UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
Campus de Jaboticabal

RECRIA DE TOURINHOS NELORE EM PASTOS DE CAPIM MARANDU SOB ADUBAÇÃO NITROGENADA

Rhaony Gonçalves Leite

Jaboticabal-SP
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Orientador: Prof. Dr. Ricardo Andrade Reis

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TÍTULO DA TESE: RECRIA DE TOURINHOS NELORE EM PASTOS DE CAPIM MARANDU SOB ADUBAÇÃO NITROGENADA

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Certificamos que o Protocolo nº 12703/15 do trabalho de pesquisa intitulado **"Management strategies to reduce environmental impacts of beef cattle production systems"**, sob a responsabilidade do Prof. Dr. Ricardo Andrade Reis está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de agosto de 2015.

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Prof.ª Dr.ª Paola Castro Moraes
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Rhaony Gonçalves Leite – nascido em Monte Alto, São Paulo, no dia 07 de dezembro de 1992, filho de Edson Sebastião Gonçalves Leite e Vera Lúcia Cardoso Leite. Formou-se Zootecnista pela Faculdade de Ciências Agrárias e Veterinárias, UNESP Campus de Jaboticabal no ano de 2014 e obteve o título de mestre pelo Programa de Pós-graduação em Zootecnia da mesma Instituição no ano de 2018, sob orientação do Prof. Dr. Ricardo Andrade Reis.

A toda minha família,

mas em especial ao meu avô Antoninho, que perdi na reta final desta jornada!

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SUMÁRIO

RECRIA DE TOURINHOS NELORE EM PASTOS DE CAPIM MARANDU SOB ADUBAÇÃO NITROGENADA

RESUMO – No presente trabalho foi avaliado os efeitos de diferentes doses de adubo nitrogenado aplicadas as pastagens de *Urochloa brizantha* cv. Marandu durante a estação chuvosa sobre a massa de forragem (MF), os componentes estruturais das plantas forrageiras, taxa de acúmulo, o índice nutricional de nitrogênio - INN, a eficiência de uso do nitrogênio, a qualidade da forragem, o desempenho animal e o fracionamento de carboidratos e proteína. O delineamento experimental foi inteiramente casualizado com quatro tratamentos (controle sem aplicação de N, e 90, 180 e 270 kg de N ha^{-1} ano^{-1}) e três repetições (piquetes por tratamento). O nitrogênio foi aplicado na forma de ureia divida em 3 aplicações. O experimento foi repetido por dois anos consecutivos (2017-2018 e 2018-2019). Massa de forragem, taxa de acúmulo de forragem, taxa de lotação e ganho por área aumentaram com o aumento das doses de nitrogênio ($P < 0,05$), enquanto carboidratos totais (CT), fibra em detergente neutro (FDNcp), fibra em detergente neutro indigestível (FDNi), a oferta de forragem e eficiência do uso nitrogênio diminuiu com o aumento da dose de nitrogênio ($P<0,05$). A proteína bruta (PB), fração A+B1, fração C e consumo de PB por kg de matéria orgânica digestível (MOD) aumentaram, e a fração C diminuiu linearmente com as doses de nitrogênio ($P <0,05$). O aumento nas doses de N reduziu os teores de FDNcp de 612 para 550 g kg^{-1} MS, de fração C da proteína de 125 para 98 g kg^{-1} PB e de EUN de 97 para 41 kg MS kg^{-1} N aplicado e aumentou os teores de proteína solúvel de 363 para 433 g kg^{-1} PB e a PB de 115 para 193 g kg^{-1} MS. Portanto, o aumento das doses de adubo nitrogenado é uma alternativa viável para aumentar a produção de capim Marandu, o valor nutritivo e a produção animal, além de aumentar as frações mais solúveis de carboidratos e proteína.

Palavras-chave: adubação, gado de corte**,** nitrogênio, pastagens, *Urochloa brizantha*.

REARING OF NELLORE STEERS IN PASTURE OF MARANDU GRASS WITH NITROGEN FERTILIZER

ABSTRACT – In the present study, the effects of different doses of nitrogen fertilization applied to pastures of Urochloa brizantha cv. Marandu during the rainy season on forage mass (FM), structural components of forage plants, accumulation rate, nitrogen nutritional index - INN, nitrogen use efficiency, forage quality, animal performance and fractionation of carbohydrates and protein. The experimental design was completely randomized with four treatments (control without N application, and 90, 180 and 270 kg of N ha-1 yr-1) and three replications (paddocks per treatment), nitrogen was applied in the form of urea divide in 3 applications. The experiment was repeated for two consecutive years (2017-2018 and 2018-2019). Forage mass, forage accumulation rate, stocking rate and area gain increased with increasing nitrogen doses ($P < 0.05$), while total carbohydrates (TC), neutral detergent fiber (NDFap), fiber in indigestible neutral detergent (NDFi), forage supply and nitrogen use efficiency decreased with increasing nitrogen dose ($P<0.05$). Crude protein (CP), fraction A+B1, fraction C and CP intake per kg of digestible organic matter (MOD) increased, and fraction C linearly decreased with nitrogen doses ($P < 0.05$). The increase in N doses reduced the levels of NDFcp from 612 to 550 g kg^{-1} DM, of protein C fraction from 125 to 98 g kg^{-1} CP and of EUN from 97 to 41 kg DM kg^{-1} N applied and increased soluble protein contents from 363 to 433 g kg^{-1} CP and CP from 115 to 193 g kg^{-1} DM. Therefore, increasing nitrogen fertilizer doses is a viable alternative to increase Marandu grass production, nutritional value and animal production, in addition to increasing the more soluble carbohydrate and protein fractions.

Key Words: fertilization, beef cattle, nitrogen, pasture, *Urochloa brizantha*, nutritional value.

Capítulo 1 – Considerações gerais

1. Introdução

Em tempos onde o consumidor final está cada vez mais exigente para adquirir produtos ambientalmente corretos e com preços acessíveis, a pecuária de corte necessita tornar-se cada vez mais eficiente para produzir de forma rápida, barata e sem prejudicar o meio ambiente. Tais resultados apenas serão possíveis aplicando conhecimentos técnicos de forma eficaz e incisiva. Sem dúvidas, a pecuária de corte é umas das principais atividades econômicas brasileira, o que representa mais de 8% do PIB nacional (Associação das Indústrias Exportadoras de Carne – ABIEC, 2020) e coloca o Brasil como maior exportador de carne bovina.

A carne bovina brasileira é mundialmente conhecida pelo preço mais acessível, que só é possível devido aos baixos custos dos sistemas de produção que utilizam forrageiras tropicais como principal fonte de alimento dos animais. Diversos fatores influenciam positivamente no desenvolvimento da pecuária, como clima, espécies forrageiras altamente produtivas e adaptadas às nossas condições edafoclimáticas, e raças bovinas adaptadas às condições climáticas brasileiras. Na maior parte do território nacional, o ano é dividido em uma estação chuvosa (primavera e verão) e uma seca (outono e inverno), o que pressiona o produtor a se planejar e extrair todo o potencial do sistema solo-planta-animal durante a estação chuvosa, que sem dúvidas é a de maior produtividade.

O solo é um sistema trifásico complexo que comporta o desenvolvimento de seres vivos, seja estruturalmente ou como fonte de nutrientes (DOS SANTOS et al., 2018). Na pecuária que utiliza a gramínea forrageira como principal fonte de alimento, o solo é base do sistema e fornece nutrientes para o desenvolvimento da planta forrageira, e consequentemente, para os animais. Por isso, para que a atividade se desenvolva e se mantenha, o solo deve ser tratado com a importância merecida e não como uma fonte inesgotável. Para tanto, é necessário o uso de técnicas como correção e adubação para reposição dos nutrientes retirados pela produção animal. Além disso, a correção do solo e a adubação potencializam a produtividade vegetal e, se houver eficiência de pastejo que preserve os mecanismos de rebrota, a persistência do pasto, resultando em forragem de alto valor nutritivo que pode aumentar a produção animal.

Mesmo que o solo seja bem manejado e tenha seus nutrientes repostos periodicamente, é necessário que a planta forrageira utilizada seja adaptada ao clima e ao tipo de solo, além de apresentar alta produtividade e características favoráveis ao consumo animal, tais como aceitabilidade, valor nutritivo elevado, resistência ao pastejo e pisoteio, além de baixa incidência de pragas e doenças. Assim sendo, há disponibilidade de diversas espécies e cultivares forrageiras, mas o principal gênero utilizado no Brasil é o *Urochloa* (syn. *Brachiaria*). Dentro deste gênero, destaca-se a espécie *Urochloa brizantha*, que apresenta várias cultivares, sendo a Marandu (*Urochloa brizantha cv. Marandu*) a mais cultivada, com aproximadamente 70% das pastagens brasileiras constituídas por esta cultivar (ANDRADE, 2001; MACEDO, 2013).

Sem dúvidas, o desenvolvimento da pecuária nacional se deu com o uso de gramíneas forrageiras do gênero *Urochloa*, mas também com a utilização de animais da raça Nelore (FERRAZ e FELÍCIO, 2010). Essa combinação do gênero *Urochloa* com animais selecionados, em associação à adaptação às condições climáticas brasileiras, demonstra o potencial de crescimento da pecuária brasileira, pois grande parte dessa pecuária, especificamente a de corte, ainda é realizada de forma extensiva ou semiextensiva. Técnicas de manejo empregadas de forma correta podem aumentar significativamente a produtividade da atividade, além de diminuir os impactos ambientes, como a produção de gases de efeito estufa (GEE), compactação e esgotamento dos nutrientes e degradação do solo.

Dentre os macronutrientes exigidos pelas plantas, o nitrogênio é o que apresenta maior potencial de resposta, quando administrado. Entretanto, a adubação nitrogenada nas pastagens cultivadas no Brasil ainda é significativamente baixa. Caso todo adubo nitrogenado utilizado em pastagens fosse dividido pela área das mesmas, seria apresentado média de apenas 7,00 kg N ha^{-1} (HEINRICHS, 2017). Portanto, o potencial uso da adubação nitrogenada é elevada na atividade pecuária brasileira. Todavia, o pastejo deve ser eficiente, para que a forragem produzida seja consumida pelo animal e não senesça, pois caso contrário, todo o investimento aplicado no pasto seja perdido.

2. Revisão de Literatura

2.1 A Pecuária de corte

A população mundial apresenta demanda cada vez maior de proteína animal de qualidade e o Brasil, como principal exportador de carne bovina, possui papel significativo nesta atual conjuntura. Além disso, a pecuária de corte possui grande importância no PIB nacional, pois a atividade representa 8,5% do total de R$ 7,3 trilhões (ABIEC, 2020), além de gerar empregos. Segundo a ABIEC (2020), o rebanho bovino brasileiro possui 213,68 milhões de cabeças das quais 43,3 milhões de cabeças são abatidas, sendo que os sistemas de terminação a pasto foram responsáveis por 87% dos bovinos abatidos dentro do território nacional. Portanto, é inegável a importância do sistema de pastejo dentro da pecuária de corte brasileira.

Por outro lado, o mercado consumidor atual é exigente e procura produtos vindos de sistemas eficientes, social e ambientalmente corretos e com preços competitivos. Em questões econômicas, a carne bovina é competitiva em termos mundial exatamente pelos baixos custos de produção, pois o principal alimento utilizado são forrageiras tropicais (CARVALHO e ZEN, 2017). Todavia, apesar de apresentar custos reduzidos, a pecuária de corte brasileira frequentemente é constituída por sistemas extensivos, o que gera índices produtivos aquém do potencial, baixa ou nenhuma reposição de nutrientes do solo e degradação do mesmo. Tal fato pode tornar as áreas utilizadas como pastagens impróprias para a atividade ao longo do tempo, devido ao esgotamento de nutrientes do solo, o que estimula a abertura de novas áreas de vegetação nativa para uso da pecuária.

Felizmente, não apenas os consumidores, mas também os produtores, cada dia mais estão preocupados com a preservação do meio ambiente. Desta forma, também está mais comum, porém ainda baixo, o uso de técnicas, como correção do solo, adubação de pastagens e manejo adequado do pastejo, que evitam degradação do meio ambiente ao mesmo tempo em que melhoram a produtividade do sistema.

2.2 O solo e a Pecuária

O solo é a fonte base de nutrientes de qualquer sistema de produção agropecuário. Portanto, é necessário que o fornecimento de nutrientes seja adequado à atividade desenvolvida na área. Cerca de 70% dos solos brasileiros, de forma geral, são ácidos e com níveis limitados de macro e micronutrientes, o que pode reduzir em até 40% a produtividade das plantas (QUAGGIO, 2000), principalmente em determinadas regiões como no Cerrado brasileiro, região onde a pecuária de corte largamente se desenvolveu. Apesar de apresentar potencial para melhorias através do uso de técnicas como calagem e adubação, a pecuária brasileira continua sendo desenvolvida em sistemas extensivos, implantados em solos de baixa fertilidade natural, ácidos e limitantes quanto à topografia, com baixo ou nenhum uso de tecnologias para reverter tal situação (MARTHA JÚNIOR e VILELA, 2002), além de apresentar resultados medianos como taxa de lotação de 1,06 UA ha^{-1} e taxa de desfrute de 20,9% (ABIEC, 2020).

2.3 Gramíneas Forrageiras Tropicais

As gramíneas forrageiras de clima tropical possuem rota fotossintética C4, podendo ser perenes e estivais. Possuem unidades fundamentais ao desenvolvimento, que são os fitômeros, unidades básicas formadoras dos perfilhos, compostos pelas seguintes estruturas: nó, gema axilar, entrenó, bainha, lígula e lâmina foliar. Segundo Casagrande et al. (2010), as tomadas de decisões quanto ao manejo adotado devem levar em consideração o conhecimento da morfologia e da morfogênese da planta forrageira, além de respeitar os limites biológicos da planta, principalmente sobre os mecanismos de controle da desfolha das mesmas.

O crescimento das gramíneas forrageiras está condicionado diretamente à morfogênese, e esta se relaciona com a disponibilidade de recursos, como água, luz, temperatura e nutrientes. Quanto à morfologia, é diretamente afetada pelas respostas morfogênicas, somadas ao manejo do pastejo adotado, representado pela frequência e intensidade de pastejo (SANTOS et al., 2011).

Para o adequado desenvolvimento de todo sistema de produção vegetal, as características físico-químicas do solo devem ser avaliadas e consideradas na escolha da espécie ou cultivar a ser explorada. Como exemplo de sucesso de adaptação e utilização nos sistemas pecuários brasileiros, há o gênero *Urochloa*, que, segundo Ferraz e Felício (2010), é aquele que alicerça toda a produção de bovinos de corte através de suas diferentes espécies e cultivares, pois apresentam elevada adaptabilidade às diversas condições de solo e clima encontrados no Brasil. Além disso, dentro do gênero existem consideráveis diferenças morfológicas entre as espécies, o que proporciona flexibilidade de manejo (JANK et al., 2013) e permite escolhas de forrageiras mais adequadas para cada situação de solo e clima. Como cultivar mais expressiva em sistemas pecuários brasileiros, tem-se a *Urochloa brizantha* (Hochst. ex A. Rich Stapf) cv. Marandu, popularmente conhecida como capim Marandu (COSTA et al., 2006). Tal fato pode ser comprovado com os dados de comercialização de sementes de forrageiras, onde 85% remetem aos diferentes cultivares do gênero *Urochloa*, sendo que o predomínio é do cultivar Marandu, com cerca de 70% de comercialização (ANDRADE, 2001; MACEDO, 2013).

O gênero *Urochloa* pertence à família *Poacea* e possui origem no continente africano, assim como grande parte das gramíneas forrageiras tropicais utilizadas em sistemas produtivos no Brasil. O gênero é amplamente difundido em regiões tropicais e subtropicais dos continentes Americano, Asiático e na Oceania (KELLER-GREIN et al., 1996). A escolha dos cultivares trazidos do seu continente de origem tinha como alvo indivíduos com alta produtividade, mesmo em solos de baixa e média fertilidade natural (EUCLIDES et al., 2008). Certamente, as *Urochloas* obtiveram destaque pela adaptação aos solos de diferentes níveis de fertilidade (SOUZA FILHO & DUTRA, 1991), além da resistência às pragas, como a cigarrinha-das-pastagens, no caso de algumas cultivares de *Urochloa brizantha*, como o cultivar Marandu (VALLE et al., 2000).

2.4 Manejo do pastejo

O controle e adequação das interações planta – animal para maximizar a eficiência do uso de forragem e do sistema de produção pecuário pode ser definido como manejo de pastejo. Quando realizado de forma correta e adequada à situação que o sistema se encontra, evitam-se sobras da forragem produzida e não consumida pelos animais. Desta forma, há menor senescência, morte de tecidos e maior eficiência de utilização da forragem, ou seja, aumenta-se o potencial para produção animal na área (OLIVEIRA, 2014).

Qualquer sistema de produção em pastejo tem como objetivo encontrar o ponto ótimo entre produção da forrageira e qualidade do alimento consumido pelos animais, pois, segundo Mott (1960), baixas taxas de lotação normalmente resultam em elevada oferta de forragem e alto desempenho individual, porém, com baixa produtividade por área. Por outro lado, altas taxas de lotação resultam em baixa oferta de forragem, comprometendo o desempenho, porém com elevada produtividade por área, desde que o ganho de peso por animal não seja demasiadamente comprometido.

Dentre as formas para o manejo do pastejo, há o critério de interceptação luminosa de 95% da luz incidente para interromper o período de descanso de pastos sob lotação intermitente (RHODES, 1973; HAY & WALKER, 1989; RUGGIERI et al., 2020). Tal critério corresponde ao índice de área foliar crítico, originalmente descrito e aplicado com sucesso em plantas de clima temperado, entretanto mostrou-se também válido também para o manejo de gramíneas tropicais, uma vez que resultou em benefícios para o acúmulo de forragem (SBRISSIA et al., 2007). Neste contexto, a taxa de acúmulo de forragem é máxima (PARSONS et al., 1988) e há maior eficiência de utilização dos pastos tropicais (GIMENES et al., 2011; DA SILVA et al., 2013; BARBERO et al., 2015).

Para facilitar tomadas de decisão no manejo do pastejo de espécies de gramíneas de clima tropical, a interceptação luminosa de 95% é correlacionada com a altura do pasto, pois é uma avaliação mais simples de ser realizada. Segundo Da Silva (2004), pastos de capim-marandu manejados sob lotação contínua devem ser manejados com alturas entre 20 a 40 cm, dependendo dos objetivos almejados referentes ao desempenho animal e produtividade. Em outros trabalhos desenvolvidos por Casagrande et al. (2013); Barbeiro et al (2015); Delevatti et al. (2018) e (2019); Koscheck et al. (2020) e Romanzini et al. (2018), a altura de manejo tida como ideal foi de 25 cm. Corroborando a informação, Santana et al. (2017) apontaram a altura de 25 cm como tendo 95% de interceptação luminosa do dossel e a mais indicada em sistemas de pastos de capim Marandu sob lotação contínua, pois proporciona relações folha:colmo acima de 0,95, massa de folhas verdes de 2.000 $kg.ha^{-1}$, e consequentemente, alto valor nutritivo. As características estruturais do perfilho associadas à oferta de forragem permite ganhos acima de 800 $g.animal^{-1}.dia^{-1}$, caracterizando alta produtividade animal (DELEVATTI et al., 2019).

2.5 Adubação nitrogenada

A adubação nitrogenada em gramíneas forrageiras promove diversos benefícios ao sistema de produção e o principal deles é o aumento da massa de forragem produzida (DE OLIVEIRA et al., 2020), através do aumento do ritmo de expansão da área foliar e no número de perfilhos (WHITEHEAD, 1995). Além disso, as gramíneas forrageiras, assim como as demais plantas, são formadas por compostos orgânicos constituídos por N, como as proteínas, aminoácidos, hormônios, clorofila e ácidos nucléicos. As principais formas de N inorgânico absorvidas pelas plantas são o nitrato (NO^{3-}) e amônio (NH^{4+}), e a maior absorção de uma forma, em relação à outra, está relacionada à variação de pH do meio (HOLFMAN e CLEEMPUT, 2004).

Desta forma, pastos adubados possuem maior capacidade de suporte, que contribui para a redução na pressão de abertura de novas áreas de pastagens. Além disso, a adubação nitrogenada possibilita outros benefícios ao sistema de forma geral, como o aumento do teor de matéria orgânica do solo ao longo dos anos (SANTOS, 2009), redução de perdas de produtividade das pastagens e mitigação de gases de efeito estufa (GEE) (MARTHA JÚNIOR et al., 2004).

Entretanto, a adubação nitrogenada, quando não realizada de forma correta, pode trazer prejuízos econômicos e ambientais ao sistema de produção animal. Essa situação pode ser evitada, definindo-se a dose, a fonte e o parcelamento mais adequados para cada solo e planta forrageira utilizada (MARTHA JÚNIOR et al., 2004). Doses de 80 $kg.ha^{-1}$ de N na forma de ureia, causam perda de 42% do nitrogênio aplicado, que, associado às perdas por lixiviação, diminui a recuperação do N aplicado no sistema solo-planta, aumenta as perdas para o ambiente, reduz a eficiência da adubação, e aumenta os impactos ambientais (MARTHA JÚNIOR et al., 2004).

Apesar de todos os benefícios, no Brasil a adubação nitrogenada das pastagens ainda é baixa. Segundo Heinrichs (2017), 520 mil ton de adubo nitrogenado são destinadas às pastagens brasileiras, que revela uma dose média de N aplicado de 3,46 kg/ha. Quando consideradas apenas as áreas de pastagens cultivadas, a dose sobe para 7,00 kg/ha, entretanto se mantém muito abaixo do que realmente deve ser utilizado, em se tratando de sistemas intensivos de produção, com elevada exportação de nutrientes do solo para as plantas e, consequentemente para os animais. Tal fato comprova um cenário com desempenho atual de $4,3@.ha^{-1}.ano^{-1}$ (ABIEC, 2020), porém, demonstra potencial para crescimento da pecuária brasileira.

2.6 Valor nutritivo

Os ingredientes utilizados na alimentação animal são compostos por duas frações básicas: água e matéria seca. Dentro da matéria seca, temos a matéria mineral, composta pelos macro e micro minerais, e a matéria orgânica, a qual é composta por todos os nutrientes e dividida em proteína bruta, extrato etéreo, fibra em detergente neutro e carboidratos não-fibrosos. As plantas forrageiras também são compostas da mesma forma, porém cada fração varia de acordo com espécie, cultivar, condições edafoclimáticas e manejo adotado, sendo este último o fator de maior controle pelo produtor. O aumento na intensidade de pastejo causa mudanças estruturais no pasto, decorrente da tentativa de se adaptar à nova condição e restabelecer a sua área foliar (LACA & LEMAIRE, 2000). Quanto às condições climáticas, a produção total de forragem é diretamente afetada pela frequência e quantidade de chuva, umidade relativa do ar, temperatura e fotoperíodo (pôr citações!). Portanto, as condições ambientais determinam alterações na produção e composição química das plantas forrageiras, devido às alterações na composição morfológica do dossel (ROMANZINI et al., 2017).

Além do aumento do acúmulo de massa de forragem, a adubação nitrogenada promove outros benefícios nos pastos, tais como melhora do valor nutritivo desde que a forragem seja colhida pelo animais antes da relação folha/colmo seja reduzida (GIMENES et al., 2011; SILVEIRA et al., 2013). O N recuperado pelas gramíneas forrageiras tende a aumentar a concentração de compostos nitrogenados no interior da mesma e, consequentemente, o teor de proteína bruta (PB) se eleva devido à maior síntese de aminoácidos, refletindo no consumo de PB pelos animais em pastejo. A ingestão de PB é um fator comumente limitante no desempenho animal em sistemas pecuários que utilizam gramíneas tropicais como alimento exclusivo. Pastos de capim

Marandu recebendo adubação nitrogenada de 270 kg N ha^{-1} ano^{-1} apresentam teores de PB de até 16% (DELEVATTI et al., 2019) e produções entre 16 e 17 t MS ha^{-1} ano^{-1} com fertilizações com 100 kg N ha^{-1} $corte^{-1}$ (DUPAS et al., 2016). Por outro lado, na ausência de adubação nitrogenada Delevatti et al. (2019) observaram pastos com 11% de PB e taxa de acúmulo menor do que 40 kg MS ha^{-1} dia^{-1}, tais dados elucidam o potencial do capim Marandu com relação à aplicação de fertilizantes nitrogenados no sistema pastoril.

Com relação à nutrição de ruminantes, a PB pode ser dividida em cinco frações, de acordo com Sniffen et al. (1992): Fração A: representa o N-não proteico (NNP), é solúvel e de alta digestibilidade no rúmen; Fração B1: parte da proteína verdadeira, também solúvel e de rápida degradação ruminal; Fração B2: parte da proteína que não é solúvel, não faz parte da parede celular e também não é NNP e apresenta degradação média; Fração B3: parte da proteína aderida na fibra em detergente neutro (FDN) e é digestível, porém com taxa de degradação muito lenta; Fração C: é a proteína indigestível contida na fibra em detergente ácido (FDA), associada à lignina e altamente resistente à degradação microbiana e enzimática. A adubação nitrogenada também afeta a distribuição de cada fração protéica, pois tende a aumentar as frações solúveis da PB e diminuir a porção de nitrogênio insolúvel em detergentes neutro e ácido (NIDN e NIDA, respectivamente). Portanto, caso a digestibilidade da FDN for baixa, resultando em forragem com baixo conteúdo de energia, há risco de perdas de N em pastagens adubadas, pois a eficiência de utilização de nitrogênio amoniacal ruminal e síntese de proteína microbiana serão menores e o N em excesso originado da alta degradação ruminal da PB será excretado pelo animal via urina. Segundo Detmann et al. (2014), quando se trata de sincronismo entre consume de energia e proteína em bovinos, a maior eficiência de uso de N no desempenho animal ocorre com consumo de 200g de proteína bruta por kg de matéria orgânica digestível.

2.7 Desempenho animal

O desempenho animal é um dos principais fatores que afeta a eficiência produtiva e econômica de sistemas pecuários, e é definido principalmente pelo consumo de nutrientes digestíveis, ou seja, a qualidade da forragem (MOTT & MOORE, 1973). O consumo de forragem se relaciona mais com os fatores não nutricionais relacionados à estrutura do pasto, como massa de forragem, altura, proporção de colmo, material morto e relação folha:colmo; do que aos fatores nutricionais relacionados ao valor nutritivo em sistemas pastoris (POPPI et al., 1987). Segundo Sollenberger e Vanzant (2011), 60 a 90% das variações referentes ao desempenho de animais em pastejo são explicados pela oferta de forragem.

Dentre as estruturas morfológicas de uma planta forrageira, a folha verde é a fração mais selecionada pelos animais durante o pastejo, pois possui alta PB (16%MS) e baixa FDN (65,6%MS), quando comparado com os colmos que possuem 8,6% de PB e 76% de FDN (PACIULLO et al., 2001) e pode representar mais de 80% da forragem consumida pelos animais (FORBES & HODGSON, 1985), quando o consumo não é limitado pela oferta de forragem. Para possibilitar a seleção de folha no consumo, tornar a utilização do pasto produzida eficiente e alcançar o desempenho animal almejado, o manejo do pastejo representa de 40 a 85% da possibilidade de ajuste no sistema produtivo (HODGSON, 1990). Sollenberger et al. (2005) afirmaram que a oferta de lâminas foliares verdes é o que permite predizer com segurança o desempenho animal, por integrar a massa de lâminas foliares com a taxa de lotação. Desta forma, a massa de forragem, a porcentagem de folhas e a taxa de lotação praticada na área interferem diretamente na quantidade e qualidade da forragem disponível (REIS et al., 2009).

Em um sistema produtivo em pastagens não se pode priorizar apenas o ganho de peso por animal e deixar o ganho por área comprometido, pois o sistema pode se tornar inviável economicamente. Casagrande et al. (2013) observaram que, ao intensificar o manejo do pastejo, aumentando a taxa de lotação e manejando pastos de capim Marandu com 15 cm de altura, foi possível aumentar o ganho por área, porém, o ganho individual pode ser comprometido, quando a suplementação não for utilizada, pois com a maior taxa de lotação e menor altura do pasto, a oferta de forragem foi reduzida. Por isso, a tomada de decisão para definir o ponto ideal entre ganho por animal e ganho por área, seguindo os conceitos de Mott (1960), é o que definirá o fracasso ou o sucesso da atividade produtiva.

2.8 Eficiência do uso de nitrogênio

A adubação de pastos, apesar dos diversos benefícios, pode acarretar problemas ao sistema, como a perda do N aplicado, que ocorre em toda adubação nitrogenada via volatilização, desnitrificação e, principalmente, lixiviação (CARDOSO et al., 2019). Essas perdas diminuem a eficiência da adubação, que prejudica a viabilidade da mesma no sistema produtivo e ainda provoca impactos ambientais (COSTA et al., 2006), como a contaminação das águas por excesso de N.

Para evitar perdas e melhorar a eficiência, é necessário empregar técnicas adequadas e que considerem as condições climáticas, de solo e as relações solo-planta. O primeiro fator a ser levado em conta na adubação nitrogenada é que os demais nutrientes estejam em quantidade necessária para suprir as exigências das plantas e não limitem seu crescimento (CORRÊA, 2000). A eficiência do uso de N pode ser medida através da recuperação de N pelas plantas forrageiras, técnica importante para o manejo de pastagens adubadas, principalmente quando são aplicadas doses elevadas de N (PRIMAVESI et al., 2006).

Ongaratto (2021) avaliou sistemas de produção em pastagens, onde o sistema intensivo foi caracterizado pela dose de N aplicada de 150 kg ha^{-1} ano^{-1}. Nesse caso, a eficiência de uso do N foi de 40,9 g N por kg N aplicado, menor do que o sistema semi-intensivo de 75 kg ha^{-1} ano^{-1} de N, que apresentou 58,3 g N por kg N aplicado. Portanto, é necessária maior atenção na tomada de decisão quanto à dose de N a ser aplicada, porque quanto maior a dose de N, menor será a eficiência de uso de N (WHITEHEAD, 1995, 2000). Todavia, a pecuária brasileira ainda possui espaço para crescimento neste ponto, pois as doses de N utilizadas em pastagens ainda são muito baixas (HEINRICHS, 2017), e também porque, segundo Martha Junior et al. (2004), a eficiência do uso do N na produção animal é de 1,45 kg de ganho de peso vivo por kg de N aplicado, considerando um potencial entre 3,5 a 4,0 kg de ganho de peso vivo por kg de N aplicado. Tal fato é evidenciado por Delevatti et al. (2019), onde foi observado 1,68 a 2,84 kg de ganho de peso vivo por kg de N aplicado em pastagens de capim Marandu adubadas com 0, 90, 180 e 270 kg N ha^{-1} ano^{-1}. Portanto, para que o sistema de produção bovino em pastagens tropicais seja economicamente viável e ambientalmente correto, é necessário que a adubação nitrogenada, assim como as demais técnicas produtivas, seja utilizada com eficiência.

Objetivou-se avaliar os efeitos da adubação nitrogenada aplicada em pastagens com *Urochloa brizantha* cv. Marandu durante o período de águas sobre as seguintes características: massa de forragem (MF), taxa de acúmulo de forragem, proporção de níveis críticos e absorvidos de nitrogênio pelas plantas (índice nutricional de nitrogênio

- INN), eficiência de uso do nitrogênio, componentes morfológicos e químicos da planta forrageira e desempenho animal.

3. Hipóteses

O presente estudo considerou como hipóteses que a aplicação de doses de nitrogênio combinada ao manejo do pastejo em lotação contínua com altura de 25 cm de altura (95% IL): aumenta a massa de forragem (MF) e Taxa de acúmulo de forragem; melhora a qualidade da forragem; aumenta a proporção de níveis críticos e absorvidos de nitrogênio por plantas (índice nutricional de nitrogênio - INN); reduz a eficiência de uso do nitrogênio; altera os componentes morfológicos e a composição química da planta forrageira, melhora o desempenho animal através do aumento do ganho de peso por área, e aumenta as frações solúveis de proteínas e carboidratos da forragem.

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Chapter 2 - Effect of nitrogen application rate on forage quality, animal performance and nitrogen use efficiency in Marandu grass pastures

Effect of nitrogen application rate on forage quality, animal performance and nitrogen use efficiency in Marandu grass pastures

ABSTRACT: The two-year-long field experiment was conducted in a continuous grazing system with variable stocking rate to evaluate effects of nitrogen levels in Marandu grass (*Urochloa brizantha* Hochstex A. Rich Stapf - marandu) on herbage mass, forage accumulation rate, forage quality, stocking rate, average daily gain, gain per hectare, and gain per kg of applied N. The experimental design was completely randomized with four treatments (control without application of N, and 90, 180, and 270 kg N ha^{-1} $year^{-1}$) and three replicates (paddocks per treatment); nitrogen was applied in the form of urea. Herbage mass, crude protein (CP), forage accumulation rate, stocking rate, gain per hectare, and the nitrogen nutrition index increased with increasing nitrogen level ($P<0.05$), where as the neutral detergent fibre (apNDF), acid detergent fiber (ADF), and nitrogen usage efficiency decreased with increasing nitrogen level ($P<0.05$). Nitrogen application rate not affected ADG ($P=0.183$). Increasing the nitrogen level is a promising way to improve Marandu grass production, nutritive value, and animal production, however with decreased nitrogen use efficiency.

Key Words: fertilization, forage quality, beef cattle, nitrogen, pasture, *Urochloa brizantha*.

1. Introduction

Brazilian livestock is an expressive producer of high quality human edible protein. The beef cattle chain is responsible for producing over 10.49 million tons of beef each year which is exported to over 124 countries, generating R$ 618.50 billion to the Brazilian economy (ABIEC, 2020), in addition to producing beef, a high quality protein source rich in high biological value proteins and amino acids (HOFFMAN & FALVO, 2004). However, despite the great economic and social importance of the beef cattle chain, there is still great potential for its growth, as beef cattle production is usually developed with low technological input (HEINRICHS, 2017), which can degrade pastures and compromise animal production.

Nitrogen fertilization appears as a suitable tool to increase the productivity of beef cattle systems. Nitrogen fertilization promotes an increase in the forage mass produced (DE OLIVEIRA et al., 2020), by increasing the rate of appearance and expansion of the leaves and the weight and number of tillers (WHITEHEAD, 1995). The nutritional value is also improved as the amount of crude protein (CP) content increases (GIMENES et al., 2011; SILVEIRA et al., 2013). However, for a better efficiency of nitrogen fertilization, the light interception criterion is used in grazing management, where 95% of the light incident on the canopy is intercepted by the leaves (RUGGIERI et al., 2020). In this condition, the forage accumulation rate is maximum (PARSONS et al., 1988) and there is greater efficiency of utilization in tropical grass pastures systems (DA SILVA et al., 2013; BARBERO et al., 2015). To be easily measurable, the light interception criterion can be related to pasture height. The authors Barbero et al (2015); Delevatti et al. (2019); Koscheck et al. (2020) and Romanzini et al. (2018) made this correlation and used a management height of 25 cm as ideal in a system with 95% light interception in Marandu grass pastures (*Urochloa brizantha* cv. Marandu).

When fertilized and properly managed, pastures offer sufficient green leaf blades to ensure animal performance (SOLLENBERGER et al., 2005), as the forage mass, the percentage of leaves and the stocking rate practiced in the area interfere in the quantity and available forage quality (REIS et al., 2009) and directly influence animal performance. In this context, nitrogen fertilization is more efficient in agronomic and livestock terms, such as Ongaratto (2021), observed nitrogen recovery of up to 58.3 gN $kgNapplied^{-1}$, and Delevatti et al. (2019), observed 1.68 to 2.84 kg of live weight gain per kg of N applied.

The aim of this study was to evaluate the effects of nitrogen fertilization applied to pastures of *Urochloa brizantha* cv. Marandu during the rainy season on animal performance, forage quality and N use efficiency. It was hypothesized that nitrogen fertilization associated with grazing management with 95% IL: increases herbage mass, forage accumulation rate and animal performance; reduces the efficiency of nitrogen use; and increases forage quality.

2. Methods

2.1 Experimental area and design

We conducted a two-year-long experiment in the Forage and Grasslands sector of São Paulo State University, "Julio de Mesquita Filho" (UNESP) (Jaboticabal, São Paulo, Brazil), during the summer growing season (December to April) of 2017/2018 and 2018/2019. Climate of the experimental area is classified as the subtropical humid type, with wet summers and dry winters. The mean annual rainfall is 1424 mm, mean air temperature is 22.3 °C, and the soil is a Rhodic Ferralsol (IUSS, 2006) derived from basalt. The pasture was established in 2001 with *Urochloa brizantha* (Hochst ex A. Rich) Stapf Marandu (Marandu grass). Precipitation and temperatures are presented in the Table 1.

Table 1. Climatic data from the trial period of palisade grass pastures under fertilization with different doses of nitrogen.

	T max	T min	T mean	Precipitation	ND
2017-2018					
December	30,9	20,4	24,7	131	16
January	30,9	20,3	24,2	271	21
February	30,5	19,7	24,0	87	12
March	32,2	20,6	25,5	56	10
April	30,8	17,3	23,2	11	3
			Total	402	37
2018-2019					
December	32,0	20,2	25,2	88	16
January	32,7	20,9	26,1	148	11
February	30,9	20,4	24,4	283	17
March	31,0	20,1	24,5	115	12
April	30,6	19,0	23,9	98	6
			Total	644	56

T max: maximum temperature; T min: minimum temperature; T mean: mean temperature; ND: number of days with precipitation. The meteorological elements used in this work were extracted from a set of data belonging to the collection of the area of Agrometeorology of the Department of Exact Sciences. The observations made at the Jaboticabal Campus Agroclimatological Station are quoted, typed in a standardized format, consistency and quality control performed. Then the daily, monthly and annual averages are obtained and passed on to users.

Experimental design was a completely randomized design, with four treatments (0, 90, 180, and 270 kg N ha^{-1}) using urea fertilizer, which were applied in a split design during 3 fertilizations per growing season, and 3 replicates per treatment, totaling 12 paddocks (experimental units). The paddock areas were 1.3 ha, 1 ha, 0.7 ha, and 0.5 ha for the treatments 0, 90, 180, and 270 kg N ha^{-1}, respectively.

At the end of the raining period prior to each experimental year, soil samples were collected to characterize the soil and the definition of maintenance fertilization was carried out previously. The values are shown in table 2. It was defined that all paddocks receive 100 kg ha^{-1} of P_2O_5 in the form of simple superphosphate (18% P_2O_5) and 200 kg ha^{-1} of K_2O in the form of potassium chloride (60% K_2O).

Table 2. Soil chemical composition and texture in the Marandu grass pasture paddocks under fertilization with different doses of nitrogen.

Treat	P resina mg dm^{-3}	MO g dm^{-3}	pH	K^{+}	Ca^{2+}	Mg^{2+}	H+Al	SB	CTC	V %
				---------------------- mmol$_c$/dm³ ----------------------						
0	13	33	5,1	1,7	21	10	30	33	62	52
90	11	32	5,0	2,0	17	8	32	28	59	47
180	12	32	5,0	1,3	20	8	31	29	60	48
270	16	29	5,3	2,2	21	9	26	31	58	55

Treatment (Treat); Soil pH in $CaCl_2$ (pH); soil organic matter (SOM); percent of base saturation (V).

2.2 Animal and grazing management

This study was conducted under grazing. Each year seventy-two young Nellore bulls (*Bos indicus*) in each year with an initial BW (mean ± SD) of 242 ± 7 kg, 220 ± 8 kg were utilized to measure animal productivity in the first- and second-year experimental, respectively.

Pasture management was put-and-take methodology under continuous stocking (Mott and Lucas, 1952). Grazing target was the pasture height of 25 cm that was chosen because at this height, the canopy intercepted 95% of the light under our experimental conditions. At this light interception, it is possible to achieve the maximal net forage accumulation, which may result in high animal performance (BARBERO et al., 2015; SANTANA et al., 2017; ROMANZINI et al., 2018). To kept pasture height stocking rate was adjusted weekly.

2.3 Forage sampling

During the experimental period, the grazing height was measured once a week. Eighty measurements per hectare were obtained using a ruler graduated in centimeters at the curvature of the upper leaves. Every 28 days herbage mass was estimated using eight samples collected per cut per paddock(BARBERO et al., 2015) at the average pasture height points of the paddock (approximately 5.0 cm of residue) using a 0.25 m^2 circular frame. Forage samples were separated into green leaves, stem + sheath, and dead material, and then dried in an oven (55 ± 5 °C for 72 h), following which, they were weighed to calculate the forage dry mass per hectare. To determine the forage composition, samples were collected from 20 points per hectare via the grazing-simulation method (BARBERO et al., 2015) with approximately 300 g of fresh matter harvested per sample. Samples were dried in a forced-air oven (55 ± 5 °C, for 72 h), ground in a mill (Thomas-Wiley Laboratory Mill Model 4, H. Thomas Co.), and taken to the laboratory for analyses. The cumulative herbage dry mass was evaluated using exclusion cages and the double pairing technique (MORAES et al., 1990). In this technique, forage samples are collected at 5 cm height from the ground and the cages are used to isolate the spots accrued because of the presence of young bulls under continuous grazing in the experimental area. On the first day of placement of the cages, two areas with similar herbage dry matter of Marandu grass were selected by visual criteria, representing the paddock condition. To estimate the initial herbage mass of Marandu grass inside the cage, we placed the cage in an area, and collected samples from a $1m^2$ area both inside and outside the cage (paired samples) at 5 cm height from the ground in the average height. After 28 days of cage placement, forage samples were collected again to obtain herbage accumulation. The samples were then weighed to obtain the fresh mass, and subsamples of approximately 300 g were separated and dried in a forced-air ventilation oven at 55 °C for 72 h to estimate the dry weight and in a forced-air ventilation oven at 55 °C for 72 h to estimate the dry weight and calculate the dry mass (kg ha^{-1}). Forage accumulation rate (kg ha^{-1} day^{-1}) was calculated by dividing the cumulative forage dry mass by the number of days between evaluations (28d). Cumulative forage dry mass was obtained by the difference between the forage dry mass of the samples collected from the interior of the cage on the date of sampling and the forage dry mass of the sample outside the cage (paired sample) on the date of the previous sampling.

2.4 Chemical composition

Dry matter (DM), organic matter (OM), and ash were estimated following the procedures described in AOAC (AOAC, 1990) (AOAC 934.01 for DM, AOAC 942.05 for OM, and AOAC 942.05 for ash). Crude protein content was estimated using a LECO® FP 528 device (Leco Corporation, Michigan, USA). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using the procedures described by ANKOM Technology (ANKOM, 2006a, ANKOM, 2006b, ANKOM, 2006c). Energy was obtained using an adiabatic calorimetric pump (PARR Instrument Company 6300, Illinois, USA), given in mega joule (MJ) per kg.

2.5 Intake and digestibility

To estimate fecal excretion, 12 g of chromium oxide (Cr_2O_3) was administered orally as an external marker (BURNS et al., 1994). Dosing of Cr_2O_3 occurred for 10 days. The internal marker used to estimate the intake of forage was indigestible neutral detergent fiber (iNDF). The iNDF was measured following the method of Casali et al. (2008), using *in situ* incubation for 288 h. During days 8, 9, and 10 of of chromium oxide intake, feces were collected from steers at 07:00, 13:00, and 17:00 h of each day. Feces were then oven dried (55 ± 5 °C for 72 h), ground in a mill (Thomas-Wiley Laboratory Mill Model 4, H. Thomas Co.), and taken to the laboratory for analysis.

The dynamic ruminal degradation of neutral detergent fiber (NDF) test began on day 10. Forage samples obtained by grazing-simulation were incubated in duplicate inside the rumen *via* the rumen cannula. Duplicate samples were incubated inside the rumen for 288 h. After which, incubated samples were washed with water, oven dried (55 ± 5 °C for 72 h) and taken to the laboratory for analysis.

2.6 NUE and nutrition index

Apparent N recovery (REC) by the forage was estimated (LEMAIRE et al., 2008) based on N uptake of the fertilized treatments and unfertilized control as follows: REC (%) = {(Uf – Uo) / Nf} where Nf = fertilizer - N rate (kg ha^{-1}) and Uf = N uptake (kg ha^{-1}) when Nf is given, and Uo = N uptake (kilograms per hectare) in non-fertilized paddocks. Apparent efficiency of absorbed N (NUE) by the plants was calculated (LEMAIRE et al., 2008) as follows: NUE (kg kg^{-1} N) = {(TFM) /Nc} where TFM = total forage mass of the treatment in kg and Nc = nitrogen uptake by the pastures in kg. Nutrient yields were calculated by multiplying the DM yield by nutrient concentration in each sampling period. Nitrogen nutrition index (NNI) of Marandu grass was calculated as: (NNI = (Na / Nc) where Nc = aW^{-b}), where Na is the actual N concentration, Nc is the critical N concentration, W is the DM yield (Mg ha^{-1}), and a and b are species-specific constants for C4 perennial grasses (3.6 and 0.34, respectively; references ALDERMAN et al., 2011 and LEMAIRE et al., 2008). Luxury N consumption was assumed for NNI values > 1 (LEMAIRE et al., 2008).

2.7 Animal production

Bulls were weighed at the beginning (0 days) of the experiment, at the beginning of the adaptation period of each year, and at the end of the experimental period, after fasting for 12 h prior to each weighing event. The bulls were also weighed every 28 days (without fasting), to adjust the stocking rate to the maintain pasture height. Animal performance variables were calculated as follows (BARBERO et al., 2015): Body weight gain - BW gain (kg) = (final BW – initial BW); Average daily gain - ADG (kg day^{-1}) = (BW gain (kg) / days); Gain per area - GPH = (ADG × number of animals per days × experimental period (days)) / area (ha)), where the number of animal days (animal day^{-1} ha^{-1}) was calculated by dividing animal stock by the mean weight of "testers". Animal stock was obtained by the sum of weights of all animals present in each paddock divided by the area of the paddock (kg BW ha^{-1}); Gain per kg N $applied^{-1}$ GPN (kg gain kg^{-1} N applied) = (GPH / kg of N applied); Stocking rate in animal unit (AU = 450 kg BW) / ha = (∑ BW mean / 450) / area (ha); Herbage mass allowance (kg DM / kg BW) = (kg DM / ha) / (∑ BW mean / area (ha)).

2.8 Statistical analysis

Data were tested for normality and equal variance using the Shapiro-Wilk normality test and Bartlett test of homogeneity of variances, respectively. Data were analyzed using the mixed model by the MIXED procedure of SAS 9.2, and the statistical model included nitrogen level as fixed effect, whereas paddock or animal was the random effect. All variables were analyzed as repeated measures, except animal performance variables. The best covariance structure used for repeated-measures analyses was chosen as the one that achieved the lowest corrected Akaike and Bayesian information. Significant effects for treatment were declared at $P < 0.05$. When a significant effect was found, orthogonal polynomial contrasts were performed to assess the effect of nitrogen dose on the variables.

3. Results and Discussion

3.1 Herbage characteristics, chemical composition, and forage accumulation

Forage mass was linearly affected (p=0.005) by nitrogen fertilization (Table 3). As all treatments were kept at the same management height, the difference was due to the tiller density (ONGARATTO, 2021). The result was expected, as the application of nitrogen increases the number of tillers in tropical grasses (LOPES et al., 2017). Forage mass values ranged from 5640 to 6449 kg of DM ha^{-1}, values similar to previous findings in other studies conducted in the same experimental area (DELEVATTI et al. 2018, DELEVATTI et al. 2019, KOSCHECK et al. 2020).

Table 3. Herbage mass, morphological composition, forage allowance, chemical composition of palisade grass pastures under fertilization with different doses of nitrogen. (Means of 2 years of evaluation, 4 evaluations per year, n=24).

	Treatment (kg N ha^{-1})						
Variable	0	90	180	270	SEM	*p-value*	Effect
Herbage characteristics							
Herbage mass (kg ha^{-1})	5740	6312	6324	6449	44,4	0,005	Linear
Green leaves (g kg^{-1})	229	274	266	282	4,3	0,026	Quadr
Stem + sheath (g kg^{-1})	255	292	278	289	20,4	0,041	Linear
Dead material (g kg^{-1})	510	444	448	429	4,2	< 0,001	Linear
Leaf / stem ratio	0,97	1,01	1,01	0,96	0,01	0,017	Quadr
Herbage allowance (kg DM kg BW^{-1})	4,99	3,50	2,99	2,87	0,03	0,028	Quadr
Green leaves allowance (kg DM kg BW^{-1})	1,17	0,92	0,83	0,81	0,02	0,021	Quadr
Accumulation rate (kg DM ha^{-1} day^{-1})	35,1	85,7	91,7	161	0,02	< 0,001	Linear
Chemical composition							
OM (g kg^{-1} DM)	908	910	913	910	0,30	0,001	Quadr
CP (g kg^{-1} DM)	115	145	173	193	1,80	< 0,001	Linear
NDFap (g kg^{-1} DM)	612	597	582	550	0,40	< 0,001	Linear
iNDF (g kg^{-1} DM)	196	185	188	172	10,3	< 0,001	Linear
pdNDF (g kg^{-1} DM)	418	411	396	380	5,20	< 0,001	Linear
ADF (g kg^{-1} DM)	353	338	331	322	11,4	< 0,001	Linear
Lignin (g kg^{-1} DM)	52	55	61	64	621	0,063	NS
NFC (g kg^{-1} DM)	157	144	140	131	1,20	0,006	Quadr
Energy (MJ kg^{-1})	16,6	16,6	17,1	16,4	0,01	0,135	NS

Nitrogen (N), dry matter (DM), body weight (BW), organic matter (OM), crude protein (CP), neutral detergent fiber free ash and protein (NDFap), acid detergent fiber (ADF), indigestible neutral detergent fiber (iNDF), potentially digestible neutral detergent fiber (pdNDF) non-fiber carboidrate (NFC), standard error of mean (SEM), quadratic effect (Quadr).

Regarding the morphological composition (Table 3), the increase in nitrogen dose caused a quadratic increase (p=0.026) in the proportion of green leaves, linear increase (0.041) in the proportion of stem + sheath and linear reduction (p<0.001) in the proportion of dead material. Such effects on the pasture morphological composition are observed, as the increasing levels of nitrogen applied leads to increases in the leaf elongation rate, leaf area and decreases in the senescence rate (LOPES et al., 2017). The greater amount of green leaves harvested by the animals resulted in an increase of the proportion of stem + sheath. In this study the stocking rate was adjusted to reach 25 cm in height and all pastures were kept at canopy height of 25 cm (SANTANA et al., 2017). Therefore, canopy leaf blades grew and were soon consumed, inhibiting stem elongation and senescence (RUGGIERI et al., 2020)

We observed a linear effect (p<0.001) on the forage accumulation rate (Figure 1), with values from 35 to 161 kg DM ha^{-1} day^{-1}. Conversely, Delevatti et al (2019) found lower values, especially for the 270 kg N ha^{-1} treatment (35 to 90 kg DM ha^{-1} day^{-1}). Likewise, values reported for the Cerrado region were also lower, presenting an average of 63 kg DM ha^{-1} day^{-1} (PAULA et al., 2012).

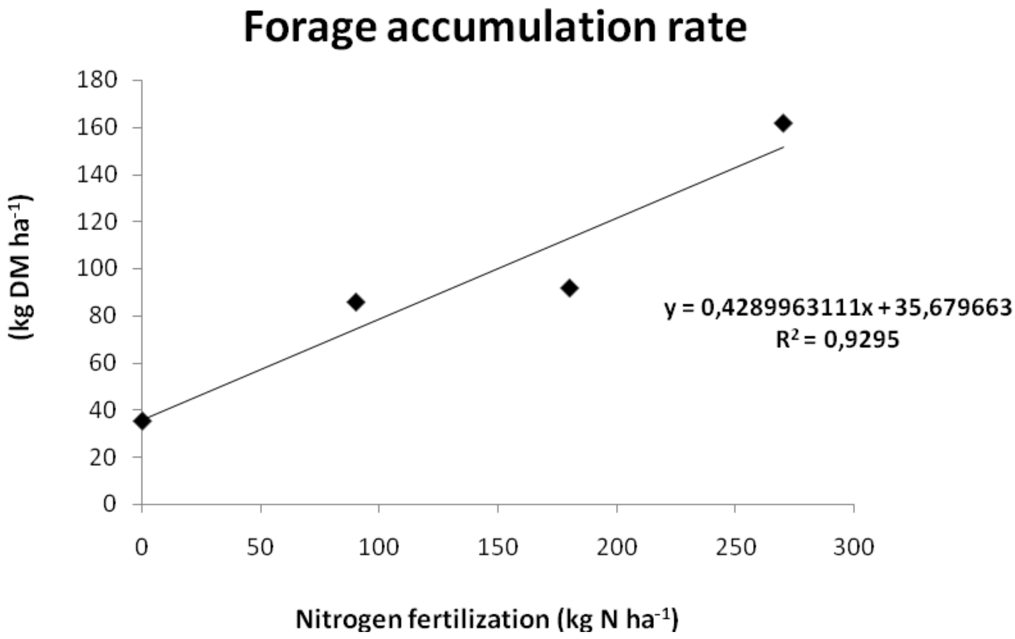

Figure 1. Forage accumulation rate (kg DM^{-1} day^{-1}) of Marandu grass under fertilization with different doses of nitrogen. ANOVA (P < 0,001). The effect of nitrogen application was linear and positive. In the linear equation, f(x) is a function of N dose (x = kg N ha^{-1}) and y = kg DM^{-1} day^{-1}

Our result of forage accumulation rate is explained by the climatic conditions and the grazing management adopted. Normally, in this evaluation, the quadratic effect is observed. The increase of the fertilizer dose, result in increase in the forage growth rate in a reduced way, defined by the Law of Diminishing Returns (MITSCHERLICH, 1912). The low nitrogen dose might have contributed to the linear effect for accumulation rate variables, if the doses applied were higher, a quadratic effect on the accumulation rate would be observed.

It was observed a linear effect ($p<0.001$) for the crude protein (CP) content of the forage (Table 3). The CP values were ranged from 115 to 193 g kg^{-1} CP. A linear increase in CP was also reported by Martins et al. (2006) and Dupas et al. (2016) evaluating Marandu grass in the subtropical region and in the Cerrado biome, respectively. However, the values reported were lower than the ones found in the present study. These even when compared with other assessments in the same area (BARBERO et al., 2015; BARBERO et al., 2017; OLIVEIRA et al., 2015) our results of CP at the highest nitrogen dose were higher. This result is mainly due to two factors: the leaf:stem ratio allowed the animals to select green leaves during grazing activities, which ensured a greater proportion of cell content when compared to the cell wall in forage available.; in addition, the doses of nitrogen (N) caused the luxury nutrition of this nutrient (NNI>1, Table 7).

The neutral detergent fiber corrected to ash and protein (NDFap), acid detergent fiber (ADF) decreased ($p<0.001$) and lignin contents increased as the N dose increased ($p=0.063$), while non-fibrous carbohydrates also decreased, but in a quadratic way ($p=0.006$). As the CP increases, the other organic compounds tend to decrease proportionally (PEYRAUD & ASTIGARRAGA, 1998), both in the digestible and indigestible fractions, which can be confirmed in the reduction of iNDF and pdNDF ($p <0.001$). Our results are consistent with the findings by Delevatti et al. (2019). The forage plant to increase the CP content, uses available carbon to reduce the inorganic N for the synthesis of amino acids instead of producing structural carbohydrates (TAIZ & ZEIGER, 2006).

3.2 Efficiency of nitrogen

The N absorbed (kg N ha^{-1}) (Table 4) by the forage showed a linear increase ($p<0.001$) with the increasing N doses applied, as it accounts for both N coming from the soil and fertilizer. While N recovered (g N kg N $applied^{-1}$) did not vary among the treatments. Results different and superior to those were observed by Delevatti et al.

(2019) who observed a linear increase in both factors and a variation of N absorbed between 110 and 175 kg N ha^{-1}. Despite not showing a significant effect, the recovered N presented consistent values to studies with similar conditions (SILVA et al., 2013) and higher values when compared with studies developed in other soil and climate conditions, such as in the Brazilian Cerrado, with recovered N of 203 g N kg N $applied^{-1}$ (DUPAS et al. 2016). The N recovery varies according to the forage species, forage management, N source, soil properties and environmental factors (STOTT, & GOURLEY, 2016). In our study, the N recovery may not have been greater regarding to losses by ammonia volatilization due to litter, which combined with the application of urea on the surface increased the losses by volatilization (SOMMER & JENSEN, 1994). Furthermore, N losses by leaching also reduce N recovery and can cause environmental problems (MARTHA JÚNIOR et al., 2004). The fractionation of N dose application aimed to reduce losses by volatilization.

Table 4. Apparent nitrogen recovery, nitrogen usage efficiency, and nitrogen nutrition index of palisade grass under fertilization with different doses of nitrogen. (Means of 2 years of evaluation, 4 evaluations per year, n=24).

	Treatment (kg N ha^{-1})						
Variable	0	90	180	270	SEM	*p-value*	Effect
N absorbed (kg N ha^{-1})	125	210	283	360	0,690	< 0,001	Linear
N rec (g N kg N $applied^{-1}$)	-	516	459	399	9,110	0,153	NS
NUE (kg DM kg^{-1} N abs)	-	42,1	35,0	30,9	0,004	< 0,001	Linear
NUE (kg DM kg^{-1} N applied)	-	97,0	54,2	41,3	0,082	< 0,001	Quadr
NNI (dimensionless)	0,99	1,38	1,74	2,04	0,015	< 0,001	Linear

Nitrogen (N), nitrogen usage efficiency (NUE), nitrogen recovery (N rec), nitrogen absorbed (N abs), nitrogen nutrition index (NNI), standard error of mean (SEM), quadratic effect (Quadr).

N use efficiency (NUE) was evaluated in two ways, production of kg of DM per kg of N absorbed and production of kg of DM per kg of N recovered (Table 4). Both treatments caused a reduction in NUE, however linear ($p<0.001$) when in kg DM kg^{-1} N absorbed (abs) and quadratic ($p<0.001$) when in kg DM kg^{-1} N applied. The N losses increased with the increasing N doses applied. Therefore, the dose of N applied must be carefully evaluated, as lower NUE causes an increase in the cost of production and environmental damage (CARDOSO et al., 2016). Therefore, the Nitrogen Nutrition Index (NNI) can be adopted as a criterion, where values below 1 represent deficiency, above 1 represent luxury nutrition, while 1 is the critical value. We observed a linear increase ($p<0.001$) in the NNI. Only the control treatment presented NNI lower than 1, however it approached the critical value (0.99). The other treatments presented values between 1.38 and 2.04, which configures luxury nutrition by N. Delevatti et al. (2019) observed, under similar conditions, lower values in the fertilized treatments, but also above 1. The 90 kg N ha^{-1} treatment is showed to be the best combination of NNI close to 1 and morphological and forage production characteristics.

3.3 Intake and digestibility

Dry matter (DM) intake in kg d^{-1} and % body weight (BW), of organic matter (OM), digestible organic matter (DOM) and NDFap were not affected by the N dose (Table 5). Irrespective of N fertilization, the grazing management of palisadegrass pastures at 25-cm height with 95% of IL criterion has been demonstrated to provide forage with high nutritional value not limiting animal intake (RUGGIERI et al., 2020). On the other hand, the CP intake increased linearly ($p=0.008$) with the increase of N applied, as the CP content of pastures also showed a linear increase, corroborating with the results also observed by Delevatti et al. (2019).

Table 5. Intake and digestibility of yearling bulls in palisade grass pastures under fertilization with different doses of nitrogen. (Means of 2 years of evaluation, 4 evaluations per year, n=24).

	Treatment (kg N ha^{-1})						
Variable	0	90	180	270	SEM	*p-value*	Effect

Intake							
DM (kg day^{-1})	5,98	5,74	5,31	6,23	0,268	0,741	NS
DM (% BW)	1,97	1,77	1,74	1,99	8,270	0,703	NS
OM (kg day^{-1})	5,43	5,24	4,86	5,71	0,249	0,745	NS
DOM (kg day^{-1})	2,81	2,97	2,70	3,31	0,104	0,540	NS
CP (kg day^{-1})	0,64	0,78	0,85	1,04	0,052	0,008	Linear
NDFap (kg day^{-1})	3,74	3,47	3,17	3,60	0,077	0,722	NS
TDN (kg day^{-1})	2,84	3,01	2,74	3,36	0,076	0,674	NS
g PB kg DOM^{-1}	226	256	303	311	0,500	< 0,001	Linear
Digestibility							
DM (g kg^{-1})	469	536	491	544	15,8	< 0,001	Cubic
OM (g kg^{-1})	518	571	523	577	25,8	< 0,001	Cubic
CP (g kg^{-1})	312	512	463	587	7,0	< 0,001	Cubic
NDFap (g kg^{-1})	525	578	550	587	10,9	0,021	Cubic

Nitrogen (N), dry matter (DM), body weight (BW), organic matter (OM), digestible organic matter (DOM), crude protein (CP), neutral detergent fiber free ash and protein (NDFap), standard error of mean (SEM).

The relationship between CP intake and DOM showed linear increase ($p<0.001$) in response to an increase N application. The ratio values ranged from 226 to 311 g CP kg DOM^{-1}, higher than those found by other studies developed in the same area (BARBERO et al., 2015; KOSCHECK et al., 2020), which ranged from184 to 193 g CP kg DOM^{-1}, and 184 and 208 g CP kg DOM^{-1}, respectively. According to Detmann et al. (2014) the maximum efficiency of N utilization in animal performance occurs when the ratio between CP intake and DOM is 200 g CP kg DOM^{-1}. Our results point to excess of N in the diet of animals in all treatments, including in the control without N fertilization. This imbalance between protein and energy can use the animal's energy to synthesize and excrete urea in order to eliminate excess of N from the body (VALADARES FILHO et al., 2009), which negatively affect the animal performance. Even without N fertilization, the CP/DOM ratio of forage consumed is above that recommended by Poppi and McLennan (1995) and Detmann et al. (2014). The main way to correct this relationship is through energy supplementation rich in soluble carbohydrates.

During the evaluation of DM and nutrients (OM, CP and NDFap) digestibility (Table 5) the effect of the N dose is not clear, as the effect found was cubic ($p<0.001$), therefore, other factors not evaluated may have affected the digestibility values. Normally, the digestibility is positively affected by the greater proportion of leaves in the pasture, as the leaves are the morphological component of forage grasses with a greater proportion of cell content in relation to the cell wall (PACIULLO, 2002). In a study conducted in the same area, Leite et al. (2021) evaluating increasing N doses, also observed an increase in the more digestible fractions of CP as the N dose is increased. Thus, the CP digestibility should have been higher in the highest N doses, but this did not occur, probably due to the amount of green leaves collected by animals in the pasture. Nitrogen fertilization modifies the composition of proteins and carbohydrates of Marandu grass (LEITE et al., 2021), leading the pastures with low fertilization doses present lower levels of digestible fractions. Treatments with the lowest N doses compensated for the worst chemical composition of pastures with greater selection of green leaves. We can prove by analyzing the forage allowance (Table 3) that it decreased quadratically ($p=0.028$) with the increase in the N dose.

3.4 Nitrogen balance

Nitrogen fertilization in pasture did not affect the N intake, N in feces and urine. N excreted in feces is composed by undigested N, and remained throughout the treatments because, as seen in Table 5, the CP intake of forage that received lower N dose was lower, but CP was composed by less digestible fractions (LEITE et al., 2021), so the undigested N remained. On the other hand, urine N (Table 6) should increase linearly with the g CP/kg DOM ratio (Table 5), as discussed earlier, as excess N from the diet and absorbed by the body is excreted in the form of urea in the urine of cattle (VALADARES FILHO et al., 2009).

Table 6. Nitrogen intake, excretion nitrogen in feces and urine, balance nitrogen of palisade grass under fertilization with different doses of nitrogen. (Means of 2 years of evaluation, 4 evaluations per year, n=24).

	Treatment (kg N ha^{-1})						
Variable	0	90	180	270	SEM	*p-value*	Effect
N intake (g day^{-1})	107,5	123,6	102,0	186,2	0,239	0,089	NS
N feces (g day^{-1})	72,1	57,8	54,9	80,2	0,385	0,355	NS
N urine (g day^{-1})	16,7	15,0	19,9	16,3	2,877	0,489	NS
N balance (g day^{-1})	18,6	50,8	27,2	89,7	0,739	0,004	Linear
N balance (g kg N intake^{-1})	159,2	375,8	233,8	470,8	8,800	< 0,001	Linear

Nitrogen (N), standard error of mean (SEM), quadratic effect (Quadr).

The N balance (Table 6) refers to the N retained in the animal's body measured in g N per day or g N per kg of N ingested. In both evaluations the N balance of bulls presented a linear increase ($p=0.004$ and $p<0.001$, respectively) when grazing on pastures with increasing N application doses. However, the N balance should have remained stable, since the average daily gain (ADG) did not vary among the treatments, which suggests same nutrient retention by animals in all treatments.

3.5 Animal performance

The initial and final BW (Table 7) of the animals did not vary since no significant effect on the average daily gain (ADG) was observed. The ADG change between 0.657 and 0.756 kg day^{-1}. According to Valadares Filho (2016), for Nellore cattle in the growing phase with the initial weight, final weight and ADG observed in the present study, the protein requirement would be 621 g day^{-1}.

Table 7. Effects of palisade grass fertilization with different doses of nitrogen on the performance of yearling bulls in the growing period. (Means of 2 years of evaluation, 4 evaluations per years, n=24).

	Treatment (kg N ha^{-1})						
Variable	0	90	180	270	SEM	*p-value*	Effect
Initial BW (kg)	229	235	229	229	0,004	0,119	NS
Final BW (kg)	319	326	314	318	0,009	0,492	NS
ADG (kg day^{-1})	0,657	0,756	0,686	0,678	0,015	0,183	NS
GPH (kg ha^{-1} day^{-1})	2,85	5,22	5,40	5,65	0,023	< 0,001	Quadr
GPH (kg ha^{-1})	340	621	644	673	0,015	< 0,001	Quadr
Stocking rate (AU ha^{-1})	2,63	4,04	4,73	5,00	0,206	< 0,001	Quadr
GPN (kg BW kg N applied^{-1})	-	2,82	1,69	1,23	0,015	< 0,001	Linear

Nitrogen (N), animal unit (AU)=450 kg, body weight (BW), average daily gain (ADG), gain per hectare (GPH), gain per kg N applied (GPN), standard error of mean (SEM), quadratic effect (Quadr).

As seen in Table 5, all treatments provided such protein intake to the animals, even in the treatment without nitrogen fertilization, enabling the observed AGD. Other studies developed in the same region presented lower values of ADG, varying among 0.510; 0.580 and 0.580 kg day^{-1} (ANDRADE, 2003; FLORES et al., 2008; HERLING et al., 2011). Conversely, Delevatti et al. (2019) who evaluated the same treatments as the present study, observed an ADG of 0.925 kg day^{-1}. The forage allowance (Table 3) and other non-nutritional factors (POPPI et al., 1987) did not limit the intake of DM (Table 5) of the animals, due to the grazing management adjusted to achieve 95% light interception with the canopy height at 25 cm, which allowed the same individual performance among the evaluated treatments (RUGGIERI et al., 2020). However, the ADG was below the potential of the treatments (DELEVATTI et al., 2019), probably due to the climatic conditions because of the low rainfall (Table 1) of the experimental period, mainly from December 2017 to April 2018 (first evaluation period). The intake of TDN (Table 5) of any treatment was sufficient to meet the nutritional requirements of the average daily gain (ADG) observed (Table 7), as according to Valadares Filho (2009) it would be necessary to consume 3.44 kg of TDN per day. This fact is probably due to the evaluation of nutrient consumption having been carried out in the final period of the performance evaluation and did not represent the entire experimental period due to climatic conditions.

The gain per hectare (GPH) and GPH per day presented a quadratic increase ($p<0.001$) among treatments. The increasing N doses N application leads to an increase in the rate of forage accumulation (Table 3), which, in order to maintain the management height, requires higher stocking rates. Accordingly, the stocking rate (Table 7) increased quadratically ($p<0.001$) with increasing N dose, ranging from 2.63 to 5.00 AU ha^{-1}, values much higher than the average Brazilian national stocking rate of 1.06 AU (ABIEC, 2020). The highest stocking rate associated with the same ADG among treatments generated the highest GPH with increasing N dose. Forage supply (Table 3) is represented by dividing the available forage mass by the total weight of animals present in the area. The forage allowance showed a quadratic reduction ($p=0.028$), since even with the increase in the forage mass with the increase in the N dose, the stocking rate showed a more substantial increase.

The productive efficiency of nitrogen use is measured by the BW gain per kg of N applied, and it is a viable criterion for evaluating the dose of N applied. In our study, we observed a linear reduction with increasing N dose. Normally the higher N dose applied, the lower productive efficiency of N use will be (DELEVATTI et al., 2019). Even with grazing management aimed at harvesting the forage produced, higher doses of N tend to increase losses due to volatilization, leaching (MARTHA JÚNIOR et al., 2004) and urinary excretion (VALADARES FILHO et al., 2009). Thus, the appropriate dose for each situation must be chosen with caution, evaluating the goals to be achieved, the additional costs and losses along the production system.

4. Conclusion

The application of N in Marandu grass pastures changed forage morphological and chemical composition, increasing the more digestible components of the forage. However, the application of high doses, above 90 kg ha^{-1} causes excessive intake of CP, imbalance between protein and energy in the diet and consequent loss of consumed protein.

Nitrogen fertilization improved resulted in a higher stocking rate and, consequently, greater weight gain per area.

In intensifying the process in the growing phase of beef cattle in Marandu grass pastures, nitrogen fertilization is an important technology, as it improves the efficiency of land use.

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Capítulo 3 - Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass

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Abstract: The effects of nitrogen (N) fertilization levels on protein and carbohydrate fractions in Marandu palisadegrass pasture [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster] were investigated in a pasture over five years. The experimental design was completely randomized with four levels of N (0, 90, 180, and 270 kg N ha^{-1}, as urea) for five years, and with three replicates. The study was conducted in a continuously stocked pasture during the forage growing season (December to April) in a tropical region. The effects of N fertilization were similar across the five years. With increasing N fertilization, the concentrations of crude protein (CP) increased from 103 to 173 g kg^{-1} ($P < 0.001$), soluble fractions (Fraction A+B1) increased from 363 to 434 g kg^{-1} of total CP ($P = 0.006$); neutral detergent fiber (NDF) decreased from 609 to 556 g kg^{-1} ($P = 0.037$); indigestible NDF ($P = 0.046$), potentially degradable NDF ($P = 0.037$), and acid detergent fiber decreased ($P = 0.05$), and total digestible nutrient (TDN) increased ($P < 0.001$). Increasing N fertilization decreased the concentrations of Fraction C ($P = 0.014$) and total carbohydrates ($P < 0.0001$), and increased CP:organic matter digestibility ($P < 0.01$). Concentrations of NDF free of ash and protein (P = 0.003), indigestible NDF ($P < 0.001$), potentially degradable NDF ($P = 0.11$), CP ($P < 0.001$), Fraction A+B1 ($P < 0.001$), Fraction B2 ($P < 0.001$), Fraction B3 ($P < 0.01$), and non-structural carbohydrates differed ($P < 0.001$) across years. Therefore, N fertilization can be used to increase CP, soluble protein, and TDN.

Keywords: grass protein, grass carbohydrate, tropical grass, soluble protein, pasture fertilization

INTRODUCTION

Grassland areas are becoming increasingly important for animal production due to the increase in cattle production in Latin America, Africa, and Asia[1]. Areas in which tropical grasses predominate are known to have forage with low protein concentrations and digestibility[2]. This low nutritional value of tropical grasses could be due to poor pasture management (e.g., inappropriate pasture height), overgrazing, an absence or a low level of fertilization, and poor soil fertility[3]. Furthermore, feed and fodder scarcity is regarded as a general problem for low livestock productivity in tropical areas. However, adequate information regarding the nutritional value of grasses in terms to improve the profile of protein fractions with fertilization is lacking.

Nitrogen (N) fertilization can increase forage production and nutritional value in tropical regions[4]. N fertilizers can increase forage production and affect forage nutritional value and quality[4,5,6,7,8] . In grasslands, the concentration of forage protein, soluble carbohydrates, and cell wall components are essential because, when consumed by the animals, these are supplied to the rumen microbes and therefore affect animal maintenance and production [9,10,11]. Protein and carbohydrate concentrations of grasses and their digestibility are affected by fertilization, species, stage of maturity, management, and climatic factors [12,13,14]. These climatic factors e.g. precipitation may vary along the year and therefore can affect the effect of N fertilization on nutritive value of grasses.

Particular fractions of proteins and carbohydrates are important; therefore, a system that classifies fractions based on their solubility has been proposed. The Cornell Net Carbohydrate and Protein System (CNCPS) was developed to account for nutrient fractionation. It was initially developed in a series of four papers [15,16,17,18] and has received many updates and modifications [10,11]. The CNCPS is not the only mathematical model to adopt the fractionation of nutrients to improve diet evaluation [19]. In the CNCPS, the detergent fiber system of feed analysis is used to fractionate carbohydrates into fiber carbohydrates [neutral detergent fiber (NDF) and lignin] and non-fibrous carbohydrates (NFC; soluble sugars, starch, and pectin). Proteins are divided into fractions based on their fermentation characteristics (Fraction A = non-protein nitrogen, Fraction B1 = easily-degradable protein, Fraction B2 = intermediately-degradable protein, Fraction B3 = slowly-degradable protein, and Fraction C = non-degradable and unavailable to the animal), as described by Sniffen et al.[17]. Therefore, to improve the nutritional value of grasses, it is essential to increase CP and reduce fiber concentrations, and identify how the fractions are available for ruminal degradation and microbial growth. Tedeschi and Fox[10,11] described additional modifications to the protein fractionation regarding the use of tungstic acid versus trichloroacetic acid to more consistently separate the non-protein fraction.

To apply the CNCPS to forages, it is necessary to characterize carbohydrates and N fractions[10,11]. Previous research has characterized protein fractions of various forage species from temperate areas[12,20], including warm grasses [such as bermudagrass, stargrass, and bahiagrass][21] and guinea grass[22]. However, information related to the effects of N fertilization and annual changes in the carbohydrate and protein fractions of *Urochloa* is limited. Furthermore, *Urochloa* is the most utilized forage for cattle production in tropical areas, and the effects of N fertilization may vary among species and regions.

Thus, we studied the effect of N fertilization and year seasonality on carbohydrate and protein fractions of Marandu palisadegrass. We hypothesized that the concentrations of CP and Fraction A would increase with N fertilization level, whereas the fibrous carbohydrate fractions would decrease. Additionally, we hypothesized that the N fertilization effect on carbohydrates and protein fractions would vary with year.

RESULTS AND DISCUSSION

In our studies with Marandu palisadegrass, a grazing management strategy with continuous stocking where 95% of the light is intercepted by the canopy resulted in forage at a height of 25 cm, a high green leaf proportion, and low amounts of dead material during the growing season[23,24]. The use of N fertilization[4], different stocking rates, and supplementation [25,26,27] are crucial for obtaining forage with a high nutritional value, resulting in a high weight gain per animal and area, and a reduction in slaughter age and greenhouse gas emissions. In the present study we did not find any interaction N doses with years. Therefore, only the significative effects of N fertilization or variation within year are presented and discussed.

Total and non-fibrous carbohydrates and total digestible nutrients

Total carbohydrate concentrations decreased linearly with increasing N levels ($P < 0.01$; Table 1). Nitrogen fertilization increases cell concentrations (soluble fractions) and changed the sugar composition and bonds established between them in the cell wall[28]. Therefore, the reduction in TC represents the reductions observed in the NDF and ADF fractions (Table 1). The fibrous compounds in forage decrease with increasing N levels because this nutrient stimulates the growth of new tissues[29]. In the present study, we managed the pasture to reduce stem growth. A sward height of 25 cm stimulates tillering and growth of new tissues[24].

Table 1. Average chemical composition for Marandu palisadegrass (g kg^{-1} dry matter) affected by nitrogen dose.

	---------Nitrogen dose (kg N ha^{-1})---------				Effect[1]
Variable	0	90	180	270	Effect[1]
TC	783.7	760.8	742.1	721.3	Linear
	(15.6)	(16.0)	(15.4)	(17.8)	
NFC	94.4	90.8	90.1	85.9	ns
	(4.5)	(5.2)	(6.6)	(8.1)	
apNDF	609.3	590.0	572.0	556.4	Linear
	(7.0)	(6.9)	(5.7)	(5.6)	
iNDF	187.8	177.1	174.1	156.5	Linear
	(2.3)	(2.4)	(1.8)	(1.5)	
NDFpd	421.5	413.0	397.9	399.9	Quadratic
	(8.2)	(6.7)	(7.2)	(8.1)	
ADF	327.0	307.0	301.6	294.7	Linear
	(6.5)	(6.9)	(7.8)	(6.5)	
Lignin	87.3	85.0	83.3	86.0	ns
	(2.1)	(2.4)	(2.5)	(1.9)	
TDN	629.9	636.0	638.5	642.2	Linear
	(11.4)	(10.3)	(15.6)	(13.4)	
CP:DOM	125	152	174	195	Linear
	(7)	(8)	(8)	(10)	
CP	103.2	128.6	150.0	172.8	Linear
	(10.1)	(11.3)	(14.4)	(14.3)	
Fraction A+B1	363.3	369.0	406.1	433.2	Linear

	(8.2)	(7.2)	(8.1)	(7.2)	
Fraction C	125.0	103.7	101.9	98.3	Linear
	(3.5)	(2.8)	(3.7)	(2.9)	

[1] Orthogonal polynomial effect of N doses. Effect probability (apNDF, $P < 0.0001$; iNDF, $P = 0.046$; NDFpd, $P = 0.037$; ADF, $P = 0.05$; Lignin, $P = 0.19$; TDN, $P < 0.0001$; TC, $P < 0.0001$; CP:DOM, $P < 0.01$; CP, $P < 0.001$; Fraction A+B1, $P = 0.006$; and Fraction C, $P = 0.014$). Within parentheses is the standard error of the means (SEM; ±).

Table 2. Average chemical composition for Marandu palisadegrass (g kg^{-1} dry matter) affected by year.

	------------------Experimental year----------------					
Variable	2015	2016	2017	2018	2019	Effect[1]
apNDF	589.6	541.3	605.4	620.4	552.9	cubic
	(5.4)	(5.7)	(6.7)	(4.9)	(4.7)	
iNDF	116.0	167.4	215.4	204.2	166.2	quadratic
	(1.0)	(6.0)	(7.6)	(4.6)	(9.6)	
NDFpd	473.6	373.9	390.0	416.3	386.6	ns
	(3.9)	(7.1)	(5.6)	(5.2)	(3.4)	
ADF	298.2	254.4	302.7	361.5	312.3	cubic
	(2.8)	(2.3)	(3.1)	(3.1)	(2.9)	
OM	911.5	922.0	919.0	911.9	908.6	quadratic
	(1.9)	(2.3)	(2.2)	(2.0)	(1.9)	
NFC	136.3	246.6	162.5	134.2	170.6	cubic
	(10.7)	(13.2)	(14.2)	(9.7)	(15.3)	

CP	123.1	130.1	127.5	142.2	170.2	linear
	(12.1)	(11.6)	(9.8)	(13.5)	(16.5)	
Fraction A+B1	325.4	302.1	367.9	382.9	586.0	linear
	(3.3)	(3.6)	(4.1)	(3.8)	(3.2)	
Fraction B2	282.5	245.3	284.0	177.8	166.6	quadratic
	(2.2)	(2.5)	(2.8)	(2.0)	(2.2)	
Fraction B3	314.0	340.4	229.6	264.5	196.1	quadratic
	(3.4)	(3.6)	(3.7)	(2.8)	(2.9)	

[1] Orthogonal polynomial effect of N doses. Effect probability (apNDF, P = 0.003; iNDF, $P < 0.001$; NDFpd, $P = 0.11$; ADF, $P < 0.0001$; NFC, $P < 0.001$; CP, $P < 0.001$; Fraction A+B1, $P < 0.001$; Fraction B2, $P < 0.001$; and Fraction B3, $P < 0.01$). Within parentheses is the standard error of the means (SEM; ±).

NFC varied according to the experimental year ($P < 0.01$; Table 2). The NFC fraction can be rapidly degraded in the rumen, and is necessary to maintain adequate carbohydrate and protein synchrony, and promote adequate microbial growth[17]. Variations in climatic conditions, such as precipitation, alter the production of leaves and stems, and, consequently, cause changes in the concentrations of soluble sugars, starches, and pectins[22]. Our results are in agreement with those of Santos et al.[22], who observed high NFC when the highest precipitation occurred. Another climatic variable related to NFC is the sunlight, which affects the amount of glucose formed during photosynthesis[30]. Only the year affected NFC (Table 2). The highest NFC occurred in 2016, when the highest precipitation and lowest sunlight hours were recorded (Fig. 1 and Table 2). Greater sunlight increases the photosynthetic rate, which stimulates stem elongation by promoting cell growth. Cloudy and warm days stimulate the growth of new tissues[29].

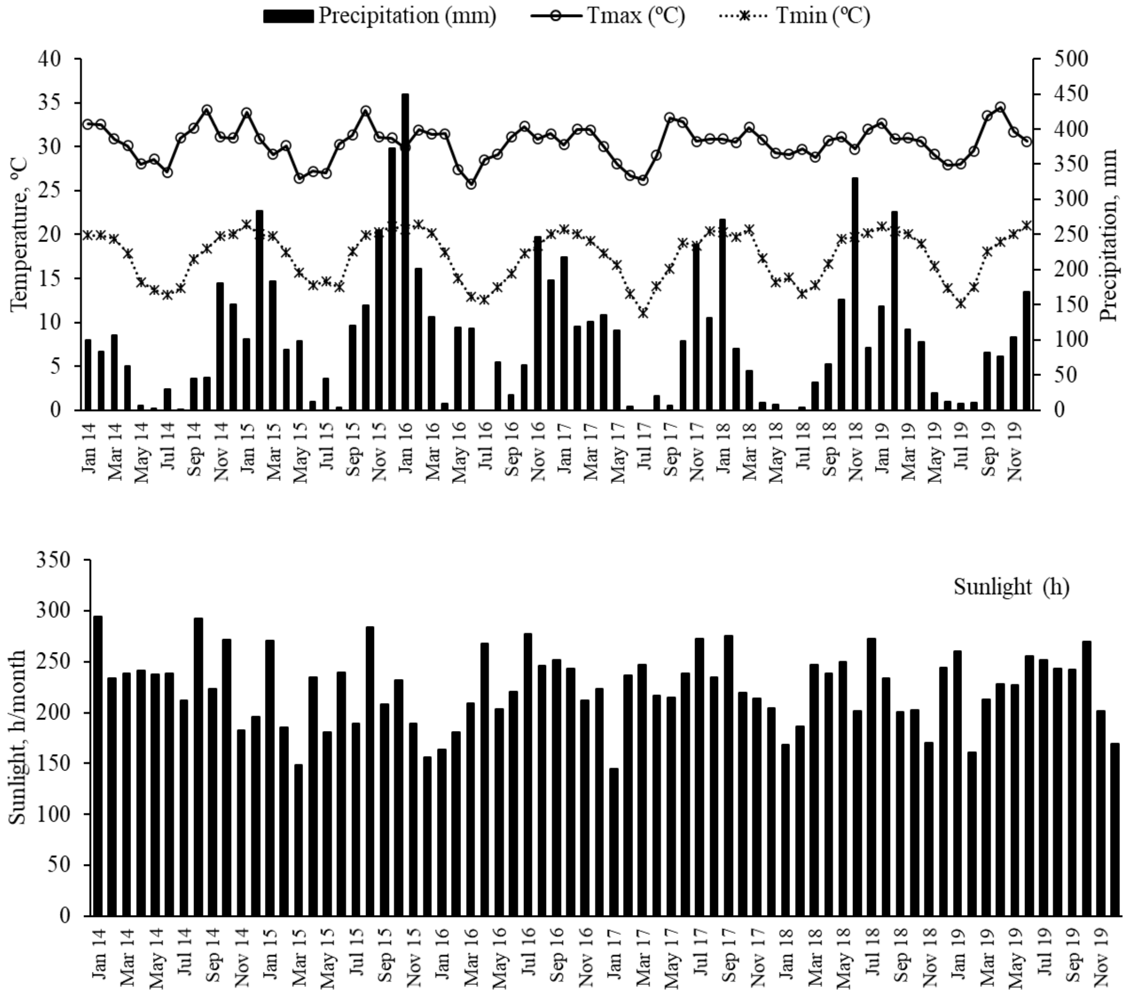

Figure 1. Total precipitation, mean temperature, and mean sunlight from 2015 to 2019 at the experimental site at the São Paulo State University Jaboticabal, São Paulo, Brazil.

Increasing N fertilization increased the TDN concentration ($P < 0.01$; Table 1). The TDN fraction is similar to OM in terms of digestibility and is usually found at a concentration of 60% in tropical grasses[28]. However, it increased on average from 62% to 65% in our study as N fertilization increased from 0 to 270 kg N ha^{-1}. Increases in variables related to forage digestibility in response to N fertilization were also observed by several authors [7,21, 22]. We found that TDN was strongly associated with NFC, NDFpd, and soluble protein (Tables 1 and 2).

Neutral detergent fiber, indigestible detergent fiber, and potentially degradable detergent fiber

The concentration of NDF decreased linearly with increasing N levels ($P <$ 0.001; Table 1). Conversely, there was a cubic relationship between NDF and year ($P <$ 0.05; Table 2). Our results corroborate other studies that found a reduction in NDF with increasing N fertilization [4,7,21]. Nitrogen fertilization can increase cellular concentrations and decrease the cell wall concentration[28]. However, this effect is climate-dependent, as observed in our study. Lower NDF concentrations occurred in 2016, when higher precipitation was observed (Fig. 1 and Table 2). Water availability in the soil is essential for N uptake by the plant. In the years that occurred greater precipitation the components of cell wall decreased probably due increases in N recovery by the Marandu palisade grass.

The iNDF concentration decreased linearly with increasing N fertilization ($P <$ 0.01; Table 1). Few publications have reported iNDF concentrations. We presented this data because fiber is the most common variable used to predict the feed energy concentration, and there is a negative relationship between fiber concentration and available energy[31]. The reduction in iNDF concentration due to increasing N fertilization suggests that high N fertilization is a potential strategy to increase available energy in tropical grasses under grazing. However, this reduction was observed when the pasture was managed under a light interception level of 95%, which limited stem elongation. Different results may occur in other forage management strategies[26].

The N fertilization had a quadratic effect on NDFpd (*P* < 0.05; Table 2). The reduction of NDFpd with the application of N occurred up to a dose of 180 kg N ha^{-1}. NDF provides energy for ruminal microbial syntheses, but also improves rumen function by adding structural carbohydrates to the ruminant diet[32]. The stabilization of the degradable fraction of NDF with increasing N fertilization suggests that the structural value, that is, the passage rate of ruminant diet, was maintained.

Acid detergent fiber

Increasing N fertilization had a negative linear effect on ADF concentration (*P* < 0.01; Table 1). Previous research found that increasing N fertilization had little or no effect on ADF [7,29]. There was a quadratic relationship between ADF concentration and experimental year (*P* < 0.05; Table 2). The plant cell wall concentration can vary due to variations in precipitation and temperature. Rainfall and temperature are the major factors that affect plant maturity. The combination of relatively cooler temperatures and the absence of rain well into the growing season can result in a forage reproductive state, which could increase the fiber fractions[2] and might explain these changes in ADF concentrations. The highest average ADF concentration (36.15%) was observed during the growing season of 2018 when lower precipitation occurred (Fig. 1).

Total protein

A linear increase in CP concentration was observed with increasing N levels, and a linear relationship between CP and experimental year was observed (*P* < 0.001; Tables 1 and 2). Increases in CP concentrations of forage due to N fertilization are known. For example, Prine and Burton (1956) observed increases in CP as annual N fertilization levels increased in warm grasses. Similar results have been observed in other studies[4,7,8,29]. Additionally, we observed a cumulative effect of N fertilization on CP concentration over the duration of the experiment. Although forage CP concentrations can vary with precipitation and temperature[28], the responses of CP to N fertilization observed could not be attributed to the climatic variables. The highest and lowest precipitation did not coincided with the experimental years that the highest and lowest CP concentrations occurred (Figs. 1 and Table 2).

The average CP concentration for Marandu palisadegrass is usually less than 10% [7,13,33]. The minimum value observed in our study was 12% (Tables 1 and 2). Our results are similar to those found previously in our experimental site (11%–14%; Barbero et al[25]; Koscheck et al.[27]) and that observed by McRoberts et al.[8], which was 13%–15%. Nitrogen concentration, together with the cell wall concentration, is the most important factor in the supply of the required quantity of nutrients[26]. Our results suggest that five years of grazing at a fixed pasture height that corresponds to the 95% light interception during the growing season can result in high CP concentrations for Marandu palisadegrass, even without N fertilization. Therefore, a high animal performance could be obtained.

Soluble protein: Fraction A and B1

The fraction of soluble protein increased linearly with increasing N levels ($P <$ 0.0001; Table 1) and with experimental year ($P < 0.01$; Table 2). Previous research [8,21,22] has indicated that increased N fertilization levels increase nitrate accumulation in the plant, which is a portion of Fraction A.

The soluble protein fraction varied from 325 to 586 g kg^{-1} throughout the experiment (Table 2). Several researchers have shown that the proportion of leaves and stems, leaf expansion rate, and tillering adapt to new grazing management targets [24,34]. In our study, the grazing management strategy allowed the growth of a greater proportion of green leaves, which probably explains the increase in soluble protein. High concentrations of Fraction A are desirable, as this fraction is rapidly degraded in the rumen and can result in greater animal performance[35]. Animal performance depends on microbial protein production that can be optimized with a greater amount of soluble N since the amount of energy does not limit microbial growth.

Fractions B2 and B3

We did not find any effect of increasing N fertilization on the protein fractions with moderate and low degradation rates. Conversely, these fractions varied quadratically with experimental year ($P < 0.01$; Table 2). Fraction B2 was approximately 40% lower during the fourth and fifth experimental years. The highest average value of Fraction B3 was observed in the year with the lowest precipitation (2016; Fig. 1). Fractions B2 and B3 are associated with membranes and extensins that are bound to hemicellulose and are dependent on the temperature. In our study, the temperature remained similar throughout the year, varying from 19 to 31 ºC (Fig. 1).

In contrast to our results, Fraction B2 has previously been found to vary with N fertilization. Rogers et al.[20] found that Fraction B2 increased by approximately 35% in bermudagrass, and Johnson et al.[21] observed an increase of up to 57% for warm-season grasses in Florida with N fertilization. Theses variations between theses studies and our findings are likely due to the pastures management differences. Berça et al.[14] showed that pasture management play essential role in the variation of fiber concentration in Marandu palisade grass. However, our results are in line with those of Santos et al.[22], who only observed an effect of seasonality, with the highest values Fraction B2 being associated with low rainfall and high NDF.

Protein Fraction C: Non-degradable nitrogen

Fraction C decreased linearly with N fertilization (*P* < 0.01; Table 1). This result differs from previous studies. Johnson et al.[22] observed that Fraction C depends of the N fertilization dose for bahiagrass and stargrass being lower or higher to the lowest dose. Rogers et al.[20] and Santos et al.[22] did not find any effect of N fertilization on Fraction C. However, similar to our results, Zhang et al.[36] found a decrease in Fraction C to 55.3% in annual ryegrass forage with increasing N fertilization.

Fraction C corresponds to N linked with lignin, tannin-protein complexes, and Maillard products, which are highly resistant to enzymes produced by the microbes in the rumen, being considered unavailable to the animal[10,11,17]. Increasing N fertilization improved protein availability in Marandu palisadegrass. Growing Marandu palisadegrass under a grazing management strategy with a pasture height corresponding to a light interception of 95% resulted in a low concentration of structural carbohydrates associated with lignin and a low concentration of Fraction C[25,27].

The crude protein:organic matter digestibility ratio increased linearly in response to N fertilization (Table 1). According to Poppi and McLennan[37], a CP:DOM of 160 g resulted in high efficiency of microbial growth; however, CP:DOM values above 210 g caused high N losses. Maximum N utilization efficiency values in post-weaning beef cattle reared in tropical grass pasture were observed below 200 g of CP/kg DOM, and losses occurred above 200 g of CP/kg DOM[38]. In the present study values above 160 g of CP/ kg DOM were observed at doses of 180 and 270 Kg of N/ha.

Conclusions

Estimations of carbohydrate and protein fractions can increase the nutrient utilization efficiency and determine the type of supplementation needed under each pasture management strategy. We observed an increase in CP and soluble protein with increasing N fertilization, leading to less protein being required in supplements. Therefore, it is necessary to include soluble carbohydrates, starch, and pectin in the diet to maintain protein and carbohydrate synchrony in the rumen and optimize microbial growth. We observed higher CP, soluble protein, and TDN concentrations when 90 kg N ha^{-1} was applied, suggesting that this dose is the most suitable for Marandu palisadegrass under a continuous stocking and a pasture management strategy with canopy target of 25 cm during the growing season. Future studies should be directed toward understanding undegradable protein supplementation, inclusion of proteins from legumes, and high degradable carbohydrate fractions in tropical diets.

METHODS

Experimental area and design

We conducted a five-year experiment in the Forage and Grasslands Laboratory of São Paulo State University, "Julio de Mesquita Filho" (UNESP) (Jaboticabal, São Paulo, Brazil), during the summer growing seasons (December to April) of 2014/2015, 2015/2016, 2016/2017, 2017/2018, and 2018/2019. The climate of the experimental area is classified as a subtropical humid climate, with wet summers and dry winters[4]. The mean annual rainfall is 1424 mm, the mean air temperature is 22.3 °C, and the soil is a Rhodic Ferralsol derived from basalt[39]. The pasture was established in 2001 with *Urochloa brizantha* (Hochst ex A. Rich) Stapf Marandu (Marandu palisadegrass). The precipitation and temperature in the experimental area are presented in Fig. 1. During the growing season, the average minimum and maximum temperatures were 19 ºC and 31 ºC, respectively, and the average monthly sunlight varied from 150 to 300 h. Mean soil chemical characteristics evaluated always on the September from 2015 to 2019 are presented in Table 3. In November of each year, maintenance fertilizer was applied to all paddocks at 50 kg P_2O_5 (superfosfate) and 70 kg K_2O (potassium chloride) per hectare. Soil texture content are 291, 123, 588 g kg^{-1} soil of clay, silte and sand, respectively.

Table 3. Means soil chemical characteristics of the experimental area at the depth of 0 – 20 cm, Jaboticabal– SP.

Year	P resin	S-SO_4^{2-}	OM	pH $CaCl_2$	K^+	Ca^{2+}	Mg^{2+}	H^+Al	Al^{3+}	CEC	V%
	-- mg/dm^3--		g/dm^3				----$mmolc/dm^3$----				
2015	12	16	25	5,2	2,6	36	11	22	0	92	62
2016	15	11	27	5,1	2,8	37	17	28	0	98	61
2017	13	12	28	5,1	3,0	35	14	24	0	95	59
2018	12	16	26	5,2	3,0	34	13	22	0	94	59
2019	11	15	28	5,2	2,9	32	13	23	0	92	57

P= phospuros, S-SO_4^{2-} = sulfate, OM = organic matter, K = potassium, Ca = Calcium, Mg = magnesium, Al = alluminum, CEC = capacity os exchange cations and V% = bases saturation.

The experiment consisted of four nitrogen doses (0, 90, 180, and 270 kg N ha^{-1}) in a completely randomized design with three replicates per treatment, totaling 12 paddocks (experimental units). The paddock areas were 1.3 ha, 1 ha, 0.7 ha, and 0.5 ha for the treatments 0, 90, 180, and 270 kg N ha^{-1}, respectively. The experimental area included a reserve area of 3 ha for the spare animals. The experimental area meets the criterion of soil homogeneity to conduct a CRD experiment. The source of N was urea, and its application was split across three times during the rainy season (Begin of December, end of January and begin of March). Animals used in research were cared for according to the rules of the São Paulo State University Animal Care and Use

Committee and the National Council of Animal Experimentation Control. The committee reviewed and approved the experiment and all procedures carried out in the study (Certificate number 12703/15). We declare that no permissions or specific requirement to collect, analyze and work with *Urochloa brizantha* are required by local and national Brazilian authorities.

Grazing management

The present study was conducted under grazing conditions. Each year, 72 young Nellore bulls (*Bos indicus*) were used to measure animal productivity (average daily gain and gain per ha). The bulls had an initial body weight (mean ± standard deviation) of 352 ± 5 kg, 334 ± 2 kg, 315 ± 6 kg, 220 ± 2 kg, and 206 ± 9 kg in the first, second, third, fourth, and fifth experimental years, respectively.

The experimental units were grazed with a continuous and variable stocking rate[40]. To maintain the pasture height, the stocking rate was adjusted weekly. The grazing target was a pasture height of 25 cm. At this height, the canopy intercepted 95% of the incident light under our experimental conditions. At this light interception, the maximal net forage accumulation is achieved, resulting in high average daily gain and gain per hectare[24,25,27].

Forage collection and preparation

Forage samples were harvested at 28-d intervals beginning in the middle of December from 20 points per hectare using the hand-plucking method[41]. In the hand-plucked method the grazing behavior of the animal is firstily observed; then herbage samples are taken manuanly mimicking the animal foraging. Approximately 200 g of fresh matter was harvested per sample to determine forage chemical compositions. Samples were dried in a forced-air oven (55 ± 5 °C, for 72 h), ground in a mill through a 2-mm screen (Thomas-Wiley Laboratory Mill Model 4, H. Thomas Co.), and taken to the laboratory for analysis.

Chemical analysis

Laboratory analyses included measurements of dry matter (DM), organic matter (OM), and ash determined using the following procedures from AOAC[42]: AOAC 934.01 for DM, AOAC 942.05 for OM, and AOAC 942.05 for ash. Crude protein (CP) concentration (AOAC 990.03) was estimated using a LECO FP 528 device (Leco Corporation, Michigan, USA). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the procedures described by ANKOM Technology[43].

The indigestible neutral detergent fiber (iNDF) was quantified by *in situ* incubation of hand-plucked samples conditioned in ANKOM brand F-57 filter bags arranged in the rumen of fistulated animals for 288 h[44]. After being thoroughly washed, oven-dried at 55 °C for 72 h, and then dried in an unventilated oven at 105 °C for 45 min, the bags were weighed to obtain the indigestible DM. Subsequently, the bags were subjected to NDF quantification using the ANKOM fiber analyzer, as cited above.

Fractions of forage carbohydrates were obtained following Sniffen et al.[17] for total carbohydrates (TC) and non-fibrous carbohydrates (NFC). Total digestible nutrient (TDN) was calculated using the equation of energy using multiple components[31]. The potentially degradable NDF (NDFpd) was calculated by subtracting iNDF from the NDF.

The fractionation of protein was determined as described by Sniffen et al.[17]. Fraction A was obtained by extracting the soluble N using trichloroacetic acid[45] and calculating the difference between the total N concentration and the non-protein nitrogen[17]. For quantification of buffer soluble protein (Fractions A and B1), 0.50 g of sample was extracted with 50 mL of borate-phosphate buffer and 1 mL of sodium azide solution. Fraction B2 was calculated by subtracting Fraction A, Fraction B1, and the neutral detergent insoluble protein (N-NDF) from the total N. Fraction B3 was the difference between the N-NDF and the acid detergent insoluble protein (N-ADF). Fraction C was the N-ADF[17].

Statistical analysis

Data were analyzed using the LME function of R for mixed models (package NLME, R version 3.4.5). The statistical model included N level, year, and their interaction as fixed effects, and paddock was a random effect. All variables were analyzed as repeated measures. The best covariance structure used for repeated measures was chosen as the one that achieved the lowest corrected Akaike or Bayesian information criteria. The statistical model was:

$\gamma ijk = \mu + \alpha i + \pi j(i) + \beta k + \alpha\beta ik + \varepsilon ijk$, where: γijk is the observation, μ is the overall mean, α is the fixed effect of N doses, $\pi j(i)$ is the random error associated with the N doses and repetitions, βk is the fixed effect of year (time), $\alpha\beta ik$ is the N doses by year interaction and εijk equals the residuals error.

When a significant effect was found, orthogonal polynomial contrasts were performed to assess the effects of N level and year on the variables.

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