).

Na busca de superar essas restrições, a técnica do gás traçador hexafluoreto de enxofre (SF_6) foi desenvolvida por Johnson et al. (1994). A técnica do SF_6 tem sido amplamente

utilizada uma vez que elimina a necessidade de confinamento do animal, permitindo que as emissões de CH_4 sejam mensuradas em animais em pastejo, além de possibilitar mensurações individuais, em um grande número de animais simultaneamente(Clark et al., 2005).

A técnica envolve a utilização de um tubo de permeação carregado com o gás SF_6, que fica inseridono retículo-rúmen dos animais. O SF_6 é utilizado por apresentar taxa de liberação constante e previsível, não intervir na fermentação ruminal, ser detectado em concentrações baixas, ser inerte e não ser tóxico (Primavesi et al., 2004;Muñoz et al., 2012).

Nessa técnica, a emissão do gás SF_6 proveniente do tubo de permeação simula a emissão de CH_4no rúmen e considera-se que a diluição desses gases na atmosfera é idêntica (Johnson et al., 1994). Assim, a taxa de emissão de metano é calculada pela seguinte fórmula: $QCH_4 = QSF_6$ x ($[CH_4]/[SF_6]$), onde QCH_4 é a taxa de emissão de metano, QSF_6 é a taxa de liberação de SF_6 no tubo de permeação e $[CH_4]$ e $[SF_6]$ são as concentrações dos gases na canga amostradora(Johnson e Johnson, 1995).

Johnson et al. (1994) validaram a técnica SF_6 constatando que a produção de CH_4correspondeu a 93% das emissões obtidas em câmararespirométrica, sem diferenças significativas. No estudo de Oss et al. (2016) a emissão de CH_4 pela técnica do SF_6 correspondeu a 81,5% da medida em câmara respirométrica (87,9 e 107,9, respectivamente). Quando as emissões de CH_4 foram ajustadas para o CMS e peso corporal (PC) dos animais não houve diferenças entre as técnicas.

As emissões de CH_4 obtidas por essa técnica podem apresentar valores inferiores aos observados em câmaras respirométricas, uma vez que aproximadamente 3% da produção total de CH_4 pode ser excretada via retal (Muñoz et al., 2012), o que não pode ser detectado pela técnica do SF_6.

Para o cálculode produção de CH_4, é necessário a aferição das concentrações de CH_4 e SF_6 do ambiente, para posterior correção das concentrações medidas nos animais(Lassey, 2013). As diferentes massas moleculares do CH_4 (16 g/mol) e do SF_6 (146 g/mol) podem fazer com que estes gases se dispersem e se acumulem diferencialmente no ambiente (Williams et al., 2011).

Vários fatores contribuem para a variabilidade nas medições de CH_4 pela técnica SF_6. Em relação aos tubos de permeação, os valores de fluxo de SF_6 são diferentes e assim a taxa de emissão do SF_6 é uma fonte potencial de variação nas emissões de CH_4 calculadas, o que contribui para a variação observada entre animais (Vlaming et al., 2007). Por isso, o cuidado para estimar essa taxa em cada tubo deve ser considerado antes de se avaliar qualquer estratégia de redução de CH_4.

A taxa de permeação (TP) do SF_6 tem efeito positivo sobre os valores de CH_4(Pinares-Patiño et al., 2008). Esses autores observaram que quando essa taxa variou de 2,62 a 5,68 mg/d, o efeito da TP na emissão diária de CH_4 foi mais importante do que o CMS, representando entre 6 e 21% da variação de CH_4. No entanto, quando a TP utilizada estava em um intervalo menor (2,21 e 3,59 mg/d) o efeito da TP sobre as emissões de CH_4 não foi significativo (4% da variação). Assim, torna-se imprescindível utilizar tubos com TP variando em um intervalo menor, para que se obtenham as estimativas mais acuradas e precisas das emissões de CH_4.

Deighton et al. (2013) avaliaram o efeito do tempo pós-calibração e da duração do tubo no rúmen sobre a taxa de liberação do SF_6 e concluíram que a queda na liberação de SF_6 não sofre interferência do ambiente ruminal, e que ela ocorre em função do tempo após a calibração. Assim, é necessário que esse declínio seja contabilizado para evitar valores superestimados das emissões de metano.

Deighton et al. (2014) demonstraram que o padrão diário das emissões de metano de vacas em câmaras respirométricas é relacionado ao padrão de consumo de ração. Por outro lado, emissão diária de SF_6 é constante e independente do padrão de emissão de metano. Assim, a técnica SF_6 não deve ser o método de escolha para investigar a dinâmica da emissão de metano diária (Broucek, 2014).

A técnica do SF_6 pode proporcionar maiores variações entre dias de coleta ou entre animais avaliados (McGinn et al., 2006; Pinares-Patiño et al., 2011). Por isso, Boadi et al. (2002) alertaram para a necessidade de maior número de animais em estudos utilizando a técnica do SF_6, na tentativa de reduzir essas variações.

Arbre et al. (2016) avaliaram a repetibilidade (R) da técnica SF_6 para mensuração das emissões de CH_4 entérico em bovinos. Para atingir um valor R de 0,70 para as emissões de CH_4 (g/kg CMS) foi necessário um período de três dias de medições. Uma outra aplicação deste trabalho foi estimar o número de animais necessários para experimentos futuros. Arbre et al. (2016) constataram que é necessário de seis a oito animais por grupo experimental para detectar uma diferença de 20% nas emissões de CH_4 entre diferentes tratamentos.

Desde o seu início, a técnica do gás traçador para estimar as emissões de metano sofreu vários ajustes (Williams et al., 2011). Os estudos que foram conduzidos comparando a técnica SF_6 às mensurações realizadas em câmaras respirométricas confirmam a sua eficiência para estimar a produção de CH_4 por ruminantes, como método de escolha para animais em pastagem. Ao longo dos anos foram sugeridas diversas modificações para a técnica original do SF_6, a fim de que os dados de emissões de CH_4 sejam mais confiáveis, além de quantificar

as emissões de GEE pelo Brasil e possibilitar que diferenças entre tratamentos possam ser encontradas.

1.6. Estratégias para mitigação da produção de metano por ruminantes

O impacto ambiental da produção de carne ao longo dos anos conferiu avanços consideráveis na eficiência produtiva dos sistemas de criação, principalmente relacionados a nutrição e a genética (Capper, 2011).

1.6.1.Manejo alimentar

A quantidade e qualidade da dieta são os fatores de maior importância na produção de CH_4, e por isso, vários modelos foram desenvolvidos para prever as emissões com base na composição da dieta (Escobar-Bahamondes et al., 2016; Mendes et al., 2016; Liu et al., 2017).

Estudos indicam que os animais terminados em confinamento emitem menores quantidades de CH_4 por kg de peso de carcaça e que os sistemas baseados em pastagem têm maiores emissões desse gás, o que é atribuído à dieta mais fibrosa, maior tempo da fase de acabamento e menor peso das carcaças (Capper, 2012; Desjardins et al., 2012; Lupo et al., 2013; Pelletier et al., 2010; Stackhouse-Lawson et al., 2012; Swain et al., 2018).

O aumento da proporção de concentrados na dieta reduz as emissões CH_4, tanto em relação ao consumo de energia quanto por unidade de carne produzida (Hristov, et al., 2013). No entanto, o aumento do concentrado pode aumentar as emissões líquidas totais, pois mais grãos devem ser cultivados, processados e transportados, levando ao aumento de fontes adicionais de emissões associadas à infraestrutura de produção e ao transporte (Beauchemin et al., 2008). A erosão do solo de terras usadas para produzir culturas para alimentação animal é um importante indicador de sustentabilidade e deve ser incorporada à contabilização da avaliação do ciclo de vida de carne bovina, mas geralmente tem sido excluída (Stanley et al. 2018).

Capper (2012) avaliou o impacto ambiental de diferentes sistemas de produção de carne bovina, baseado no metabolismo e na exigência de nutrientes, além da quantificação dos insumos e produção de resíduos. O sistema convencional (terminação em confinamento) exigiu 56,3% dos animais, 24,8% da água, 55,3% da terra e 71,4% da energia necessária de combustíveis fósseis para produzir $1,0 \times 10^9$ kg de carne bovina em comparação com o sistema alimentado com capim. A pegada de carbono para $1,0 \times 10^9$ kg de carne bovina foi menor no sistema convencional ($15,989 \times 10^3$ t) do que no sistema de pastagem ($26,785 \times 10^3$ t), mas todos os sistemas de produção de carne bovina foram potencialmente sustentáveis.

Os sistemas de pastejo intensivo consistem de intervalos de curto pastoreio com alta densidade animal. Os potenciais benefícios desses sistemas incluem as reduções no pastoreio excessivo e erosão do solo, melhor utilização de forragem e produtividade animal, além do aumento do sequestro de carbono de C do solo, o que pode reduzir as emissões líquidas de GEE (Beauchemin et al., 2008; Teague et al., 2016).

Estudos anteriores não conseguiram demonstrar de forma conclusiva que a melhoria do manejo de pastagens intensivas e bem manejadas reduziria as emissões de CH_4. No entanto, trabalhos recentes utilizando a avaliação de ciclo de vida sugerem que o manejo intensivo do pasto facilita o sequestro de C no solo além de reduzir significativamente as emissões de GEE (Cardoso et al., 2016; Griscom et al., 2017; Stanley et al., 2018).

Em um estudo de metanálise, Dawson et al. (2011) compararam a pegada de carbono de dois sistemas de produção, um sistema intensivo e um sistema baseado em forragem. Os valores encontrados foram semelhantes para ambos os sistemas, indicando que é possível reduzir a pegada de carbono da produção de carne bovina pela utilização ótima de forragem.

As emissões de diferentes tipos de forragem (expressas como energia metabolizável (EM)/kg de MS) foram medidas por Waghorn e Clark (2006). Quando expressos em CO_2 equivalente/ganho de carcaça (g/dia), os resultados demonstraram que, à medida que a qualidade da pastagem melhorou, as emissões de metano por ganho de carcaça diminuíram.

De acordo com Soussana et al (2010) várias práticas de manejo reduzem as perdas de C e aumenta o armazenamento de C nos solos, entre elas evitar o revolvimento do solo e a intensificação das pastagens. Em conclusão, o armazenamento de C tem um forte potencial para mitigar o balanço de GEE dos sistemas de produção de ruminantes a pasto.

1.6.2. Composição racial

Quanto às diferenças genéticas, os trabalhos são escassos e abordam principalmente a criação seletiva de animais que utilizam os alimentos de forma mais eficiente ou produzem menos CH_4 por unidade de CMS (Hegarty e McEwan, 2010; Hegarty et al., 2010; Martin et al., 2010; Wall et al., 2010).

Eckard et al. (2010) sugeriram que a seleção de animais poderia causar uma redução de aproximadamente 10 a 20% no rendimento de metano (em função do CMS). No entanto, é necessário avaliar com cautela a seleção visando menor produção de metano, uma vez que pode haver correlações desfavoráveis entre produção de metano e características de produção, por exemplo (Eckard et al. 2010; Hegarty e McEwan 2010; Wall et al. 2010).

Além disso, os microrganismos ruminais foram influenciadas pelo genótipo, e consequentemente afetam as emissões de metano, o que foi demonstrado nos estudos de Wallace et al. (2015) e Roehe et al. (2016).

Estimar precisamente a emissão de CH_4 das principais raças de bovinos de corte criadas no Brasil irá contribuir para o desenvolvimento de estratégias de mitigação de GEE, reduzindo o impacto da produção de carne nas mudanças climáticas.

1.7. Emissão de óxido nitroso a partir da deposição de excretas no solo

As emissões de N_2O provenientes da agropecuária podem ser divididas em emissões diretas e indiretas. As fezes e a urina dos animais contribuem de forma direta, enquanto as emissões indiretas são relacionadas com a proporção do N adicionado aos solos que é volatilizada como amônia (NH_3)(Alves, 2010).

Além do N_2O, outros importantes GEE também são produzidos por fermentação anaeróbia das excretas quando em contato com o solo. O carbono disponível, adicionado ao solo via excretas de bovinos, fornece substrato para a produção do CO_2 e CH_4 por microrganismos do solo (Boon et al., 2014). O CH_4 é produzido principalmente pela presença das fezes, devido à matéria orgânica existente e das condições anaeróbicas logo após sua deposição no solo (Mazzetto et al., 2015).

Existem evidências de que a urina possa ser uma fonte mais importante de N_2O do que as fezes, devido à diferença na excreção de N entre essas excretas (Lessa et al., 2014). O menor valor encontrado para as fezes pode estar relacionado ao N não estar prontamente disponível para a produção de N_2O como o N da urina (ureia). A quantidade de N excretada na urina é aproximadamente 65% maior do que nas fezes (Rodrigues et al., 2008).

Sordi et al. (2014) avaliaram o impacto da urina e fezes de bovinos nas emissões de óxido nitroso (N_2O) em pastagens, uma vez que informações específicas sobre essas emissões ainda são escassas em regiões subtropicais e tropicais brasileiras. Os picos de emissão de N_2O ocorreram em média 17±9 dias após a aplicação, tanto para urina quanto fezes, reduzindo para as concentrações basais após 41 dias para a urina e 49dias para as fezes.

As quantidades de N_2O emitidas a partir dos solos são geralmente proporcionais às entradas de N, mas também são dependentes das interações entre os fatores climáticos, as propriedades do solo e as práticas de manejo (Saggar et al., 2004a).

As variáveis do solo e clima são essenciais para explicar os fluxos de GEE do solo. Dentre as condições meteorológicas, a precipitação e a temperatura média do ar estão fortemente relacionadas às emissões de N_2O (Zanatta et al., 2014). Estudos têm encontrado

uma relação estreita entre as variações diurnas da temperatura do ar e os fluxos de N_2O, com um padrão de fluxo mais alto durante o dia e menor durante a noite, uma vez que a temperatura do solo acompanha as flutuações da temperatura do ar (Akiyama et al., 2000, Livesley et al., 2008).

De acordo com Alves et al. (2012) a temperatura média diária do ar corresponde a mesma mensurada logo após o nascer ou o pôr do sol. Se a temperatura do ar é um importante fator atuante nas mudanças dos fluxos de N_2O observados durante as 24 horas do dia, pode-se supor que nesses dois momentos há maior chance do fluxo de N_2O observado representar a média diária de N_2O.

As condições dos solos, tal como a quantidade de poros preenchidos com água, carbono disponível, temperatura, pH e nitrato afetam as emissões de N_2O (Whitehead, 1995). Outros fatores, relacionados principalmente com a perda de C e N nos solos contribuem direta e indiretamente para aumentar as emissões de GEE na atmosfera (Metay et al., 2007). Na maioria dos ambientes, a formação de N_2O no solo é controlada principalmente pelo C disponível e N mineral, concentração de O_2 no solo, temperatura e espaço de poros preenchidos por água (Granli e Bøckman, 1994).

Saggar et al. (2004b) avaliaram a influência da umidade dos solos, temperatura, C solúvel e disponibilidade de N na forma de amônio (NH_4^+) e nitrato (NO_3^-) nas emissões de N_2O em diferentes tipos de solos. Os resultados mostraram que a entrada de excreta e/ou fertilizante na forma de N e os espaços de poros preenchidos por água foram as variáveis que mais influenciaram os fluxos de N_2O.

De modo geral, o fluxo de N_2O aumenta exponencialmente com a temperatura do solo, o que pode ser explicado pela combinação da expansão em zonas anaeróbias desencadeada pela aceleração da respiração do solo e pela crescente taxa de desnitrificação por unidade de volume anaeróbio (Smith et al., 2003). A saturação de água nos poros do solo também leva à alteração exponencial nos fluxos de N_2O no solo, mas o efeito parece não ser tão rápido (Russow et al., 2000), como demonstrado para mudanças na temperatura do solo.

Em áreas em pousio, a estrutura física dos solos afetou significativamente as emissões N_2O, sendo as maiores emissões ocorridas em solos argilosos e as menores para os arenosos. No entanto, em áreas de pastagens, a diferença nas emissões de N_2O entre os tipos de solo torna-se menos pronunciada, apresentando menor emissão (2,4 a 6,2 vezes) em comparação aos solos sem pastagens. A presença da vegetação resulta em quantidades reduzidas de água e nitrogênio disponível nos solos e, portanto, condições menos favoráveis para a desnitrificação (Jamali et al., 2016).

O estudo de Uchida et al. (2011) mostrou que as emissões de N_2O pela deposição de urina dos ruminantes sofrem interferência da temperatura do ar e da presença de vegetação no solo. Em áreas de pastagem e em temperaturas mais elevadas, as emissões de N_2O foram maiores, o que foi atribuído à maior desnitrificação em resposta às maiores quantidades de C oriundos da vegetação.

Muitos questionamentos ainda persistem, sendo necessários mais estudos para elucidar o impacto das condições climáticas, fatores dos solos e das plantas nas variações das emissões de N_2O induzidas por excretas depositadas em solo. Além disso, as emissões provenientes das excretas dos animais em solos de confinamento permanecem desconhecidos.

1.8. Fator de emissão do óxido nitroso e a técnica decâmaras estáticas

O fator de emissão do N_2O para uma dada fonte de N é o percentual do N aplicado que é emitido como N_2O e, portanto, permite a comparação entre estudos realizados em diferentes condições ambientais e agronômicas (Sordi et al., 2014). De acordo com o IPCC, o fator de emissão para excretas bovinas depositadas em pastagens é de 2% (sem distinção entre urina e fezes), com uma incerteza de 0,7 a 6% (IPCC, 2006). Esse fator é baseado em estudos realizados em condições temperadas e, podem não ser apropriados para regiões tropicais e subtropicais, uma vez que a maioria das pastagens brasileiras está em solos bem drenados, onde a produção de N_2O não é tão favorável, devido à melhores condições de aeração (Sordi et al., 2014).

É importante ressaltar que devido às diferenças nos teores e compostos de N entre fezes e urina, foram encontrados distintos fatores de emissão de N_2O para as excretas (Van Der Weerden et al., 2012; Sordi et al., 2014). No estudo de Sordi et al. (2014), o fator médio de emissão para as fezes (0,15%) foi menor do que para a urina (0,26%), devido ao N da urina ser mais prontamente disponível para a hidrólise do que os compostos de Norgânico das fezes. Estes resultados sugerem a necessidade da avaliação dos fatores de emissão para fezes e urina separadamente e que estes dois excrementos devem ser tratados de forma independente nos inventários de GEE nacionais.

Além disso, é provável que os fatores de emissão para os sistemas mais intensivos sejam maiores do que os sistemas menos intensivos, em função da melhoria da dieta e utilização de fertilizantes (Cardoso et al., 2016).

Em função do aumento das emissões de N_2O e da necessidade de se estabelecer os fatores de emissão do N_2O em condições brasileiras, a Embrapa Florestas criou protocolo para medição de fluxo de gases de efeito estufa dos solos utilizando câmaras estáticas (Zanatta et

al, 2014). Essas câmaras têm sido utilizadas como padrão para a avaliação das emissões de GEE do solo, devido ao custo elevado dos demais dispositivos destinados a este propósito (câmara dinâmica e estações micrometeorológicas) (Zanatta et al., 2014).

O procedimento para as medições de fluxos de GEE dos solos utilizando câmaras estáticas envolve a amostragem manual do gás produzido (Jantalia et al., 2008). Os fluxos de N_2O do solo são frequentemente avaliados com uma única amostragem diária. Para os cálculos das emissões diárias de N_2O é realizada uma extrapolação dessa única medição diária durante um curto período para representar o fluxo médio para um total de 24 horas. Essa extrapolação foi confirmada por Alves et al. (2012) que avaliaram o tempo de amostragem mais adequado para estimar o fluxo médio diário de N_2O a partir de solos.

Alves et al. (2012) monitoraram os fluxos de N_2O de solos em dois locais com condições climáticas contrastantes, Edimburgo, no Reino Unido, e Seropédica, no Rio de Janeiro. Para ambos os locais, as noites (entre 21:00 e 22:00h) e as manhãs (entre 09:00 e 10:00h) foram os momentos em que a medição apresentou melhor representatividade da média diária do fluxo.

Assim, como vários fatores climáticos, dos solos e das plantas interferem nas emissões de N_2O, espera-se que diferenças nos fatores de emissão também sejam observados. Fatores de emissão de N_2O encontrados para urina e fezes na estação chuvosa foram 1,93 e 0,14%, respectivamente, e 0,01 e 0% para urina e fezes na estação seca. A adoção desses fatores separados por excreta e por época do ano teve grande impacto na redução das estimativas de emissões de N_2O obtidas, uma vez que apresentou valor inferior ao proposto pelo IPCC (2006) (Lessa et al., 2014).

A época do ano para a deposição de excretas não teve impacto no fator de emissão em solos arenosos, mas os valores médios foram maiores no verão (1,59%) do que na primavera (1,14%) e outono (0,55%) nos solos argilosos (Rochette et al., 2014).

Assim, o padrão de 2% do fator de emissão proposto pelo IPCC (IPCC, 2006) para os excretas de bovinos são superestimados para as condições brasileiras, o que ressalta a importância de se calcular o fator de emissão com base na mensuração da emissão de N_2O pelas fezes e urina separadamente.

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CAPÍTULO II– ARTIGO I: PUBLICADO NA PLOSONE

Could the breed composition improve performance and change the enteric methane emissions from beef cattle in a tropical intensive production system?

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2.1.ABSTRACT

Crossbreeding has been used to improve performance in beef cattle, however the effects of breed composition on methane (CH_4) production, yield and intensity from cattle raised in tropical intensive and integrated systems remain unknown. To assess how breed composition affects performance and methane emissions, 70 animals of two breed compositions, Angus x Nellore crossbred (AN) and Nellore (Nel), were compared in an intensive production system - rearing in integrated crop-livestock (ICL) system and finishing in feedlot. The animals grazed on ICL system in the rearing phase (stocking rate 5.5 AU/ha,

herbage mass 4,884 kg DM/ha, forage allowance 5.9 kg DM/100kg BW). In finishing phase, the animals were fed with 35% corn silage and 65% concentrate. Eight different animals of each breed composition were selected in each period within each year to measure CH_4 production. Enteric CH_4 measurements were collected using a sulfur hexafluoride (SF_6) tracer technique and DMI was determined using titanium oxide in both periods. Compared with Nel, AN had both superior total gain and ADG in the grazing period. Also, the AN presented greater ADG in the feedlot with a shorter finishing period, and resulted in greater carcass yield and carcass ADG. Methane production (kg/period) was lower in Nel (19% less) than AN in grazing (P<0.01), and no difference in the feedlot was observed. Nel had greater CH_4 intensity (g CH_4 per unit of ADG) compared to AN in the feedlot. Breed composition did not influence the CH_4 yield (g CH_4 per unit of DMI) in the pasture phase or in the feedlot, despite the difference in DMI (kg/day) in feedlot. In conclusion, crossbreeding may be an option to improve performance and reduce the CH_4 emission intensity in intensive and integrated system under tropical climate conditions, resulting in lower methane emission per kg of meat produced.

Keywords: Greenhouse gas emission, Ruminants, Sustainable intensification, Grazing, Feedlot, Integrated systems

2.2.INTRODUCTION

The population around the world has been growing rapidly and has a corresponding increase in food demand. The improvement in environmental efficiency of beef production systems seems to be, at least for the foreseeable future, part of the solution for the issue of global food security [1]. Notwithstanding, ruminant livestock systems are under continued political pressure to reduce their greenhouse gas (GHG) outputs.

Cattle production is an important driver for Brazil's economy, and ranks second worldwide, with approximately 212 million head [2]. Additionally, Brazil is the largest beef exporter, maintaining trade relations with 180 countries [3]. Traditionally, the national herd is created mainly in an extensive system of production, being the main source of feeding constituted of pastures that occupy great extensions of earth. In the last thirty years, there has been a notable change in beef production systems in Brazil, with livestock farming gradually occupying less area with higher production and productivity gains [3].

The modern, intensive livestock systems, like beef production in grain-finishing systems, offer both substantially lower land requirements and greenhouse gases (GHG)

emissions per kilogram of meat than traditional, extensive ones [4]. However, the GHG emissions reduction by ruminants using adaptive grazing systems has been shown in some studies [5, 6]. This decrease was attributed to the quality and productivity of the pastures, and potentially increase soil carbon sequestration thereby negating emissions into the atmosphere [7]. Therefore, the best option could be a system that mix grass-fed and grain-fed in the different cattle growth phases.

On the other hand, genetic improvement in beef cattle has a potential for reducing CH_4 emissions [8, 9]. The Zebu (*Bos indicus*) animals, for example, are quite resistant and adaptable to tropical climates and, because of that, the Nellore is the most prevalent breed in Brazil. However, *Bos taurus*animals demonstrates greater yield potential, especially under appropriate conditions [10]. Thus, crossing breeds could be a viable alternative to improve the production rates of cattle purebred herds in this climate conditions. Faster-growing animals can be more efficient in quantity of product produced, because they should theoretically partition relatively more feed nutrients into production. Thus the output of polluting excretion products on a per unit product basis should be less for these animals [11].

Due to the contribution of livestock in GHG, there is a strong motivation for the measurement of enteric CH_4 to be accurately performed. Besides this, methane emission inventories are based on models developed in temperate climates and, therefore, precise methane measurements of tropical region production systems are crucial to reduce the uncertainties of these inventories and evaluate GHG mitigation strategies [12].

The objective of this trial was to examine the animal performance and enteric CH_4 production, yield and intensity from two breed compositions in a Brazilian beef cattle production system– rearing in integrated crop-livestock system and finishing in feedlot. Our hypothesis was that: *(i)* Performance of crossbred animals would be superior than Nellore in a Brazilian beef cattle production system; and (*ii*) CH_4 yield and intensity would be lower for crossbred animals compared to Nellore.

2.3.MATERIALS AND METHODS

Treatments and Experimental Design

The experiment was conducted at Brazilian Agricultural Research Corporation – EmbrapaMilho e Sorgo (SeteLagoas, Minas Gerais, Brazil; 19°28′S; 44°15′W, at 732 m altitude). Climate data for the experimental period was obtained at the meteorological station located at Embrapa and are presented in figure 2.1.

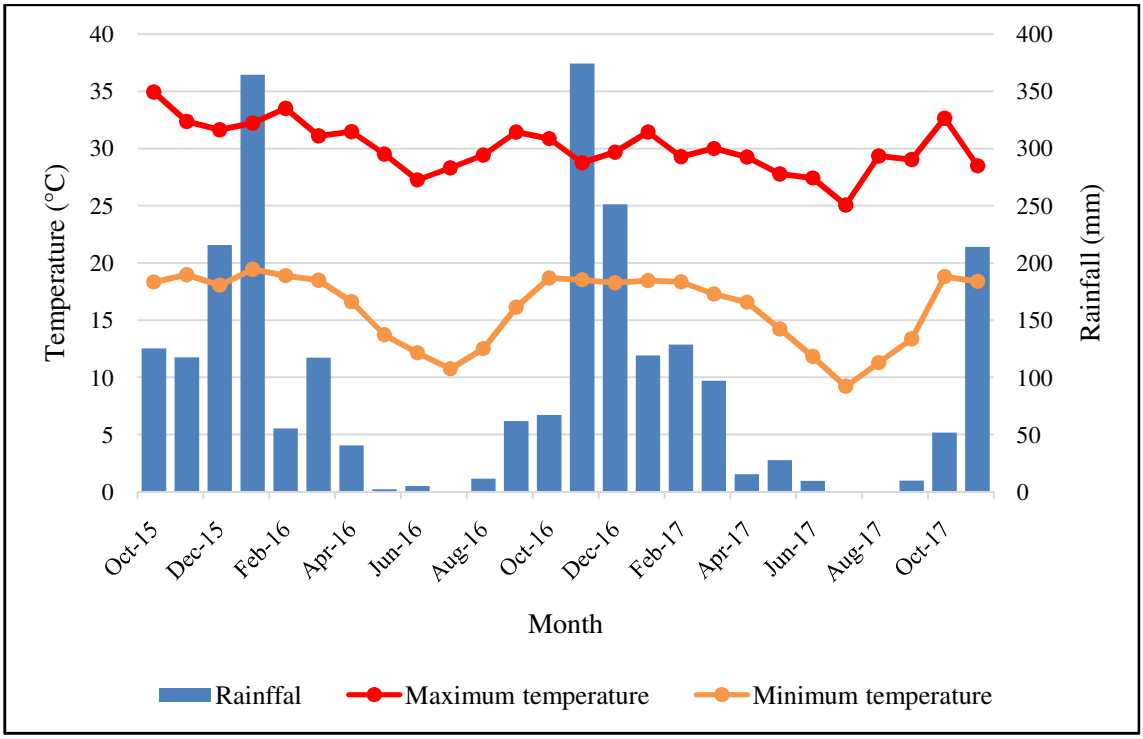

Figure 2.1. Climate data for the experimental period from October 2015 to November 2017, measured at the Embrapa Maize and Sorghum Research Centre meteorological station, SeteLagoas, MG, Brazil

All experimental procedures used in this experiment were approved by the Ethics Committee for Animal Use of Universidade Federal de Minas Gerais (UFMG, protocol number 326/2014).

At trial onset, 10 mo old steers were divided into two groups according to their breed composition as follows: Nellore (171.5 ± 19.47 kg, n=10), Angus x Nellore crossbred (214.2 ± 26.41 kg, n=10) in the first year and Nellore (215.8 ± 32.34 kg, n=25), Angus x Nellore crossbred (242.5 ± 32.26 kg, n=25) in the second year.

Grazing Management

The animals were evaluated in the rearing period, with initial age of 10 months, in the integrated crop-livestock (ICL) system under no-tillage system adopted since 2005.

The pasture consisted of *Megathyrsus maximus* cv. Mombaça and the total pasture area of 5.5 hectares (ha) was subdivided into five sub-paddocks of approximately 1.1 ha each, used as a rotational grazing system with seven days grazing period and 28 days of rest. The experimental grazing period lasted 230 and 216 days in the first and second year, respectively. All animals were drenched with an anthelmintic prior to the start of grazing.

The energetic-protein supplement (Table 2.1) was offered *ad libitum* throughout the grazing period in a collective feeder. Supplement daily intake was estimated by dividing the total supplement consumed by the number of animals for each day in each period. As the supplement was offered *ad libitum* in a collective feeder, consumption might be different among animals related to self-intake regulations.

Table 2.1. Percentage of ingredients of the energy-protein mineral supplement used in pasture test and TMR diet used in feedlot

Ingredients (%DM)	PastureSupplement		Feedlot
	Year 1	Year 2	TMR diet
CornSilage	-	-	35
Corngrain, ground	-	-	54
Cornglutenmeal	84	86	-
Soybeanmeal	5	7	5
Mineral Salt*	11	7	6

* Amountsofminerals (per kg ofsupplement):Year 1: phosphorus (P), 9 g; calcium (Ca), 20 g; sulfur (S), 16 g; magnesium (Mg), 2 g; sodium (Na), 37 g; zinc (Zn), 600 mg; copper (Cu), 150 mg; manganese (Mn), 140 mg; cobalt (Co), 20 mg; iodine (I), 17 mg; selenium (Se), 3 mg; iron (Fe), 100 mg. Year 2: P, 6 g; Ca, 20 g; S, 16 g; Mg, 1.4 g; Na, 9 g; Zn, 450 mg; Cu, 100 mg; Mn, 100 mg; Co, 14 mg; I, 12 mg; Se, 2 mg; Fe, 100 mg. Feedlot: P, 18 g; Ca, 50 g; S, 10 g; Mg, 20 g; Na, 30 g; Zn, 1303 mg; Cu, 375 mg; Fe, 500 mg; Mn, 520 mg; Co, 50 mg; I, 50 mg; Se, 9 mg; Fe, 500 mg; lasalocid sodium, 450 mg

Available herbage mass (AHM) was sampled within each paddock by cutting 5 randomly selected quadrats (1.0 m × 1.0 m) to ground level (5-cm stubble height) using hand shears before grazing. All collected herbage from each strip was collected, weighed and subsampled. A subsample (fresh weight) of the herbage sample from each quadrats was dried for 72 h at 65°C and was taken for subsequent chemical analysis.

A further subsample was manually separated in leaf, stem, and dead content, and was dried for 72 h at 65°C. Leaves were used to characterize the composition of the food ingested by the animals. It was decided to evaluate only leaf, since it represented almost all the forage sampled (above 60%).

The forage allowance (kg dry matter [DM]/100 kg BW/day) was calculated by the ratio of forage production (kg DM/day) to total body weight of animals. In year one, there were 20 additional testers animals, that did not belong to the evaluated genetic groups and remained on pasture throughout all the experimental period.

Feedlot Management

In the feedlot, the animals were divided into groups according to the breed composition. The feedlot period began in June of each year, and the animals were allocated to pens measuring 20 x 12 m each and equipped with feed lanes and drinkers. The pens had enough space to ensure adequate animal well-being, with the minimal 18.5 m^2 area per animal, observed in pens with 13 animals (year 2). All animals were drenched with an anthelmintic prior to the start of feedlot.

The cattle were fed three times per day – at 0700, 1100 and 1600 h. The amount of food supplied was adjusted daily to maintain 5 to 10% refusals. The amount of feed given was recorded per pen, and refusals were weighed daily. Feed samples were taken monthly for chemical analysis.

The animals were adapted to the experimental diets for 21 days. Initially, 60% corn silage and 40% concentrate diet were supplied, the amount of concentrate was increased until the ratio of roughage: concentrate was 35:65 (DM base). The diet was formulated to allow for 1.4 kg average daily weight gain [13] and consisted of corn silage, ground corn, soybean meal, and trace mineral mixture (Table 2.1).

A gain of 200 kg BW during the feedlot period was stipulated as the slaughter criterion. Animals remained in feedlot for 111 and 105 days (AN) and 138 and 127 days (NEL) in the first and second year, respectively.

Animal performance was determined monthly by recording body weight (BW) following a fast of food and water for 16 hours. The average daily gain (ADG) was calculated as the difference between the final body weight (FBW) and the initial body weight (IBW) of each period (grazing and feedlot), divided by the total number of days

On the day of slaughter, animals were weighed in the morning, before being sent to the slaughterhouse, where they were kept fasting for 24 hours with only *ad libitum* water intake. All the animals were slaughtered in a commercial slaughterhouse, according to the humanitarian procedures required by Brazilian legislation. The weight of hot carcass (WHC) was recorded immediately after the carcass was cleaned. Carcass yield (CY) was calculated by the ratio of WHC to FBW. The mean daily weight gain of carcass (ADGc) was calculated according to Eq. (1):

$$\boldsymbol{ADGc} = \frac{[\boldsymbol{WHC} - (\boldsymbol{IBW} \times \mathbf{50\%})]}{\boldsymbol{daysinfeedlot}} \quad (1)$$

Methane Production Measurement

Enteric CH_4 emissions were measured using the sulfur hexafluoride (SF_6) tracer technique reported by Johnson [14] and modified by Deighton [15] during three periods - feedlot in first year, grazing and feedlot in second year. Technical problems prevented the measurement of methane in the first year of grazing.

Eight animals from each breed composition were evaluated in each period. Enteric CH_4 emissions were measured for at least 3 days per animal. Animals that did not meet this requirement were not used in the statistical analysis. According to Arbre [16] a 3-days period is necessary to achieve an R of 0.70 for CH_4 emissions by SF_6 technique and the number of required animals to detect a difference of 20% in CH_4 emissions among treatments is 6–8 animals per group.

Ten days before the beginning of each measurement, a SF_6 permeation tube was introduced directly into the rumen of each animal via the esophagus. The permeation rates were 4.44 ± 0.28; 4.60 ± 0.39 and 4.29 ± 0.06 mg/d (mean ± SD) in feedlot first year, grazing second year, and feedlot second year, respectively, as given by an 8-weeks calibration assay in a controlled environment at 39°C.

Expired gases were collected with a sampling apparatus containing a collection canister made of polyvinyl chloride (PVC) equipped with a capillary tube (0.127 mm diameter). The capillary was calibrated to allow the vacuum inside the canister remaining at 40-60% of the initial vacuum after 24 h of measurement. If the pressure inside the canisters was below or above the 40-60% range, gas samples were not collected. Additionally, an identical set was used to collect background air samples at two points at the same time canisters were collected from animals.

Canisters were removed daily at 0900 h, evacuated, and replaced then the contents were sampled. Animals were moved to a chute area for each canister evacuation, and total time to sample and replace canisters for all animals in both breed compositions groups was approximately 1 h. To collect enteric CH_4 and SF_6 samples, the canisters were vacuumed to approximately −12 PSI with vacuum pump. After the collection period, canisters were individually connected to dilution system, and the final pressure was recorded. Nitrogen was then added slowly until canister pressure reached +13 PSI. Pressure readings were recorded to calculate the dilution factor [17]. After pressurization, the contents of the canisters were transferred under positive pressure to four pre-evacuated 20 mL Exetainers vials (Labco Limited, Lampeter, UK) for each animal.

The breath gas samples collected were analyzed immediately after the end of the experimental period. Analysis of CH_4 and SF_6 concentrations were determined by gas

chromatography at the Laboratory of Gas Chromatography, Embrapa Dairy Cattle, in Juiz de Fora, Minas Gerais, Brazil. The SF_6 (ppt) and CH_4 (ppm) concentrations in the sampling canisters were determined using two separate gas chromatographs; models 6890 N plus and 7820A, respectively (Agilent Technologies, Santa Clara, CA). Both chromatographs were equipped with a split-splitlessinjector, but a μECD detector (electron capture) was used to measure SF_6 and a FID (flame ionization detector) was used to measure CH_4 concentration.

For SF_6 analysis, a column (HP-Molsieve, Agilent Technologies, Santa Clara, CA) was used with N_2 as carrier gas at a flow rate of 5.0 mL/min with N_2 as the makeup gas at 40 mL/min, with μECD detector. The gas chromatograph was calibrated weekly using SF_6 (White Martins, São Cristóvão, RJ) standards ranging in concentrations from 30, 100, 500, 1500, 3000 ppt. The CH_4 was analyzed using two columns, (HP-Plot/Q and HP-Molsieve, Agilent Technologies, Santa Clara, CA) with H_2 as carrier gas at a flow rate of 7.0 mL/min, with FID detector. The gas chromatograph was calibrated using CH_4 (Linde AG, Rio de Janeiro, RJ) at 4.8, 9.7, 19.6, 102, 203 ppm.

The CH_4 emission rate (RCH_4, g/d) for each animal was calculated using the SF_6 and CH_4 mixing ratio (μmol/mol) sampled by the canisters on the animals (SF_6 and CH_4 canister, respectively) and those used for background (SF_6 and CH_4 background, respectively), and the predetermined SF_6 release rate (RSF_6, g/d) from the permeation tubes, where molecular weights (MW) of the gases is $MWCH_4 = 16$ and $MWSF_6 = 146$, as described by [18], using Eq. (2):

$$\boldsymbol{RCH_4 = RSF_6 \times \left[\frac{(CH_4 canister - CH_4 background)}{(SF_6 canister - CH_6 background)}\right] \times \left[\frac{MWCH_4}{MWSF_6}\right] \times 100} \quad (2)$$

Individual animals methane emissions were expressed as methane production (g CH_4/animal/day, kg CH_4/year, and kg CH_4/period), methane yield (g CH_4/kg DMI) and methane intensity (g CH_4/kg ADG), besides g CH_4/kg $BW^{0.75}$.

Intake Measurement

Individual DMI was determined for eight animals from each group in each period (grazing or feedlot, year 1 and 2), the same animals used for the methane measurement. Titanium dioxide (TiO_2) was used as intake marker, and 10 g were administered to the animals once daily for 12 days during each period. TiO_2 was stored in paper cartridges and

introduced directly into the esophagus of the animals at 0900 h with the aid of a PVC applicator.

Fecal samples were collected once daily during the last 5 days of the dosage period. Samples of feces corresponding to the different collection times composed a sample for each animal. Feces were dried at 65 ºC until constant weight. Dried feces were ground through a 1mm screen with a Wiley mill and analyzed by atomic absorption spectrophotometry.

TiO_2 content was determined according to Myers [19]. The standard curve was prepared using 2, 4, 6, 8 and 10 mg TiO_2 and the spectrophotometer readings were recorded at a wavelength of 410 nm. For the calculation of fecal production (FP) estimated by TiO_2, the following formula was used (Eq. 3):

$$FP = \frac{TiO_2 supplied}{TiO_2 in feces/DM\ 105°C} \quad (3)$$

where FP = fecal production obtained by TiO_2, g DM/day; TiO_2 supplied = amount of TiO_2 supplied to the animals per day (10 g); TiO_2 in feces = percentage of titanium in feces, %; DM 105ºC = the dry matter of feces at 105 ºC.

Fecal Production and indigestible NDF (iNDF) were used to estimate dry matter intake (DMI, kg/day) for each animal. Indigestible NDF was used as the internal marker and obtained after *in situ* incubation of a diet (iNDF diet) and feces (iNDF feces) samples for 288 hours in the rumen of a fistulated bovine [13]. Follows equation (Eq. 4) used for DMI:

$$DMI = FP \times \left(\frac{iNDF feces}{iNDF diet}\right) \quad (4)$$

Average daily DMI during the methane measurement period and CH_4 emission rate were used to calculate methane yield (g CH_4/kg DMI).

The average BW, ADG, DMI and feed and conversion efficiency were calculated over the same CH_4 measurement period in both grazing and feedlot.

Chemical Analysis

Forage samples, supplements, diets, and refusals of foods were collected, oven-dried in a forced-ventilation oven at 65°C, for at least 72 hours, and ground in a Willey mill (Alpax, Diadema, SP, Brazil) through a 1-mm sieve.

The constituents were determined as described by Latimer Jr. [20], according to the following methods: dry matter (DM), 934.01; crude protein (CP), 984.13 (Leco FP-428,

Australia Pty Ltd., Castle Hill, New South Wales, Australia); neutral detergent fiber (NDF), 2002.04; acid detergent fiber (ADF), 973.18; ether extract, 920.85; and ash (500°C furnace for 6 h), 938.08.

Statistical analysis

To evaluate the animal performances a completely randomized design was used. Data for daily DMI were averaged per animal per 5-d period. The methane production data was averaged per animal per 3-d period minimum.

Breed composition, year and the interaction between year and breed composition were included in the model, as fixed effect. The distribution of model residuals was tested for normality using Shapiro-Wilk test and for uniformity using the Cochran test.

The mathematical model used was: $Y_{ijk} = \mu + B_i + Y_j\ (BY)_{ij} + \varepsilon_{ijk}$, in which: Y_{ij} is the observation of the animal k, from the breed i, in year j, μ is the mean effect; B_i is the fixed effect of the breed composition i, (i = 1, 2); Y_j is the fixed effect of the year j, (j = 1, 2); $(BY)_{ij}$ is the interaction effect breed i and year j and ε_{ijk} is the random error associated with each animal.

Statistical analysis was performed using PROC GLM from SAS software (version 9.2; SAS Inst. Inc., Cary, NC). Means were compared using the Fisher's test. Treatment differences were considered significant at $P<0.05$.

2.4.RESULTS

GrazingandFeedlot Diet Characteristics

Forage production during the grazing period was satisfactory and corresponded with average herbage mass (AHM) of approximately 3,884 kg DM/ha. Stocking rate was higher in the second year (2880 versus 2025 kg BW/ha in the first year), and forage allowance (kg DM/100 kg BW) was 6.9 and 4.9 in the first and second year, respectively (Table 2.2).

Table 2.2 Forage characteristics and productivity for grazing and feedlot system for each year in an intensive beef cattle production system

System	Item	Year 1	Year 2
Grazing	N° animals	40	50
	Days in grazing	230	216

	Herbage Mass, kg DM/ha	3,824.2	3,944.5
	Stocking Rate, kg BW/ha	2025	2880
	Forage Allowance, kg DM/100 kg BW	6.9	4.9
	Total Gain, kg/animal	166.7	156.3
	Total Gain, kg BW	6660	7800
	Days in feedlot	125	116
Feedlot	Total Gain, kg/animal	175.8	189.2
	Total Gain, kg BW	7020	9450

DM = dry matter; BW = body weight

Supplement consumption was different between years, 0.534 and 1.239 kg/animal/day in first and second year, respectively.

The same diet composition was used in feedlot for the two years and the chemical composition (Table 2.3) showed a similarity between the diet fed to the animal independent of the breed composition, pens and year evaluated.

Table 2.3. Chemical composition of *Megathyrsus maximus* 'Mombaça' pasture, of the supplement and of the TMR diet offered in the feedlot for the two breed compositions during experimental period

Item	GrazingPeriod				FeedlotPeriod			
	Year 1		Year 2		Year 1		Year 2	
	Forage	Supplement	Forage	Supplement	NEL	AN	NEL	AN
DM (%)	25.49	86.78	27.8	90.29	59.94	60.09	57.94	58.61
Ash[2]	8.06	26.27	7.24	23.95	3.60	3.50	4.23	4.34
OM[2]	88.44	65.77	86.04	72.75	92.28	92.46	86.81	86.75
CP[2]	12.7	20.68	13.24	20.87	15.31	15.52	16.03	16.02
EE[2]	1.78	4.09	2.05	3.41	3.75	3.71	4.09	4.28
NDF[2]	64.34	27.23	67.01	28.74	26.40	25.97	27.48	27.30

ADF[2]	44.79	8.40	35.51	8.02	12.19	12.06	11.81	11.65
Hem[2]	34.48	18.83	35.56	20.72	14.21	13.99	15.47	15.66
Cel[2]	41.36	7.41	32.54	7.13	10.39	10.32	11.22	11.13
Lignin[2]	3.43	0.99	2.97	0.89	1.80	1.74	0.59	0.52
CC[2]	28.30	72.76	28.91	71.26	73.60	75.55	72.51	72.69
P[2]	0.22	0.88	0.20	0.84	0.33	0.33	0.37	0.37
Ca[2]	0.64	4.18	0.69	3.43	0.39	0.40	0.55	0.53
TDN (%)	56.95	70.00	55.84	74.00	75.93	76.18	75.31	75.42

[1]The grazing period was 1st year – 10/29/2015 to 06/15/2016 and 2nd year – 11/16/2016 to 06/20/2017; [2]%DM; DM: dry matter; OM: Organic matter; CP: Crude protein; EE: Ethereal extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; Hem: Hemicellulose; Cel: Celulose; CC: Cell content; P: Phosphorous; Ca: Calcium; TDN: Total digestible nutrients was estimated using the formula recommended by Capelle et al [46]: TDN (%) = 83.790 – 4171 x FDN (forage) and TDN (%) = 91.0246 − 0.571588 x NDF (FL diet); NEL: Nellore; AN: Angus x Nellore crossbred

Animal Performance

The difference between initial weights at the start of data recording between the two breed compositions was expected, with superiority for AN animals (Table 2.4).

Table 2.4. Effects of breed composition on animal performance of beef cattle in grazing and feedlot tests (where NEL = Nellore, AN = Angus x Nellore crossbred)

	NEL	AN	SEM	P Value		
				Breed	Year	Breed *Year
Grazing						
InitialWeight	203.13	234.44	5.55	<0.01	<0.01	0.28
Final Weight	351.71	404.41	7.94	<0.01	<0.05	0.15
Total Gain	148.58	169.97	4.19	<0.01	0.09	0.14
ADG	0.675	0.772	0.01	<0.01	>0.10	0.19
Feedlot						
InitialWeight	337.74	418.38	6.40	<0.01	<0.01	>0.10
Final Weight	509.41	617.45	9.72	<0.01	<0.01	0.16

Total Gain	171.67	199.07	4.88	<0.01	<0.05	<0.05
ADG	1.320	1.869	0.04	<0.01	<0.01	<0.05
CarcassWeight	284.23	352.43	5.79	<0.01	<0.05	0.10
CarcassYield	55.79	57.08	0.27	<0.01	<0.01	0.18
Carcass ADG FL	0.886	1.344	0.03	<0.01	<0.01	<0.05
Carcass ADG Total	0.521	0.721	0.01	<0.01	0.06	<0.05

ADG = average daily gain; FL = feedlot; SEM = standard error of the mean

Total gain and ADG in the grazing period were higher for the AN animals (P<0.01) and, consequently, they presented greater weight at the end of this period (P<0.01). Total weight gain in grazing period was 6660 kg BW in the first year and 7800 kg BW in the second year (Table 2.2).

In the feedlot, there was a significant difference between the two breed compositions for all of variables evaluated. NEL animals, although they remained in the feedlot longer, had lower total weight gain. Breed composition had significant effect on carcass yield and carcass ADG (P<0.01), with AN animals being greater than NEL. Carcass ADG in feedlot was 35% higher for AN than NEL, while carcass ADG total (considered throughout the experiment period) was 28% higher for AN. The productivity gain in the feedlot added 7020 and 9450 kg BW to the system in the first and second year, respectively (Table 2.2).

Methane Emissions

When the effects of breed composition were analyzed, CH_4 production (g/day and kg/year) were lower in NEL than AN animals in both grazing and feedlot systems (P<0.01). Methane production emitted per period was calculated, according to grazing and feedlot days. Note that due to the technical issues the methane measurement in grazing was performed only in year 2. The NEL emitted 19% less CH_4 than AN in grazing, but no differences between breed composition in feedlot were observed (Table 2.5).

Table 2.5. Effects of breed composition on methane emissions of beef cattle in grazing and feedlot tests (where NEL = Nellore, AN = Angus x Nellore crossbred)

	NEL	AN	SEM	P Value		
				Breed	Year	Breed *Year
Grazing						

DMI, kg/day	5.95	6.23	0.31	>0.10	-	-
BW average, kg	314.6	336.6	9.33	0.07	-	-
ADG, kg/day	0.680	0.729	0.03	0.22	-	-
Feed Conversion	8.98	8.81	0.50	>0.10	-	-
Feed Efficiency	0.119	0.122	0.007	>0.10	-	-
CH_4, g/day	79.69	98.05	4.45	<0.01	-	-
CH_4, kg/year	29.08	35.78	1.62	<0.01	-	-
CH_4, kg/period	17.21	21.17	0.85	<0.01	-	-
CH_4, g/kg DMI	14.31	16.76	1.32	0.17	-	-
CH_4, g/kg $BW^{0.75}$	1.06	1.26	0.05	<0.05	-	-
CH_4, g/kg ADG	119.53	140.03	8.09	0.07	-	-
Feedlot						
DMI, kg/day	9.29	12.44	0.39	<0.01	0.10	<0.01
BW average, kg	386.2	488.6	4.87	<0.01	<0.01	0.25
ADG kg/day	1.49	2.26	0.07	<0.01	0.13	<0.05
Feed Conversion	7.17	5.93	0.36	0.06	0.05	<0.01
Feed Efficiency	0.167	0.193	0.009	0.09	>0.10	<0.01
CH_4, g/day	168.72	209.84	7.78	<0.01	<0.01	>0.10
CH_4, kg/year	61.58	76.59	2.84	<0.01	<0.01	>0.10
CH_4, kg/period	22.34	22.67	0.98	>0.10	0.05	>0.10
CH_4, g/kg DMI	18.52	17.83	0.89	>0.10	<0.05	<0.05
CH_4, g/kg $BW^{0.75}$	1.93	2.01	0.08	>0.10	<0.05	>0.10
CH_4, g/kg CW	0.079	0.067	0.10	<0.01	0.16	0.52
CH_4, g/kg ADG	122.76	97.49	6.86	<0.01	>0.10	0.06
CH_4, g/kg ADGc	192.34	174.54	7.67	<0.05	<0.05	0.28

DMI = dry matter intake; BW = body weight; $BW^{0.75}$ = metabolic body weight; ADG = average daily gain; CH_4 = methane; CW = carcass weight; ADGc = ADG of carcass; SEM = standard error of the mean

It was found that there was no difference in DMI between breed compositions in pasture, but in the feedlot AN presented higher DMI than NEL (P<0.01). Despite the difference in DMI, breed composition did not influence the CH_4 yield (g CH_4 per unit of DMI) neither in pasture nor in feedlot.

It was found that there was no difference for BW during the grazing season, but significant differences for CH_4 emission (g CH_4/kg $BW^{0.75}$) were detected with AN emitting

more. However, methane emission rate was similar between the breed compositions in the feedlot even with differences in BW (P<0.01) for the animals in this finishing stage.

Regarding CH_4 per unit of ADG, no difference was observed between the two breed compositions in pasture (P=0.07). In contrast, in feedlot the CH_4/ADG or CH_4/carcass ADG was significantly lower (P<0.01) in AN than NEL animals.

2.5.DISCUSSION

Identifying efficient cattle breeds and adopting appropriate production systems is an important challenge for meat production worldwide with the growing concern about beef productions impact on the environment [21].

Effect of Different Breed Compositions on Animal Performance

There was an effect of the breed composition on the performance variables in both grazing and feedlot periods. The higher initial body weight to AN in relation to NEL was due the combination of different kind of features from the breeds and the hybrid vigor of the crossed AN animals.

Regarding forage production, our results indicated that the pastures presented good DM production and composition, leading animals to obtain high gains during the grazing period (Tables 2.2 and 2.3). Forage analysis was performed on the leaves only. Leaves are preferentially grazed by the cattle when the availability of forage was not limiting. It was assumed that the animals had the opportunity to select and eat leafy material with nutritional composition more similar to that found in the leaves which justifies the use of this type of forage sampling for analysis.

The high herbage availability and CP during the experimental period may have resulted from nitrogen fertilization (150 kg/ha N) during the beginning of the experiment and from the use of the ICL system, which may be attributed to the recent forage planting. As the ICL system has been improved over the years, the stocking rate was higher than that obtained in previous study executed in the same area during 2013/2014 [22]. The stocking rate were 1093.5 and 1431.0 kg BW/ha in the dry and rainy seasons, respectively [22], which was lower than in the present study during the rainy period (2880 and 2025 0 kg BW/ha in the first and second year, respectively). This difference was attributed to the greater number of animals used in the current study.

Cardoso et al [23] simulated scenarios for beef production in Brazil and found the best scenario was similar to the system presented in the current study (Nellore and Nellore crosses animals in rearing phase in pastures of *Panicum maximum* in rotational grazing), and resulted in a lower stocking rate (1237.5 kg BW/ha), which attests the potential of ICL systems to increase the animals' performance.

The weight gains obtained in the current study were higher than those reported by Oliveira et al [24]. These authors evaluated Nellore animals (initial weight of 373 kg) in continuous grazing put-and-take stocking of *Urochloabrizantha*Stapf cv. Marandu and the animals obtained DMI of 5.93 kg/day and ADG of 0.447 kg/day.

During the current experiment, the voluntary intake of forage was estimated by use of external and internal markers. The estimation of feed intake in pasture-raised animals continues to be costly and highly variables, despite advances in the experimental and analytical procedures over time [24]. However, in this study, the DMI values obtained for grazing animals are in accordance with Kamali et al [25].

The energy-protein mineral supplement offered in the second year had lower proportion of mineral salt, which allowed higher animals consumption. In addition, the lower forage allowance in the second year (4.9 kg DM/100 kg BW), consequence of the greater stocking rate, may also have contributed to higher supplement intake.

Our results showed the capacity of greater animal production per area in ICL systems. Although the beef cattle sector in Brazil is still characterized by regions with low efficiency indexes [26], ICL system could improve animal production and reduce environmental impact from livestock in pasture-based beef production systems in the tropical regions.

In the feedlot period, significant differences between the two breed compositions for all variables evaluated were observed, with AN animals having better performance.

The AN animals achieved the stipulated weight gain with 111 and 105 days in first and second year, respectively. The NEL, however had a total weight gain of 172 kg in 138 and 127 days in feedlot in first and second year, respectively. In this study, NEL had lower efficiency of gain at the end of feedlot period and because of that, did not achieve the criterion stipulated for slaughter.

Average finishing weights in the feedlot were similar to those reported for Angus cross and Nellore cattle [27, 28]. The differences observed for carcass weight in this current study were related to differences in slaughter weight of the animals. Higher weight at slaughter was observed in AN animals when compared to NEL. This observed increase in productivity

results in fewer finished animals needed to produce a given quantity of meat [29], which may contribute to reducing the environmental impacts of beef production.

Crossbred animals showed greater performance throughout the experimental period (total gain of 383 kg versus 306 kg for Nellore animals), but the growth rates reached by both breeds were satisfactory. High gains can be explained by the animals' physiological conditions (non-castrated) and age (up to 24 months old) [30, 31], beyond the effect of cross breeding animals alone [32, 33], in addition to the high concentrate diet in the finishing phase.

Animal performance is not only a direct effect of the quality and quantity of the diet but also animal genetic potential [34, 35]. We observed that in appropriate conditions of feeding, AN animals obtain greater performance.

Effect of Different Breed Compositions on Methane Emissions

The AN animals had a higher CH_4 production (g/day) and consequently higher kg CH_4/year in both grazing and feedlot. Lager and fast-growing cattle will generally eat more and produce more enteric methane than smaller, slower-growing cattle under the same feeding regimen [36].

Although grazing methane measurements were performed only in year 2, the focus of our study is not the comparison between years and the design of the study and the statistical analysis allowed us to discuss these data without leading us to partially erroneous conclusions. Methane production (g/day and kg/year) measured in grazing period were lower than those reported by Oliveira et al[24]. The higher methane emission reported by these authors compared to the values obtained in this study may be attributed to continuous grazing system used, where forage presents greater fiber content and therefore provides higher production of CH_4.

When comparing to continuous grazing, multi paddock (MP) grazing can improve forage quality as well as forage production; thus, MP grazing is potentially a good option to reduce GHG emission [6]. According to these authors, total GHG emissions could be reduced by as much as 30%, only by increasing forage quality and digestibility.

Methane production (g/day and/or kg/year) measured in feedlot were similar to those reported by other studies [37-39]. Feedlot diets generally do not exhibit many discrepancies in nutritional composition, and therefore lower methane emissions variations are observed at that stage.

In the grazing period, no difference in either BW or DMI was observed. As the ADG of the AN animals was higher than NEL in the grazing and feedlot period (Table 2.4) the

difference between the BW of the two breed compositions was higher in the feedlot, and in this period differences were also observed for DMI in the feedlot.

The AN consumed more feed in feedlot, however when CH_4 volumes were compensated for feed intake, there were no significant differences between breeds. Methane production expressed as g/kg DMI in the current study was similar to previously observed production rates in Nellore animals by Fiorentini et al [37] (17.1 g de CH_4/kg DMI). DMI in our study was 2.4 and 2.5% BW for NEL and AN, respectively, and no differences between methane yield could be due to the similarity in intakes between the two breed compositions.

The AN animals were more efficient and obtained lower CH_4 per ADG compare to NEL in feedlot. Previous studies did not support the hypothesis that an increase in feed efficiency decreases CH_4 production [40, 41], however Hegarty et al [42] showed that more efficient animals produce less enteric CH_4 production than less efficient animals, especially when these animals are fed a high concentrate diet, which agrees with our results.

Even though AN animals showed higher methane emission (g/day), the total methane emission during the finishing phase was the same for both breed compositions, because these animals spent less time in feedlot. The intensification of beef cattle production systems leads to a reduction in emissions of GHGs per unit of product, and greater reductions may theoretically be possible if animals of higher performance were utilized [23], as confirmed in this study by the Angus x Nellore cattle.

Previous research has focused on the use of feedlots as a strategy to reduce CH_4 emissions per kg of meat produced compared with grazing system. However, the majority of studies evaluated the continuous grazing management system and assumed steady-state soil carbon (C) to model the grass-finishing environmental impact [7]. In these studies, the ADG is generally below what can be achieved in well managed intensive pasture systems and because of that a substantial reduction in net GHG emissions can occur in pasture systems, even when requiring double the land of feedlot systems, as a consequence of increased sequestration of organic carbon in the soil, challenges existing conclusions that only feedlot intensification reduces the overall beef GHG footprint through greater productivity [43, 44, 7].

Concerning total methane production (adding the methane emitted in both grazing and feedlot), it was observed that the CH_4 production was higher for AN animals compared to NEL (43.84 kg versus 39.55 kg). However, methane production per kg of carcass was 0.124 versus 0.139 for AN and NEL, respectively. These results suggest that the methane production of crossbred animals is compensated by better performance, resulting in lower CH_4 per kg of meat produced, when this intensive production system is used in tropical climate conditions.

In addition to the benefit of reducing enteric CH_4 emissions/kg of meat produced, the great advantage of intensification is associated with the reduction of the area required to produce the same amount of product. This change in the efficiency of productive systems has the potential to reduce the degraded area and, in addition, contribute to the non-opening of new areas and therefore avoid deforestation [45].

This discussion might have relevant considerations for other developing countries, which have large area of low-productive pasture, like in Brazil.

2.6.CONCLUSIONS AND IMPLICATIONS

The present study proposed to compare the GHG mitigation potential of two breed compositions in established Brazilian intensive beef cattle production system. Our data shows that emission intensity might be altered depending of breed and diet composition, and AN animals in feedlot contributes to the reduction of methane intensity. Overall, the AN animals were more efficient and had greater weight gain compared to Nellore, resulting in lower methane per kg of meat produced over the whole experimental period.

The data generated could contribute to the development of methane mitigation policies, assuming standard systems that combines pasture use in the rearing phase and grain-based diet for finishing the animals. The integrated systems could enable high gains per unit of land, and feedlot finishing contributes to increased productivity of the whole system.

Therefore, associating these two systems for beef cattle breeding in a tropical climate conditions with extensive pasture areas seems to be in line with new GHG reduction policy.

2.7.ACKNOWLEDGMENTS

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CAPÍTULO III –ARTIGO II

Nitrous oxide andmethaneemissionsfrombeefcattle excreta depositedonfeedlotlands in tropical condition

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3.1.ABSTRACT

Although increasing attention to the importance of greenhouse gases (GHG) emissions due to livestock activities it is being given, data from animal excreta at beef feedlots are not well established for feedlot raised in tropical conditions. Our objective was to investigate the effects of excreta type deposited in feedlot soils on nitrous oxide (N_2O) and methane (CH_4) emissions and N_2O emission factor (EF). The sample' pool of each excreta were obtained from 25 steers in feedlot (Average BW = 393 kg). Urine (1.3 l) and dung (1.3 kg) were

applied once in a feedlot pen and after excreta application fluxes were monitored lasted 92 days, by using static chambers technique. The N_2O fluxes had two peaks for the urine treatment, the first at 1st day after application (DAA) of excreta and the second after the rainfall events (70 DAA). Also, the N_2O fluxes for the dung had a peak at 70 DAA. The CH_4fluxes were unstable and presented several pulses throughout the measurement period and was altered between positive and negative flow values. Soil CH_4 emissions remained near zero and all treatments showed low levels up CH_4 uptake (-8.4, -3.2 and -14.8 $\mu gC\ m^{-2}\ h^{-1}$ for dung, urine and control, respectively). The excreta presence increased soil moisture by 44.5 and 55.4% for dung and urine, respectively. The high mineral N concentration in the urine caused that high values in the soil and significant difference of ammonium (NH_4^+) and nitrate (NO_3^-) compared to dung and control. The NH_4^+ and NO_3^- soil concentrations in the cattle urine treated soils peaked at 13 DAA, while for dung treated soils peaked at 42 DAA. The N_2O EF from urine was significantly ($P<0.0001$) higher than the EF from feces (2.83 vs. 0.32%, respectively), resulting in a combined excretal EF of 1.83%, which is <8.5% of the IPCC default EF for excretal returns.

KEY WORDS: bovine excreta, emissionfactor, greenhousegasemission, N_2O emissions

3.2.INTRODUCTION

Nitrous oxide (N_2O) is an important greenhouse gas (GHG), since it has a global warming potential 298 times higher than carbon dioxide and represent approximately 6% of global GHG emissions. About 90% of N_2O emissions are derived from agricultural practices, as nitrogen (N) fertilization of soil and excretion of N by animals (WMO, 2015). Although the number of available studies about N_2O fluxes is currently growing, Methane (CH_4) data from animal' excreta is not evident yet. High temporal and spatial variability from CH_4fluxes may contributes to this considerable uncertainty (Nicoline et al., 2013; Rahman et al., 2013).

The N_2O is produced mainly by microbial denitrification, which is an anaerobic process that reduces nitrate (NO_3^-) to N_2 with formation of N_2O as an obligatory intermediate (De Klein and Eckard, 2008). Soil GHG emissions from animal's excreta decomposition are influenced by several factors, including the weather, time, species, housing, manure handling system, feed type, and management system (Broucek, 2018).

Most previous studies have been conducted on grassland soils and showed the effects of soil or climates conditions on N_2O emissions from cattle excreta (Sordi et al., 2014; Rochette et

al., 2014; Lessa et al., 2014; Mazzetto et al., 2014). However, N_2O emission fluxes from beef feedlot pen excreta are not well established in the literature. According Redding et al. (2015) quantifying GHG from feedlots could be difficult due to the small variations of N_2O and CH_4 concentration in free air, and climatological effects.

Studies have quantified the emission of GHG from feedlot manure (Parker et al., 2017; Parker et al, 2018a, 2018b). However, the manure (a mixture of feces, urine, soil, dropped feed, and scurf) that accumulates in pens is heterogeneous and dynamic, consisting of both freshly excreted and older material that is continually changing compositionally (Waldrip et al., 2016).

Although the amount of N_2O emissions from the surface of animal pens are small (Bai et al., 2015), some factors present in feedlot may increase N_2O emissions. The high animal stocking density, besides leading to trampling and soil deformation (Houlbrooke et al., 2009), can increase soil compaction creating anaerobic conditions which favor the increase of N_2O emissions (Van Groenigen et al., 2005; Uchida et al., 2011). Furthermore, the vegetation absence in feedlot areas (Jamali et al., 2016) as well as urine and dung deposition could significantly increase N_2O emission (Monaghan et al., 2013; Van der Weerden et al., 2011).

According to the IPCC (2006), the percentage of N lost as N_2O, defined as N_2O emission factor (EF), is 2% for the animal' excreta in pastures or in open confinement area, without distinction between urine and dung. Studies have shown that there are differences for EF between dung and urine deposited in grazing lands, and the value was lower than the standard factor proposed by the IPCC (Van Der Weerden et al., 2012; Lessa et al., 2014; Rochette et al., 2014; Sordi et al., 2014).

It is likely that the emission factors for the more intensive systems are higher than the less intensive systems, due to improve diet and fertilizer use (Cardoso et al., 2016). Although the Brazilian beef production system is based on pasture, the number of feedlot-finished animals in Brazil is increasing at a rate of 7% per year, and the number of cattle confined in 2018 was 5.58 million (ABIEC, 2019), which suggests that greater attention should be given to the GHG emissions by the deposition of urine and dungs in feedlot lands in tropical condition.

The GHG measurement onto feedlot land could better determine the contribution of animal excreta to GHGs emission worldwide and is needed for inventory purposes. Thus, we hypothesized that GHG emissions from dung will be lower than urine and the emissions factor proposed by IPCC currently can overestimated. The objectives of this trial were to determine

N_2O and CH_4 emissions and the associated emission factor (EF; percentage of urine-N lost and dung-N lost as N_2O-N) for beef cattle dung and urine deposited onto a feedlot land.

3.3.MATERIAL AND METHODS

All experimental procedures were approved by the Ethics Committee for Animal Use of Universidade Federal de Minas Gerais (UFMG, protocol number 326/2014).

Site description

The experiment was conducted at Brazilian Agricultural Research Corporation – EmbrapaMilho e Sorgo (SeteLagoas, Minas Gerais, Brazil; 19°28′S; 44°15′W, at 732 m altitude). The regional climate is Cwa, with dry winters and wet and rainy summers (Alvares et al., 2013).

The trial was located on feedlot area and it had been used for beef cattle finishing. A pen was fenced off and stock excluded at least three months prior to the start the field trials to avoid interference from fresh dung and urine inputs and reduce spatial variability from the previous uneven deposition of dung and urine.

Treatments

Excreta treatments, including beef cattle urine and dung, were collected at 34 and 35 days of the feedlot. Twenty-five Nellore steers (393 kg average BW) were used for fecal and urine sample collection.

No animal-excreta treatment was also included as control. The treatments were assigned to the plots in a completely randomized design with 4 replicates of each treatment. Plot size was about 4x3 m for each repetition. The dung and urine patches were established for the N_2O chamber measurements and another areas on each plot were treated with either dung or urine at the same rate, allowing multiple soil sampling occasions for carbon (C), nitrate (NO_3^-), ammonium (NH_4^+) and moisture soil. The following treatments were established: Urine, Dung and Control (no excreta addition).

Excreta collection

Dung were sampled immediately after defecation in pens or directly from the rectum while the animals remained in the yarding area. Urine was collected from the same animals, on the same day, brought to a yarding area and after eliciting micturition by manual stimulation. Dung and urine were collected for two days and were stored at 4°C between the

two collection days and removed from cold storage at least 12h before application onto soil, allowing them to attain ambient temperature prior to application.

Dung samples were dried at 55 ºC until constant weight, ground with a Wiley mill through a 1-mm sieve and stored for subsequent total N, C and volatile solid determination. Aliquots of urine (10 mL) were diluted in sulfuric acid (40 mL, 0.036 N) and stored at -20°C for subsequent total N determination and Total N was extracted by the method of Kjeldahl. Dung and urine characteristics are shown in Table 3.1.

Table 3.1. Nitrogen concentration (N) of urine and dry matter (DM), carbon (C) and N of dung

Excreta type	N (g L^{-1})	DM (g kg^{-1})	C (g $kg^{-1}$of DM)	N (g $kg^{-1}$of DM)
Urine	9.3	-	-	-
Dung	-	249.2	393	26.0

Excreta N application rate

The application of the excreta was done only once, at the beginning of the experiment. Trial commenced in dry season (winter 2017), at 36 days of the feedlot. The amount of dung and urine used for each chamber was 1.3 kg and 1.3 L, respectively. Dung and urine treatments were applied in the center of the chamber base using a PVC circle measuring 20 cm diameter to allow fecal shaping and to facilitate infiltration (rather than runoff of urine). The mass of fresh feces for each treatment was transferred to inside the ring and gently molded to simulate the contact with the soil, as naturally occurs after animal defecation.

Nitrous oxide and methane measurement

GHG emissions from beef cattle excreta in feedlot were measurements using a static chamber technique, and the methodology was based on that used the previous published studies on excreta N_2O emissions (Saggar et al., 2004a; Luo et al., 2013, 2015; Van Der Weerden et al., 2016). Two weeks before the trial began, static chamber bases were inserted into the soil to a depth of 8 cm in each plot and left for the whole experimental period. A trough was made around the top of the frame, and filled with water in the collect moment to ensure the seal after coupling the top portion of the chamber.

Gas samples were taken manually from each chamber and measurements were carried daily during the first four days after treatment application to account for possible instant emissions from excreta, and subsequently every 2 and 3 days in the second and third week,

respectively and thereafter weekly. The measurements continued until day 92 after excretes deposition. Extra samplings conducted when rainfall exceeded 10 mm in 24 hours, during weekly phases of N_2O flux measurement. On each sampling day, gases measurements were carried out once between 09:00 and 10:00am, a period that allows extrapolation to a daily flux without bias (Alves et al., 2012). Gas samples from chamber head space were collected during a cover period of 45 min at times 0, 15, 30 and 45 minutes and transferred to previously vacuumed vials.

The gas sampling schedule agrees with those recommended in the guidelines for N_2O chamber methodology (De Klein and Harvey, 2012).

Nitrous oxide and methane concentration of gas samples were analyzed by gas chromatography using a Shimadzu GC-17a gas chromatograph equipped with a ^{63}Ni-electroncapture detector (oven, valve and detector temperatures were operated at 65, 100 and 280 ºC, respectively) using oxygen-free N as a carrier gas and connected to an automatic sampler, which is capable of handling up to 120 samples using an SRI 8610 automated gas chromatograph.

The increase in N_2O and CH_4 concentration within the chamber headspace, for the gas samples collected at 0, 15, 30 and 45 were generally linear (R^2> 0.90). Therefore, the hourly N_2O and CH_4fluxes were calculated (Mosier and Mack, 1980) using liner regression and the ideal gas law according to Eq. (1):

$$\boldsymbol{F = \frac{\delta GHG}{\delta T} \times \frac{M}{V_m} \times H} \quad (5)$$

where, F is the hourly N_2O or CH_4 fluxes (µg N or C/m^2/h); δGHG is the increase in head space N_2O or CH_4 over time (µL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O or C in CH_4; Vm is the molar volume of gas at the sampling temperature (L/mol); H is the height of head space (m).

The hourly flux data were integrated over time, for each enclosure, to estimate the total emissions over the measurement period. Emission factors (EF, N_2O-N emitted as % of excreta N applied) were calculated using Eq. (2):

$$\boldsymbol{EF = \frac{(N_2O - N_{excreta}) - (N_2O - N_{control})}{excretaNapplied} \times 100} \quad (6)$$

where, EF is emission factor (N_2O-N emitted as % of dung-N or urine-N applied), $(N_2O - N_{excreta})$ and $(N_2O - N_{control})$ are the cumulative N_2O-N emissions from the dung or urine

and control plots, respectively, during the 92-days period (μg N m^{-2}), and excreta N applied is the rate of dung or urine N applied (μg N m^{-2}).

Soil and climatic variables

At trial onset, a bulk soil sample comprising ten soil cores from two depths (0-10 and 10-20 cm) were collected randomly from trial site and composited into 1 sample for each depth for soil chemical and physical analysis (Table 3.2).

The soil moisture was determined by weighed (fresh weight) before oven drying at 105°C overnight, and then reweighed, according to AOAC (1990) using the method of n°. 934.01. Particle size analysis (clay, silt, and sand) was assessed using sedimentation and soil pH was measured potentiometrically in a 1:2.5 soil water suspension, with buffer solutions of pH 4 and 7.

Inorganic nitrogen forms, nitrate (NO_3^-) and ammonium (NH_4^+) extracted from the soil samples (0–10 cm) were taken from the dedicated sampling areas (without chamber) of each plot on 6, 13 and 42 days after application of the excreta and were analyzed using the steam distillation method.

Soil water content was measured for all plots when gas samples were collected. The depth of all the soil samples was 10 cm, and diameter of soil samples was 2.5 cm. Daily rainfall and air and soil (0–5cm) temperature were recorded for the entire trial period. Daily rainfall data were obtained at weather station located at Embrapa (within 1 km).

Table 3.2. Chemical and physical attributes and granulometry of soil, at 0 to 10 and 0 to 20 cm depth layer, before the experiment implantation

Attributes	Depth (cm)	
	0-10	10-20
Soil pH in H_2O	5.7	5.9
Nitrogen, %	0.22	0.22
Phosphorus[1], mg dm^{-3}	112.0	34.9
Potassium, mg dm^{-3}	960.1	684.8
Calcium, cmolc dm^{-3}	4.0	2.8
Magnesium, cmolc dm^{-3}	2.3	1.4
Hydrogen + Aluminum, cmolc dm^{-3}	4.4	4.4
Base saturation, cmolc dm^{-3}	8.8	5.9
Cation exchange capacity (CEC), cmolcdm^{-3}	13.2	10.3
Base saturation, %	66.7	57.2
Aluminumsaturation, %	0	0
Granulometry (g kg^{-1})		
Coarsesand	170	100
Fine sand	110	90

Silt	130	120
Clay	590	690

[1]Extracted with the Mehlich-1 solution. The methodologies used for the analysis of all attributes were based on Silva (2009)

Statistical analysis

The distribution of model residuals was tested for normality and homogeneity using Shapiro-Wilk and Cochran tests. When necessary, the data were transformed from the Box-Cox.

Emissions from the Control treatment were subjected to statistical analysis to assess the differences in background emissions from dung and urine. Descriptive statistic of data was performed. Pearson product member correlations among N_2O and CH_4fluxes and air and soil temperature and soil moisture were performed. Daily means of N_2O and CH_4flux, air and soil temperature and soil moisture were calculated from the measured data in each day.

Data on EF_3 values, calculated from the emissions, were used in the statistical analyses for comparing between the excreta type. EFs were calculated by subtracting cumulative N_2O emissions from control plots from treatment plots.

Excreta type effect was evaluated using the F test in analysis of variance (ANOVA) using R program.

The model was as follows:

$$\gamma = \mu + d_k + e_i + de_{kj} + \epsilon_{ijk},$$

where μ is the overall mean; d_k is the fixed effect of day after application (DAA) of excreta; $e_i$is the fixed effect of excreta type; de_{kj} is the interaction between the DAA and excreta type; ϵ_{ijk} is the residual error. Differencesbetweentreatmentsweresignificantat $P \leq 0.05$.

3.4.RESULTS

Weather conditions

Total rainfall observed throughout the experimental period was 33mm (Fig. 3.1). Only 3 rainfall events occurred on 67, 68 and 70 days after application (DAA) of excreta.

Daily mean air temperature increased along the DAA, as well as the soil temperature (5 cm depth) (Fig. 3.1). Direct correlation between soil and air temperature (R^2 = 0.88) was significant (Fig. 3.2).

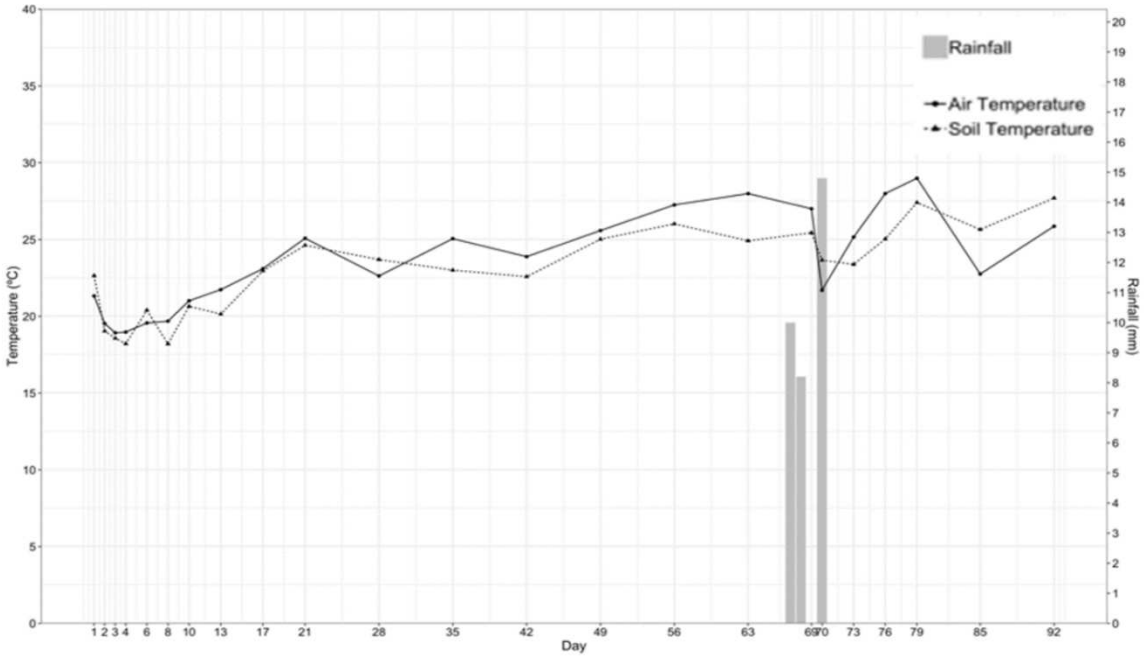

Figure 3.1. Soil and air temperature and rainfall measured at 92-day period following the application of dung and urine deposited in feedlot lands

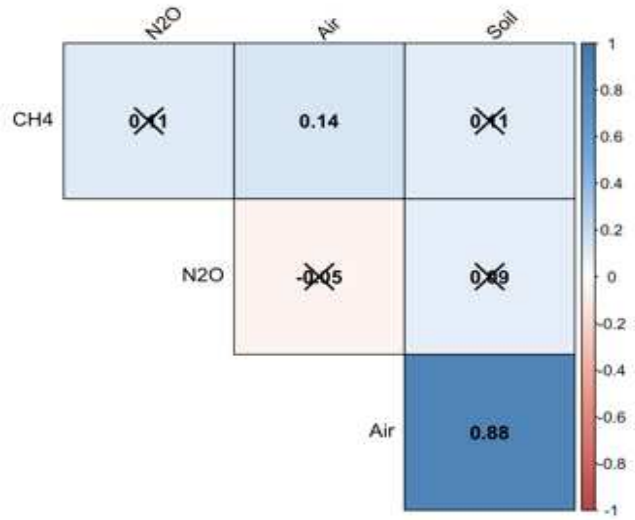

Figure 3.2.Pearson product-member correlation among N_2O and CH_4fluxes, soil and air temperatures. Positive correlations are shown in blue and negative correlations in red. Non-significant correlation are marked by x ($P > 0.05$)

Nitrous oxide and methane emissions

The N_2O fluxes presented mean values of 239.4, 287.5 and 173.4 µgN m^{-2} h^{-1} to dung, urine and control, respectively, over the 92 DAA (winter/spring) measurement period (Fig. 3.3a). There were interaction between the excreta type and DAA for N_2O and CH_4 fluxes (Table 3.3).

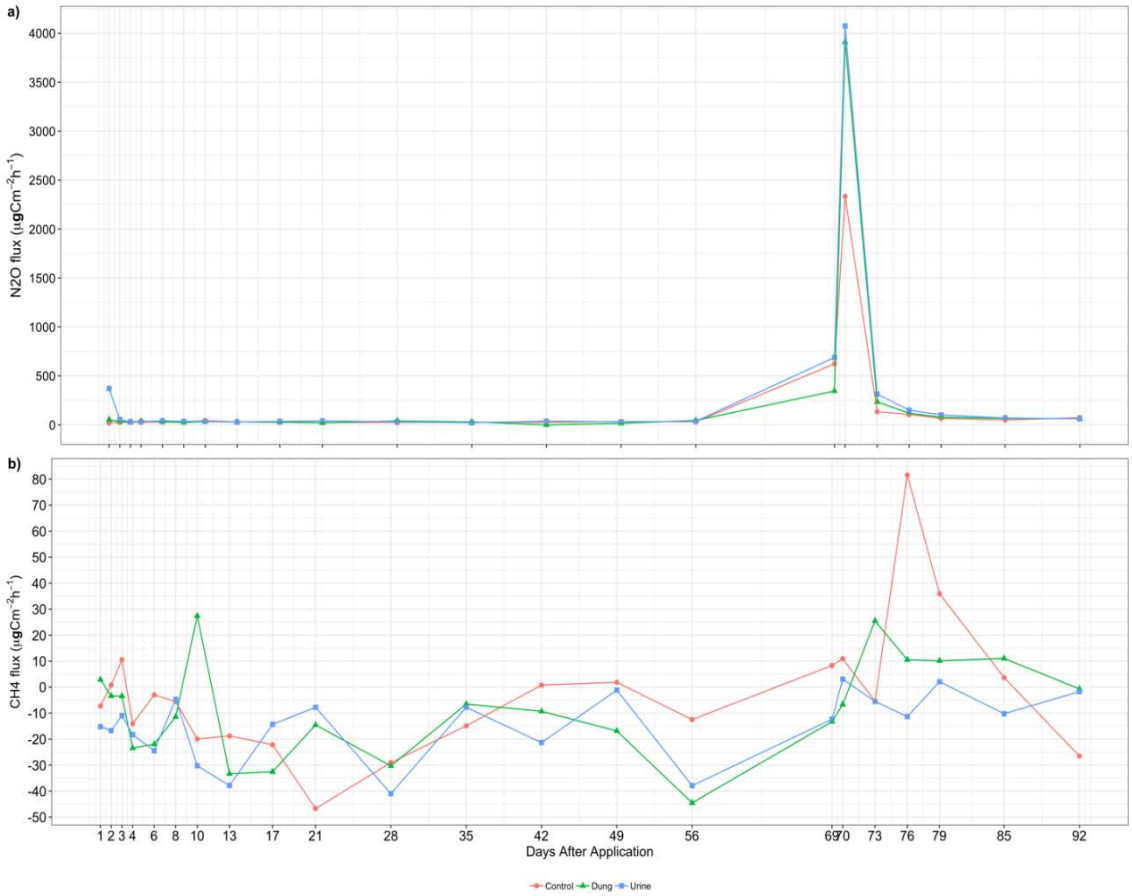

Figure 3.3.Soil N_2O and CH_4 fluxes measured at 92-day period following the application of dung and urine deposited in feedlot lands. Each point represents the mean of four replications

During the first DAA, N_2O fluxes were greater for urine (370.9 µgN m^{-2} h^{-1}) than control and differences were observed for dung and control, as well (52.6 and 19.8 µgN m^{-2} h^{-1}, respectively). In the second DAA, urine N_2O flow decreased dramatically (55.5 µgN m^{-2} h^{-1}), but the difference compared to control (25.2 µgN m^{-2} h^{-1}) was maintained (Table 3.3). From the third day until 42 DAA of excreta, no differences between treatments were observed. The urine N_2O flux returned to low levels and showed flow similar values to dung and control. In 42 DAA, it was observed difference between urine and dung. There was an increase in emission after the rainfall events for treatments, however, there was no difference between it. At 70 d, after the second rain event, the values of N_2O fluxes reached the maximum value and presented a fall at 73 d (Fig. 3.3a). At 73 DAA, after the rainfall events, there was an increase in emissions for the excreta treatments as well as in control treatment, but the differences were only observed between each excreta type compared to the control.

Table 3.3. Nitrous oxide (N_2O) and methane (CH_4) emissions means (μg m^{-2} h^{-1}) for excreta type and days after application (DAA) of excreta and their interaction

DAA	Dung (D)	Urine (U)	Control (C)	Fisher' test - P-values		
	N_2O (μg m^{-2} h^{-1})			D x U	D x C	U x C
1	52.6	370.9	19.8	0.02	0.94	0.01
2	33.8	55.5	25.2	0.16	0.71	0.04
42	0.17	38.5	25.0	0.01	0.11	0.46
73	235.2	313.9	133.8	0.15	0.06	0.002
	CH_4 (μg m^{-2} h^{-1})					
1	2.9	(15.2)	(7.25)	0.02	0.20	0.34
10	27.4	(30.3)	(19.9)	0.02	0.07	0.84
56	(44.5)	(37.9)	(12.5)	0.82	0.04	0.10
76	10.5	(11.4)	81.6	0.50	0.01	0.002

N_2O: nitrous oxide; CH_4: methane; DAA: days after application;Numbers within parentheses mean negative values

The CH_4fluxes were unstable and presented several pulses throughout the measurement period and was altered between positive and negative flow values. Soil CH_4 emissions remained near zero and the treatments showed low levels up CH_4 uptake (negative flux). Mean values for dung, urine and control were -8.4, -3.2 and -14.8 μgC m^{-2} h^{-1}, respectively (Fig. 3.3b).

During the first DAA, the CH_4 fluxes were greater for dung (2.9 μgN m^{-2} h^{-1}) than urine (-15.2 μgN m^{-2} h^{-1}). Significant difference between excreta type was observed again in the 10 DAA. It was no observed difference between treatments containing excreta from 10 until 56 DAA of excreta, however, at 56 DAA, was observed difference between dung and control (P=0.04). After the rainfall events, at 76 DAA, there were difference between urine and control (P=0.002) and to dung and control (P=0.01), as well.

Although the correlations between N_2O fluxes and soil and air temperatures were not significant, the soil CH_4 fluxes had significant correlation with air temperature (Fig. 3.2). Also, the CH_4 pattern as a function of rainfall events along the excreta DAA was not observed.

Soil mineral-N, Carbon-C and moisture conditions

There was no interaction between the excreta type and DAA for all the variables measured from the soil. The soil moisture means values to the three periods measurements (6, 13 and 42 DAA) were 12.9, 12.0 and 8.3% for dung, urine and control, respectively. There was no difference between the excreta type for soil moisture (P=0.95), but it was observed difference between each excreta type and control (for dung, P=0.01 and for urine, P=0.02), showing that the excreta presence increased soil moisture by 44.5 and 55.4% for dung and urine, respectively.

For soil carbon concentration, there was no significant difference when the dung and urine were compared (P>0.05), the mean values were 37.8, 32.9 and 23.7 g kg^{-1} dry soil for dung, urine and control, respectively.

It was no observed difference for ammonium (NH_4^+-N) (P=0.63) and nitrate (NO_3^--N) (P=0.62) soil concentrations between the dung and control. Both presented average values of 64.7 and 52.5 mg N kg^{-1} dry soil for NH_4^+-N and 95.0 and 77.4 mg N kg^{-1} dry soil for NO_3^--N, respectively, over the measurement period (Fig. 3.4c and 3.4d).

The high mineral N concentration in the urine caused that high values of NH_4^+-N and NO_3^--N throughout the experimental period. There was a significant difference between urine and control (P<0.001) and urine and dung (P=0.01) for NH_4^+-N, as well as between urine and control (P<0.01) and urine and dung (P=0.03) for NO_3^--N. Overall, the average concentrations for urine were 104.1 and 144.0 mg N kg^{-1} dry soil for NH_4^+-N and NO_3^--N, respectively.

NH_4^+-N and NO_3^--N soil concentrations in the cattle urine treated soils peaked at 13 DAA, while for dung treated soils peaked at 42 DAA.

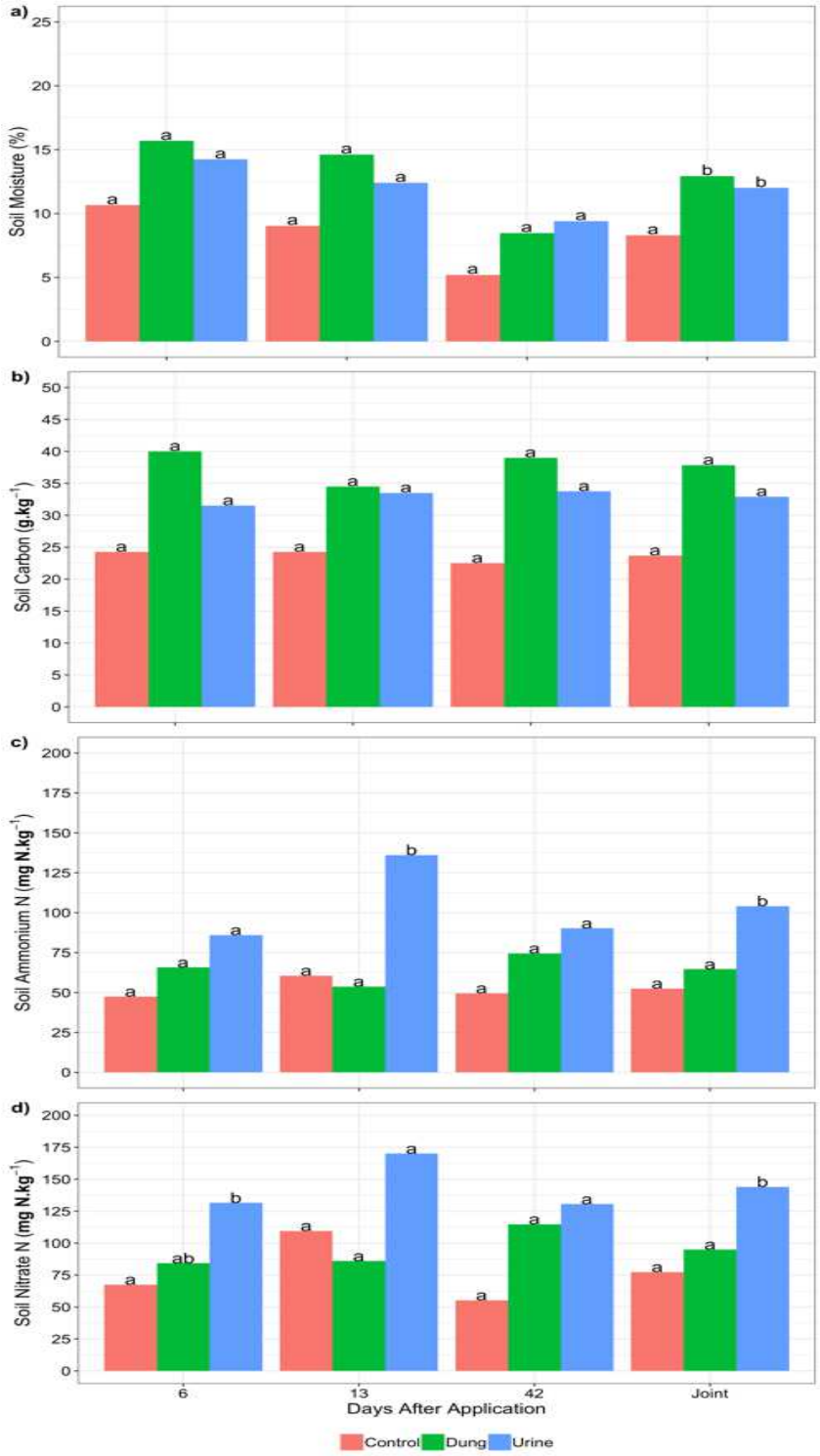

Figure 3.4. Soil moisture (a), soil carbon (b), soil ammonium (c) and soil nitrate (d) measured at 6, 13 and 42 days after application (DAA) from dung and urine deposited in feedlot lands and joint analysis of days 6, 13, and 42. Low-case different letters represent significative differences by Tukey Test ($P < 0.05$)

Nitrous oxide emission factors

Nitrous oxide emission factors (N_2O EF) for animal excreta are presented in Table 3.4. It was observed statistically significant differences in urine or dung EF values ($P < 0.05$).

Table 3.4. Nitrous oxide (N_2O) emission factor mean (% of applied N) from different excreta type and standard error

Excreta type	Emission Factor
Dung	0.32 (± 0.51)
Urine	2.83 (± 0.73)

Fisher' Test ($P<0.0001$)

3.5.DISCUSSION

Our data has shown that cattle excreta are indeed sources of direct N_2O emissions when deposited in open confinement area. Besides, rainfall affect the magnitude and rate of GHG emissions from urine and dung patches, creating optimal environments for the production of N_2O and CH_4 (Van der Weerden et al., 2011; Wang et al., 2013).

Two peaks of N_2O emissions were observed for urine, the first peak at 1 DAA and the second after the rain event. For feces, only the second peak was observed. The first peak for urine in our study is comparable with peak emission rates from Barneze et al. (2014). According these authors, the first emission peak may be associated with nitrification, due to the increase in ammonium nitrogen concentrations in the soil after urine deposition. For the second peak, it is probably that denitrification was the predominant process leading to N_2O emissions due to the rainfall and increasing the soil water content.

The N_2O fluxes data are compared with other studies. De Klein et al. (2003) recorded maximum emission rates from 300 to 4,900 µg N_2O-N m^{-2} h^{-1} from cattle urine applied to grass. Simon et al. (2018) reported emissions rate from 1,880 to 3,700 µg N_2O-N m^{-2} h^{-1} from urine and from 80 to 460 µg N_2O-N m^{-2} h^{-1} for dung applied in kikuyu grass pasture over a haplic Cambisol, in southern Brazil.

Simon et al. (2018) showed that N_2O fluxes are related with soil ammonium and nitrate concentrations in urine patches. They increased, peaked and returned to background level in less than 40 days. In our study the nitrate and ammonium peaks happened in 13 and 42 DAA for urine and dung, respectively. In the literature, N_2O emission peaks after excreta

application occur within from 5 to 45 DAA, and fall to background levels within 90 days or earlier (De Klein et al., 2003; Van Groenigen et al., 2005; Hoeft et al., 2012).

Our data showed that soil moisture is a key factor for N_2O peaks to occur. Even with the substrate peak (ammonium and nitrate) occurring at 13 and 42 DAA for urine and dung, respectively, there was no significant emission until the first rain event. This may be related to increased activity of microorganisms with soil hydration. Increasing soil moisture content raises liquid diffusion rates, providing microorganisms with C and N substrates that are key factors structuring microbial communities and activities ((Blagodatsky and Smith, 2012; Barnard et al., 2013). Although soil N sources have not been measured after the rainfall event, concentrations of NH_4^+ and NO_3^- are expected to peak and then decrease over time, as observed by Hoeft et al. (2012) and Simon et al. (2018).

Peaks of soil NH_4^+-N and NO_3^--N concentrations from feces were delayed compared to urine. These results agree with the results observed by Sordi et al. (2014), and can be explained by the smaller amount of N applied per area and the organic N forms of dungs, which are not readily available for hydrolysis such as urine N-urea. Another possibility is that a greater amount of N was still kept inside the dung, while in practice every N of urine enters the soil immediately after application.

About CH_4 emissions, under tropical conditions, some studies have reported conflicting results. In the tropical region of Brazil, CH_4 emissions from excreta were substantially higher than in temperate conditions (e.g., The Netherlands) (Van Groenigen et al., 2005). Mazzeto et al. (2015) showed that CH_4 emissions were approximately 2.7 times higher in summer than in winter in Brazil.

The CH_4 is mainly produced by the presence of dung, due to the existing organic matter and the anaerobic conditions soon after its deposition in the soil (Angel et al., 2011; Mazzetto et al., 2015). The CH_4 production of dung showed positive values at 1 and 10 DAA in the currently study. On all other days until 76 DAA, the values were negative, which is expected in aerobic soils. Over the days, the dung dried and the oxygen of the air is permeating the dung, with that the emission ceases and the negative flows appear. Urine treatments were CH_4 sinks during the dry season, which can occur in some soils, which is similar to earlier studies (Saggar et al., 2014b; Tully et al., 2017).

Urine N_2O EFs were significantly greater than the dung N_2O EFs, signifying the importance of the N content as a substrate for the soil processes, nitrification and denitrification, responsible for N_2O production. Urine and dung N_2O EFs are similar to some of those measured by others (Sordi et al., 2014; Cardoso et al., 2016). For deposition of

excreta in open confinement area, in currently study, N_2O emission factor corresponded to 1.83%, which is 8.5% lower than that proposed by the IPCC. We calculated a provisional excretal N_2O EF in this study, assuming a 60:40 split between the total N excreted in urine and dung (Webb and Misselbrook, 2004).

The EFs from excreta varied seasonally and also dependent on soil type. According to Krol et al. (2016), indeed, relationships between the magnitude of N_2O EFs with season of deposition should be interpreted with caution, as soil and environmental conditions can vary markedly within a season. In Brazil, beef cattle feedlots are mainly carried out in the dry season, so the effect of rainfall and high temperatures seems to be less relevant in N_2O emissions. However, even the first rains have occurred only at 67 DAA, the emissions of N_2O to dung and urine had a considerable peak.

Our study focused on cattle urine and dung where applications were made to feedlot soils, and where urine and dung were collected from cattle fed feedlot diets and the results showed differences between the literature results. In fact, studies suggest that higher dietary protein levels may increase N_2O emissions, since greater amounts of N may be lost by dung and urine. Feed composition can affect the C/N ratio in excreta, which in turn affects N_2O and CH_4 emissions (Cardenas et al., 2007; Cardoso et al., 2017). Therefore it is fundamental to seek a higher efficiency of the animals, that is, a higher average daily gain in the feedlot, in order to reduce the GHG emission per kg of meat produced.

The key factor for regulating N_2O emission from soil remain unknown. Oenema et al. (1997) suggested that the N availability in the soil is the most useful indicator for evaluating total emissions from a certain area. However, Mazzetto et al. (2014) argued that the soil mineral N concentration regulate N_2O emission from soil, because when soil mineral N reaches levels as high as those found in urine patches, it no longer limits the amount of N_2O released.

Van Groenigen et al. (2005) reported a significant effect of soil compaction on N_2O emissions from applied urine. They observed that with soil compaction, N_2O emissions increased by a factor of 2.2 (from 1.30% to 2.92% of applied N) and that when dung was added, N_2O production was augmented by a factor of 1.8 (from 1.60% to 2.82%). The dung applied had this C:N ratio, which combined with moist conditions and N availability, probably stimulated microbial activity and created an ideal environment for higher N_2O emissions.

3.6.CONCLUSIONS

Application of cattle excreta to a feedlot soil increased N_2O emissions. In tropical condition, the net cumulative N_2O emission represented 1.83% of the applied excreta N, lower than the current IPCC default emission factor for open confinement area.

Many questions remain, and further studies are needed to elucidate the impact of excreta type deposited in feedlot land on N_2O emissions and which factors influence greatly this emissions.

3.7.ACKNOWLEDGEMENTS

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