FERNANDA DE KÁSSIA GOMES

CARBOHYDRATES AND PROTEIN METABOLISM IN BEEF CATTLE GRAZING FERTILIZED OR INTERCROPPED TROPICAL PASTURE

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Tese apresentada à Universidade Federal de Lavras – UFLA, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para obtenção do título de Doutora em Zootecnia.

Prof. Dr. Daniel Rume Casagrande

Orientador

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APROVADA em 28 de fevereiro de 2019.

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ABSTRACT

This study was developed to determine the metabolism of nitrogen and carbohydrates of animals feeding on two types of pastures: brachiaria grass (monoculture) or intercropped with forage peanut, which contains condensed tannin. The experiment was carried out at the University of Lavras, in Lavras, MG. Two different areas were used in the experiment: 0.8 ha of *Brachiaria brizantha* cv. Marandu (brachiaria grass) intercropped with *Arachis pintoi* cv. Mandobi (forage peanut) and 0.6 ha of *Brachiaria brizantha* cv. Marandu fertilized with mineral nitrogen, applied at 150 kg $N.ha^{-1}$ per year. Six pure breed zebu heifers cannulated on rumen with an initial body weight of 404 kg performed grazing. Those animals had a fistula located in the rumen. Experimental treatments had two types of pastures: monoculture brachiaria grass, fertilized with mineral nitrogen (FP), and intercropped with forage peanut (IP). The design used was double cross-over, with four experimental periods. Means with $P \leq 0.10$ was statistically different. DM and OM intakes were 11% greater at treatment FP in relation to IP. N intake was 15.0% greater at treatment IP in relation to FP. Besides no effects on NFC intake, IP tended to be 11.2 % greater than treatment FP. Condensed tannin intake was about 63.4 $g.d^{-1}$. The $N\text{-}NH_3$ on ruminal fluid was 28% greater at treatment FP in relation to IP. The VFA (M*m*) was greater in IP than FP. The A: P ratio was greater at treatment IP in relation to FP. The microbial CP was 22.9% greater at treatment IP in relation to FP. Similarly, the efficiency of microbial protein supply and CP intake: digestible OM ratio were 47.8% and 32.5% greater at treatment IP in relation to FP. N ruminal passage rate was 12.3% greater at treatment IP in relation to FP. The DM and NDF ruminal digestion rates were 21.3% and 39.2% greater at treatment FP in relation to IP, respectively. The DM, OM, N and NDF apparent intestinal digestibility was 33.8%, 41.9%, 23.3% and 36.5% greater at treatment IP compare to FP. Effects on N and non-ammonia nitrogen (NAN) flows only were observed between treatments, which were greater 14.1% and 15.7% on treatment IP in relation to FP. Fecal nitrogen output was 15.9% greater at treatment IP in relation to FP. However, urine nitrogen output did not differ between treatments. The nitrogen balance was greater at treatment IP in relation to FP. The protozoa population on FP treatment was greater in relation to IP, which averaged 7.05 (x104/ml) and 3.40 (x104/ml), respectively. The forage peanuts used in our study, which contain condensed tannin, altered the ruminal environment. Ruminal fiber degradation was lower and there was a decrease in apparent dry matter digestibility. The protein had lower ruminal degradation, as a result of binding with condensed tannin, generating an increase in the N flow to the post-rumen. There was higher production of microbial protein with the use of legumes. As a result of these facts, N balance was higher in the animals that fed on forage peanut, indicating that this plant provided more retained nitrogen. Lastly, CT was able to manipulate the ruminal environment in a way that decreased the protozoa population.

Key words: Arachis pintoi. Nitrogen balance. Intestinal and ruminal digestibility. Digestion rate. Forage intake.

RESUMO GERAL

Amendoim forrageiro possui tanino condensado (TC), composto que se liga à proteína formando complexos estáveis que não são degradados no rúmen, entratanto, degradados no pós-rúmen, conferindo característica de *bypass*. Objetivou-se determinar o metabolismo de nitrogênio e carboidratos de animais alimentados com pastagem de monocultivo de capim braquiária ou consorciados com leguminosa, a qual continha tanino condensado. O experimento foi realizado na Universidade de Lavras, em Lavras, MG. Foram utilizadas duas áreas para realizar o experimento: 0,8 ha de *Brachiaria brizantha* cv. Marandu (capim-braquiária) consorciada com *Arachis pintoi* cv. Mandobi (amendoim forrageiro) e 0,6 ha de *Brachiaria brizantha* cv. Marandu a qual foi fertilizada com nitrogênio mineral aplicado a uma dose de 150 kg N.ha-1 por ano. O pastejo foi realizados por seis novilhas zebuínas de raça pura com peso corporal inicial de 404 kg. Os tratamentos experimentais foram pastagem de capim braquiária em monocultura, adubada com nitrogênio mineral (PA) e consorciada com amendoim forrageiro (PC). O delineamento utilizado foi Crossover duplo com quatro períodos experimentais. Médias com $P \leq 0,10$ foram estatisticamente diferentes. O consumo de MS e MO foram 11% maiores no tratamento PA comparado ao PC. O consumo de N foi 15,0% maior no tratamento PC em relação ao PA. O consumo de NFC no PC tendeu a ser 11,2% maior que o tratamento PA. A ingestão de tanino condensado foi de cerca de 63,4 g.d-1. O $N\text{-}NH_3$ no líquido ruminal foi 28% maior no tratamento PA em relação ao PC. O VFA (Mm) foi maior em PC que em PA. A relação A:P foi maior no tratamento PC em relação ao PA. A proteina microbiana foi 22,9% maior no tratamento PC em relação ao PA. Da mesma forma, a eficiência de produção de proteína microbiana foi 47,8% maior no tratamento PC em relação ao PA. A taxa de passagem ruminal foi 12,3% maior no tratamento PC em relação ao PA. As taxas de digestão ruminal da MS e FDN foram 21,3% e 39,2% maiores no tratamento PA em relação à PC, respectivamente. A digestibilidade intestinal aparente da MS, MO, N e FDN foram 33,8%, 41,9%, 23,3% e 36,5% maiores no tratamento PC comparado ao PA, respectivamente. Efeitos sobre os fluxos de nitrogênio e N foram 14,1% superiores no tratamento PC em relação ao PA. A produção de nitrogênio fecal foi 15,9% maior no tratamento PC em relação ao PA. No entanto, a produção de nitrogênio urinário não diferiu entre os tratamentos. O balanço de nitrogênio foi maior no tratamento PC em relação ao PA. população de protozoários no tratamento de PA foi maior em relação à PC, com média de 7,05 (x104 / ml) e 3,40 (x104 / ml), respectivamente. Os amendoins forrageiros utilizados em nosso estudo, que contêm tanino condensado, alteraram o ambiente ruminal. A degradação da fibra ruminal foi menor e houve redução na digestibilidade aparente da matéria seca. A proteína apresentou menor degradação ruminal, como resultado da ligação com o tanino condensado, gerando um aumento no fluxo de N para o pós-rúmen. Houve maior produção de proteína microbiana com o uso de leguminosas. Como resultado desses fatos, o balanço de N foi maior nos animais que se alimentaram de amendoim forrageiro, indicando que esta planta proporcionou maior nitrogênio retido. Por fim, a TC foi capaz de manipular o ambiente ruminal de forma a diminuir a população de protozoários.

Palavras-chave: Arachis pintoi. Balanço de Nitrogênio. Digestibilidade Ruminal e Intestinal . Taxa de Digestão. Consumo de Forragem.

RESUMO INTERPRETATIVO E RESUMO GRÁFICO

O uso de pastos consorciados de leguminosas com gramíneas possui dois grandes benefícios: diminuição do impacto ambiental e aumento no valor nutritivo. Do ponto de vista ambiental, o uso das leguminosas surge como alternativa de introdução de nitrogênio (N) no sistema de forma mais sustentável, pela capacidade de fixação biológica de N. Além dos aspectos ambientais, as leguminosas forrageiras possuem melhor valor nutritivo em relação às gramíneas. Além disso, a presença de tanino condensado (TC), composto que se liga à proteína formando complexos estáveis que não são degradados no rúmen, entretanto, reversíveis no pós-rúmen, aumenta a proteína não degradável no rúmen (PNDR). Isso é vantajoso pois, há situações em que a quantidade de amônia liberada no rúmen a partir da degradação das proteínas pelos microrganismos ruminais é maior que a capacidade de utilização e incorporação como proteína microbiana. Essa amônia excedente é removida do rúmen, excretada na urina ou reciclada para o rúmen. Se parte desse nitrogênio excedente liberado no rúmen for transformado em PNDR e absorvido no intestino, influenciaria positivamente o balanço de nitrogênio pelos ruminantes, aumentando o N retido como produto animal e diminuindo as perdas desse nutriente para o ambiente. Neste trabalho, foram estudados dois cenários de produção animal: pastagem de capim-marandu consorciada com amendoim forrageiro e pastagem de capim marandu fertilizada com nitrogênio mineral aplicado a uma dose de 150 kg $N.ha^{-1}$ por ano. O pastejo foi realizados por novilhas de corte. A degradação ruminal da fibra diminuiu e houve redução na digestibilidade aparente da matéria seca. A proteína foi menos degradada no rúmen, o que foi atribuído à sua ligação com o TC e, assim, houve aumento do fluxo de N para o pós-rúmen. Além disso, houve maior produção de proteína microbiana com o uso de leguminosas. Devido a esses eventos, o balanço de N foi maior nos animais que consumiram amendoim forrageiro, ou seja, esta planta proporcionou maior N retido nos animais.

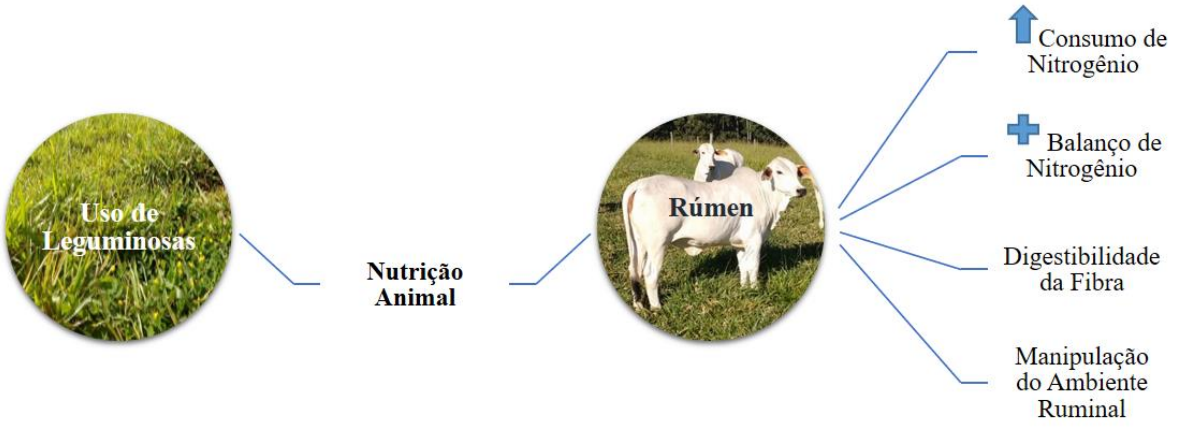


TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. REFERENCIAL TEÓRICO

A importância da pecuária para a economia brasileira é um fato reconhecido, visto que o Brasil ocupa posição de destaque no mundo e possui o maior rebanho comercial bovino, com 220 milhões de cabeças (IBGE, 2017). É uma atividade que tem como base de sua alimentação a pastagem e, diante disso, o cultivo de plantas forrageiras assume papel importante para a cadeia produtiva de carne e leite. A expansão da atividade, nos últimos anos, reflete não somente a competitividade do setor no contexto mundial, mas também a demanda crescente por produção de alimento de qualidade e em grande quantidade para uma população que ainda não parou de crescer.

Para manter a produtividade de um sistema de produção animal à pasto é necessário que a entrada de nutrientes na pastagem seja mantida e que seja feito manejo correto. O nitrogênio (N) é um dos principais nutrientes que contribui na produtividade das gramíneas forrageiras, visto que é um fator fundamental na formação das proteínas, cloroplastos e outros compostos que participam ativamente da fotossíntese. Esse nutriente é responsável por características ligadas ao porte da planta, tais como o tamanho das folhas, taxa de aparecimento foliar, tamanho dos colmos, formação, desenvolvimento e longevidade dos perfilhos (WERNER, 1986), aumentando a capacidade de suporte das pastagens.

Nos últimos anos, aumentou muito a preocupação com a preservação ambiental e com as possíveis consequências da atividade humana sobre a sustentabilidade da produção de alimento no mundo (Boyazoglu, 1998; Gibon et al., 1999; Hochmana et al., 2013; Nardone et al., 2010). A discussão segue persistente e, diante disso, é difícil contestar a ideia de que há que se produzir alimento, em abundância, de qualidade, seguro, a um custo baixo e com menor impacto ambiental possível.

A adubação nitrogenada causa impactos negativos ao ambiente quando feita em elevadas doses devido às perdas por lixiviação na forma de nitrato e contaminação do lençol freático (Sekhon, 1995). Assim, o uso das leguminosas surge como uma alternativa de introdução de N no sistema de forma mais sustentável. A maioria dessas plantas tem a capacidade de estabelecer simbiose com bactérias diazotróficas, conhecidas como rizóbios, que induzem a formação de nódulos radiculares onde ocorre a fixação biológica de nitrogênio atmosférico (FBN), conferindo às leguminosas uma posição ecológica

relevante (Peix et al., 2015). Os microrganismos fixadores de N fornecem compostos nitrogenados às plantas, os quais são "trocados" por carboidratos fornecidos por estas.

A leguminosa no sistema em consórcio contribui, portanto, com o aporte de nitrogênio ao sistema, por meio da reciclagem e transferência desse nutriente para a gramínea, melhorando a produção de forragem e a dieta do animal. Além disso, traz benefícios ao ambiente, a partir do momento em que reduz o uso de adubos e, consequentemente, diminuindo a poluição ambiental.

Amendoim forrageiro (*Arachis pintoi*)

Em condições tropicais, a leguminosa que tem merecido destaque referente às pastagens consorciadas é o *Arachis pintoi* (amendoim forrageiro). Essa espécie é nativa do Brasil, cuja principal área de dispersão é a região Central do País (Purcino et al., 2004). Esse gênero pertence à família, subfamília Papilionoideae, tribo Stylosanthinae e subtribo Aeschynomeneae (Gregory et al., 1980). A característica típica dessas plantas é o desenvolvimento de frutos subterrâneos (geocarpismo), a partir de flores localizadas na parte aérea. É uma forrageira de porte baixo, dificilmente ultrapassando 30-40 cm de altura, possui raiz pivotante, podendo alcançar 1,60 m de profundidade (Gregory et al., 1980). Por ser uma planta estolonífera, possui hábito de crescimento rasteiro, não obstante a presença de um eixo central ascendente nas plantas propagadas por semente, do qual partem os primeiros estolões, que se ramificam e podem emitir raízes em cada nó. O caule em contato com o solo ao enraizar dá origem a outras plantas através das gemas laterais, formando ramos secundários e assim sucessivamente, assim formando vários pontos de crescimento (Pereira et al., 2009). Portanto, não depende do surgimento de novas plantas provenientes de sementes para sua persistência no pasto. Isto evita o estresse competitivo que as plântulas poderiam sofrer em um dossel fechado, visto que a reposição das plantas ocorre também de forma vegetativa. Esta característica é fundamental para a leguminosa resistir ao pastejo, visto que são plantas com boa aceitabilidade e bom valor nutricional. Segundo Humphreys (1980), a maior aceitabilidade pelos animais é uma particularidade compatível somente com plantas de crescimento prostrado, com pontos de crescimento protegidos, que possuem alta tolerância ao pastejo.

O amendoim forrageiro possui numerosas estruturas de regeneração (rizomas, estolões e sementes), que se mantêm bem protegidas. Como foi dito anteriormente, a produção de semente é subterrânea, portanto esta via de propagação fica protegida do pastejo pelos animais. Essas características morfológicas, juntamente com sua tolerância

ao sombreamento, são determinantes para a sobrevivência e persistência do *Arachis* em pastagens consorciadas (Valentin et al., 2004). Os cultivares desenvolvidos para o uso forrageiro têm-se revelado persistentes e com elevado valor nutritivo nos locais com melhor oferta ambiental para o cultivo, com benefícios sobre a produtividade do animal e das pastagens. Um fator importante, que deve ser ressaltado também na interação das leguminosas e gramíneas em pasto consorciado, é a plasticidade morfológica. É um elemento que estabelece alterações adaptativas na estrutura das plantas em resposta ao pastejo e proporcionando uma reação positiva face às adversidades ao consórcio. O *A. pintoi* é uma leguminosa que possui alta plasticidade morfológica que confere a esta planta a capacidade de alterar sua morfologia, de acordo com as condições ambientais e de manejo.

As plantas de leguminosas no geral possuem alto valor nutritivo (Norton, 1994; Dewhurst et al., 2009). O valor nutricional do *A. Pintoi* é considerado superior em relação à maioria das leguminosas tropicais forrageiras, com teores de proteína bruta em torno de 18.%, teores de FDN de 40.7% e digestibilidade in vitro da matéria seca (DIVMS), de 67.6% (Gomes et. al., 2018).

O valor nutritivo das leguminosas é decorrente de sua organização estrutural ou da anatomia dos órgãos da planta e de seus tecidos constituintes. São plantas com o metabolismo fotossintético C3, que têm como o primeiro produto da fixação de CO_2 um composto de três carbonos (3-fosfoglicerato), e possuem somente a enzima Rubisco (ribulose bifosfato carboxilase/oxigenasse) para fixar o carbono. Como o próprio nome já diz, a Rubisco realiza também atividade de oxigenação (fotorrespiração), resultando na perda de CO_2 (biomassa) e na diminuição da eficiência fotossintética (Buchanan et al., 2000).

As plantas C3 possuem o feixe vascular das folhas circundado por uma bainha de parede espessada na parte interna. Os feixes vasculares são separados por uma grande proporção de mesofilo constituído por células com arranjo que permitem espaços entre elas (Buchanan et al., 2000). Essas características disponibilizam para os microrganismos do rumem grande quantidade de substratos prontamente digestíveis, conferindo ás espécies C3 maior digestibilidade. Entretanto, as leguminosas possuem quantidades variadas de tanino, que é um dos principais fatores antinutricionais das plantas forrageiras. A presença deste polifenol afeta a aceitabilidade das plantas pelos animais e, além disso, este composto pode formar complexos fortes com as proteínas, carboidratos e minerais, tornando-os indisponíveis para degradação ruminal.

Tanino Condensado

As plantas produzem uma grande variedade de compostos químicos, os quais são, comumente, divididos em metabólitos primários e secundários. O metabolismo primário é essencial ao ciclo de vida das plantas e as substâncias produzidas por ele tem distribuição geral. Por outro lado, os metabólitos secundários não são essenciais à sobrevivência das plantas, e seus componentes são formados a partir de compostos intermediários do metabolismo primário (Buchanan et al., 2000).

Os metabólitos secundários têm como função garantir vantagens à planta quanto à sua sobrevivência e perpetuação no ecossistema em que habita, protegendo-as contra condições adversas do meio ambiente como o ataque de patógenos e proteção contra herbivoria, além de atraírem polinizadores e dispersores de sementes. Esses metabólitos compreendem uma série de produtos químicos tais como saponina, terpeno, alcaloide, flavonoide, lignina e tanino (Buchanan et al., 2000).

Os taninos são compostos fenólicos encontrados nas plantas solúveis em água (Bate-Smith e Swain, 1962), presentes em madeiras, cascas, folhas e frutos. São responsáveis pela adstringência de muitos frutos e outras partes das plantas. Têm-se distinguido dois grupos de taninos, os quais são diferentes tanto estruturalmente quanto geneticamente, sendo eles os taninos hidrolisáveis e condensados (Singleton e Kratzer, 1973).

Os taninos hidrolisáveis (Figura 1) são constituídos por uma parte poli alcoólica, normalmente a glicose, e por uma parte fenólica, ligados através de uma ligação éster (Khanbabaee et al. 2001). A presença desses fenóis é o que caracteriza cada tanino hidrolisável, podendo ser os galotaninos, caso a parte fenólica seja o ácido gálico, ou elagitaninos, caso seja o ácido elágico. Geralmente, são encontrados em baixas concentrações nas plantas, e podem ser hidrolisados facilmente por bases, ácidos e ou até mesmo algumas enzimas (Mueller-Harvey, 2006). Os taninos hidrolisáveis são encontrados em algumas espécies de angiospermas dicotiledôneas tais como *Terminalia, Phyllantus* e *Caesalpina* (Cronquist, 1968). São sintetizados nas plantas através da rota do ácido chiquímico e tem como molécula precursora a pentagalloilglicose.

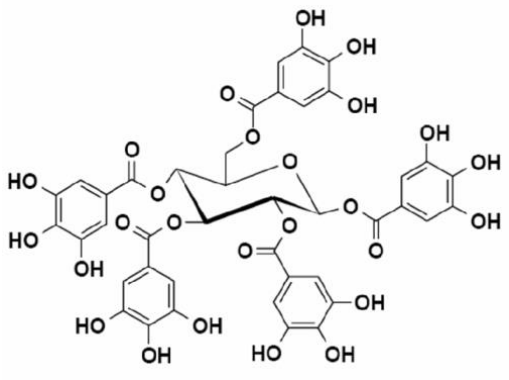


Figura 1. Estrutura de taninos hidrolisáveis: galactotanino.

Os taninos condensados (TC) são amplamente encontrados nas angiospermas e gimnospermas das plantas. Dentro de angiospermas, são mais comuns em dicotiledôneas do que em monocotiledôneas. São moléculas grandes constituídas por polímeros de flavonoides (Figura 3), sendo sintetizadas pela rota do ácido chiquímico e pela rota do ácido malônico (Figura 2) (Taiz e Zeiger, 2009). A leucocianidinas (Flavan-3-ol) são as moléculas precursoras dos TC, as quais são unidas por ligações de carbono-carbono que não são susceptíveis a quebra por hidrólise (Buchanan et al., 2000). Geralmente, têm um peso molecular mais elevado do que os taninos hidrolisáveis (Mueller-Harvey, 2006). Embora o termo tanino condensado seja amplamente usado, a denominação como proantocianidina também é aceita (Dixon, 2005). Proantocianidinas são compostos que produzem pigmentos antocionidínicos que, após oxidação em meio alcoólico-ácido quente, produzem composto de coloração vermelha (Porter, 1989).

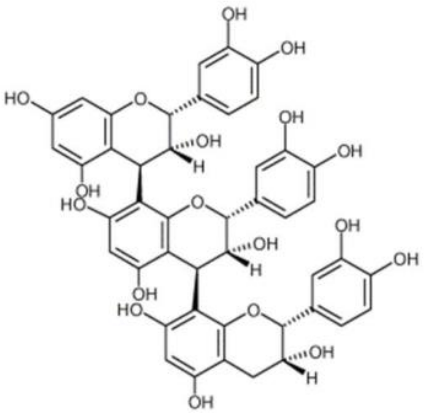


Figura 2. Estrutura do tanino condensado: epicatequina

Efeito do Tanino na Digestão Ruminal

O destino dos taninos depois de ingeridos varia com o seu tipo. Os taninos hidrolisáveis se decompõe completamente no rúmen, liberando proteína, aminoácidos, e alguns compostos fenólicos que, provavelmente, são eliminados na urina (Van Soest, 1994). Já os TC, como não são hidrolisados, quando ingeridos em grandes quantidades, têm efeitos negativos, comparativamente, aos taninos hidrolisáveis, reduzindo a digestibilidade dos alimentos ingeridos. Esse efeito ocorre, principalmente, sobre as proteínas, mas também afetam outros componentes como celulose, hemicelulose, amido e minerais, conferindo a esse composto suas propriedades antinutricionais (Mueller-Harvey, 2006).

Os taninos são mantidos em compartimento separado na célula das folhas para evitar a sua interferência com o próprio aparato metabólico das plantas. Após a mastigação das folhas, os taninos ficam expostos às proteínas salivares, as quais atuam como primeiro sistema de defesa contra a ingestão de tanino (Shimada et al., 2006). As glândulas salivares parótidas produzem saliva que contém diversos tipos de proteínas, e entre elas estão as proteínas com elevada capacidade de ligação ao fenol, por exemplo, as proteínas ricas em prolina (Lamy et al., 2011). Essas proteínas possuem papel importante na ligação aos polifenóis e, em particular, os taninos (Lamy et al., 2011).

A interação entre taninos e proteínas tem sido estudada por autores que têm proposto diversos modelos de interação (Hagerman e Klucher, 1986). Nesses modelos os aspectos mais discutidos são o tipo de ligação (hidrofóbica e/ou por pontes de hidrogênio) envolvida na formação dos complexos. As interações proteína-tanino (Figura 4) são afetadas por diversos fatores estruturais e pelas condições do meio (Asano et al., 1982). O núcleo polifenólico tem uma estrutura molecular favorável à interação com proteínas de dois modos. O primeiro seria pela presença de zonas apolares, como o anel benzênico, que podem interagir com zonas apolares das proteínas, tais como as cadeias laterais de aminoácidos, como a prolina. O segundo modo de interação seria a presença de zonas hidrofílicas, como os grupos de hidroxila, que podem participar nas ligações de hidrogênio com os grupos carbonila e amina das proteínas. No rúmen, estes complexos de proteína-tanino são estáveis, entretanto, quando o pH cai abaixo de 3,5 (tal como no abomaso) ou maior que 8 (tal como no duodeno) o complexo dissocia-se (Mangan, 1988). Esta ligação reversível da proteína com o tanino, protegendo-a da degradação ruminal confere à proteína a característica de *bypass*, ou seja, proteína não degradada no rúmen.

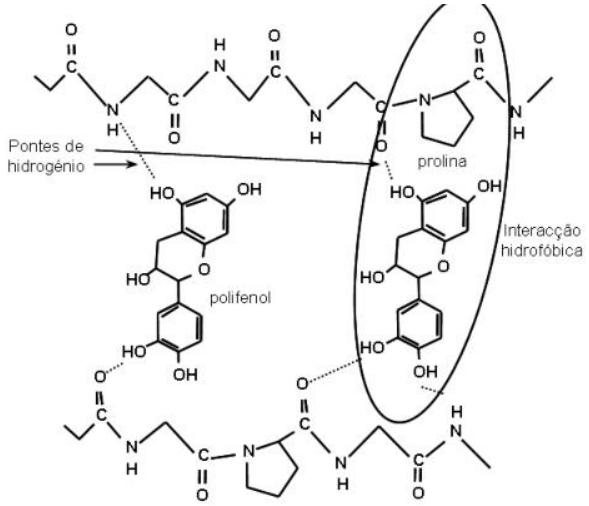


Figura 3. Modelo representativo das possíveis interações entre proteínas e polifenóis (Asano et al., 1982)

O efeito do tanino na menor digestão da fibra é considerado secundário em relação ao efeito sobre a degradação da proteína no rúmen. Entretanto, estudo tem demonstrado que a degradação da fibra no rúmen é reduzida em animais consumindo leguminosas tropicais as quais contêm tanino condensado (Barry et.al., 1986, Waghorn et al., 1987; Palmer e MCSweeney, 2000). Taninos podem reduzir a digestão da fibra pela complexação com lignocelulose impedindo a digestão microbiana ou atuando diretamente sobre os microrganismos celulolíticos (MCSweeney, 2001).

O consumo de ração pelos animais é, normalmente, reduzido em dietas com alta concentração de taninos, o qual é devido a uma redução na aceitabilidade e na diminuição da taxa de degradação no rúmen (Mueller-Harvey, 2006; Waghorn, 2008). Da mesma forma, plantas forrageiras com altos níveis de tanino são menos consumidas pelos ruminantes (Mokoboki et al., 2011). Vários estudos sugerem que quantidades de TC acima de 50 $g.kg^{-1}$ MS reduz significativamente o consumo dos ruminantes (Aerts et al., 1999; Barry e Manley, 1984; Waghorn, 1994; Barry e Dunca, 1984), enquanto que, quantidades mais modestas, de 20 a 50 $g.kg^{-1}$ MS, tem menor efeito sobre o consumo (Aguerre et al., 2016). O problema da diminuição da aceitabilidade das plantas contendo tanino é a adstringência. Esta sensação é resultado de interações entre proteínas salivares com o fenol (Breslin et al., 1993; Kallithraka et al., 1998).

Além disso, a interação da dieta com os taninos pode influenciar o consumo. A exemplo disso, foi demonstrado que a utilização de TC de quebracho fornecidos na quantidade de 89,3 $g.kg^{-1}$ MS reduziu o consumo de matéria seca (CMS) dos cordeiros alimentados com ervilhaca

(*Vicia sativa*) comparado aos cordeiros alimentados com a mesma forragem sem taninos. Enquanto que, o fornecimento do mesmo TC não afetou o CMS dos cordeiros alimentados com dieta à base de concentrado (Vastra et al., 2009). O efeito nutricional dos TC na dieta de ruminantes depende da sua concentração, do peso molecular e do tipo de TC presente nas plantas forrageiras, e esses fatores influenciam no CMS e no desempenho dos animais (Vastra et al., 2009).

A predominância de *prodelphinidins* (polímeros de taninos compostos por catequina) extraídos de *Calliandra callothyrsus* foi associado com maior consumo de ração e maior digestão, em comparação com as amostras que tinham a predominância de *procyanidins* (Lascano et al., 2003). Sem dúvida, diferentes espécies de plantas contêm misturas complexas de taninos, e nem todos os taninos têm os mesmos efeitos na alimentação.

Outro efeito do tanino sobre o consumo é relacionado a digestibilidade da dieta, que na maioria das vezes é menor quando o TC é consumido (Barry e Manley, 1984). Como há o efeito inibitório da degradação dos nutrientes no rúmen, afetando a taxa de degradação do alimento no rúmen, a taxa de passagem do alimento é afetada, portanto o fluxo do alimento é mais lento. Isso afeta diretamente no consumo dos animais.

Proteínas solúveis degradam-se rapidamente no rúmen (Mangan, 1988), a menos que sejam protegidas. A ocorrência dos taninos nas plantas forrageiras pode ter um benefício nutricional, protegendo a proteína das folhas da degradação ruminal, podendo ser degradadas no intestino delgado. Dentro desse contexto, diversos estudos comprovaram que o tanino na dieta dos ruminantes reduz as concentrações de amônia ($N\text{-}NH_3$) no fluido ruminal, melhorando a utilização das proteínas no pós-rúmem e reduzindo a excreção de N no ambiente (Barry et al., 1986, Mandal et al., 2016, Aguierre et al., 2016). De acordo com Mandal et al., (2016), os taninos podem diminuir as concentrações de $N\text{-}NH_3$ no fluido ruminal tanto pela proteção da proteína na formação do complexo, quanto pela ação inibitória do tanino sobre os microrganismos proteolíticos. Apesar dos benefícios, foi demonstrado em estudos *in vitro* que TC originado de diversas fontes (sorgo, feijão, quebracho e acácia) têm afinidades por diferentes proteínas e com diferentes graus de complexação (Asquith e Butler, 1986; Zelter et al., 1970). Portanto, nota-se que os TC de diversas espécies têm diferentes propriedades físicas e químicas e um dos fatores que pode interferir na complexação é o peso molecular do tanino (Mangan, 1988).

Muitos estudos demostram efeitos adversos desse complexo tanino-proteína, diminuindo a digestibilidade desse nutriente e, como consequência, ocorre o aumento da

excreção fecal de N (Aguierre et al., 2016; Kariuki et al., 2008; Komolong et al., 2001). A exemplo disso, Silanikove et al., (2001) estudaram a adição de TC de Alfarrobeira (*Ceratonia siliqua*) na dieta de ovelhas na quantidade de 50 $g.kg^{-1}$ de MS consumida e observaram que os animais perderam peso e excretaram mais proteína em suas fezes do que o consumido. É importante perceber, que as consequências da ingestão de taninos incluem também o aumento da secreção de proteínas endógenas, tais como glicoproteínas salivares, muco e enzimas digestivas, e aumento da descamação de células intestinais (Silanikove et al., 2001). Este aumento de nitrogênio fecal pode ocorrer a partir do aumento do N de origem endógena.

Em estudos mais recentes foi constatado a maior excreção de N fecal pelos animais (Aguierre et al., 2016). Nesse caso, os autores trabalharam com vacas em lactação submetidas a dietas com 45 a 180 $g.kg^{-1}$ de MS de TC originado do quebracho (*Schinopsis lorentzii*) e do castanheiro-europeu (*Castanea dentata*). Contudo, houve menor excreção de N pela urina. Esses autores observaram resultados positivos em relação ao desempenho dos animais que consumiram 45 $g.kg^{-1}$ de MS de TC. Os animais que foram alimentados com dietas de menor % de proteína bruta não diminuíram a produção de leite quando foi adicionado o tanino e, além disso, aumentou a eficiência de utilização de N (N no leite/ N consumido), além de diminuir o teor de $N\text{-}NH_3$ no leite e no fluido ruminal, indicando efeitos benéficos. A maior parte das proteínas se tornam rapidamente solúveis no rúmen e são degradadas, resultando em níveis de amônia excedentes que são absorvidas pelo rúmen e excretadas na urina.

Efeito do Tanino Condensado no Pós-Rumen

Embora haja amplo conhecimento sobre os efeitos do TC na degradação dos nutrientes no rúmen, poucos estudos têm investigado seus efeitos na digestão pós-ruminal. O processo de absorção depende de quanto o complexo tanino-proteína é revertido no pós-rúmen. Ocasionalmente, os complexos de tanino-proteína não podem ser dissociados totalmente, dependendo do pH e da formação do mesmo, que pode ser atribuído às diferenças nas estruturas dos taninos e das proteínas (Mueller-Harvey, 2006).

Estudos demonstram que TC de *Lotus pedunculatus* e de *Desmodium ovalifolium* formaram complexos com proteínas no rúmen, e foi sugerido que esses complexos seriam dissociados no abomaso e no intestino (Makkar et al., 1995b). Os resultados indicam que o pH ácido do abomaso resultaria em aumento da quantidade de proteínas disponíveis para serem digeridas no intestino. Utilizando-se outra fonte de TC, Calliandra, foi observado o mesmo resultado em estudos *in vitro* (Tiemann et al., 2010).

Estudos *in vivo* investigaram a digestão do complexo tanino-proteína no pós-rúmen de ovelhas fistuladas no abomaso e no intestino (Kariuki et al., 2008). Foi infundido no rúmen dos animais o complexo albumina-tanino contendo iodo como marcador e utilizou-se TC de *Leucaena pallida* e de *L. Leucocephala*. Observou-se que a digestibilidade verdadeira do complexo tanino-proteína de *L. pallida* foi menor em relação a *L. leucocephala* entre o abomaso e íleo terminal, o que indica diferenças na capacidade de inibição de cada TC com a proteína. A maior excreção de N fecal ocorreu quando se utilizou TC de *L. Pallida*.

Apesar dos resultados utilizando o TC de Leucena, foi demonstrado que a inclusão de TC de Quebracho nas quantidades de 10 e 20 $g.kg^{-1}$ de MS em dietas para bovinos (Beauchemin et al., 2007) e na suplementação de caprinos com TC de *O. viciifolia* (Aufrere et al., 2008) diminuíram a digestibilidade aparente da proteína.

Segundo Komolong et al, (2001), concentrações crescentes de TC de Quebracho em dieta de alfafa em ovinos reduziu a digestibilidade aparente e absorção de aminoácidos no intestino delgado. Além disso, aumentou a excreção de N fecal em consequência dessa redução da digestibilidade aparente da proteína. A maior excreção fecal de N em dietas contendo taninos ocorre onde os complexos tanino-proteína provavelmente não foram completamente dissociados no abomaso ou no intestino (Grainger et al., 2009). O que acontece é uma inversão na rota de excreção de N quando se alimenta os ruminantes com TC. Como já descrito, há um aumento na excreção de N pelas fezes, entretanto, a menor absorção de NH_3 no rúmen do animal promove menor excreção de N pela urina.

Embora tenha sido sugerido que o TC pode aumentar o fluxo de nutrientes para o intestino (McSweeney et al, 2001), os taninos podem exercer um efeito negativo na absorção de nutrientes no intestino delgado, o que pode ser devido à estabilidade do complexo tanino-proteína no intestino que não foi dissociado no abomaso, formação de complexos de tanino com enzimas digestivas ou até mesmo novos complexos do tanino com outros nutrientes (Silanikove et al., 2001). Além do mais, pode ocorrer alterações na absorção intestinal devido à interação de taninos com a mucosa intestinal (Waghorn e McNabb, 2003).

Embora complexos de tanino-proteína dissociam a pH <3,5 (o pH do abomaso), o pH no início do intestino (≈5,5) pode permitir que os complexos de proteína-tanino se restaurem, e, em consequência disso, inibem sua digestão (Waghorn e McNabb, 2003). Kumar e Singh (1984) sugerem que os taninos são capazes de inibir as enzimas digestivas devido à capacidade de se ligarem de modo a formar complexos insolúveis (ou complexos solúveis, mas inativos), no entanto, esse fato é questionável. Os taninos têm a oportunidade de formar complexos com uma vasta variedade de proteínas alimentares muito antes de entrar em contato com as enzimas

digestivas (Mehansho et al. 1987). Apesar das observações realizadas por esses estudos, é importante ter em mente que a maioria deles foram realizados *in vitro*, não levando em conta fatores como a presença de sais biliares.

Efeito do Tanino na Eficiência da Síntese Microbiana

A proteína de origem microbiana é a fonte de proteína de maior importância para o ruminante. A maximização da síntese de proteína microbiana é a forma mais barata e eficiente de fornecer proteína de alta qualidade ao bovino, sendo os microrganismos a principal fonte deste nutriente para ruminantes (Van Soest, 1994).

A produção de proteína microbiana é influenciada pela proporção de N no alimento que é solúvel e degradável no rúmen, e em adição a disponibilidade de energia digestível, enquanto que, a disponibilidade de proteína para absorção pós ruminal é influenciada pela proporção do N que é resistente a degradação ruminal mais a proteína microbiana (Van Soest, 1994). Parte da proteína oriunda da dieta é degradada no rúmen para ser utilizada na síntese de proteína microbiana e outra parte, que escapa da degradação ruminal, é chamada de proteína *bypass* ou proteína não degradada no rúmen (PNDR). Utilizando-se moderadas concentrações de TC nas dietas de ruminantes, ocorre o aumento do fluxo de proteína *bypass* devido à sua capacidade de formar complexos reversíveis com as proteínas (Van Soest, 1994).

Taninos em níveis moderados têm o potencial de modular a fermentação do rúmen para a maximização da síntese de proteína microbiana (Getachew et al., 2008; Salem et al., 2007). Embora os taninos diminuam a disponibilidade de nutrientes no rúmen, a maior proporção dos nutrientes disponíveis é direcionada para a síntese de massa microbiana e menos para a produção de ácidos graxos de cadeia curta (Makkar et al., 2003).

A diminuição da taxa de digestão de alimentos causada pelos taninos poderia ajudar a sincronizar a disponibilidade de vários nutrientes, que por sua vez, podem ser responsáveis pelo aumento da eficiência microbiana (Makkar et al., 2003). Além disso, a proporção mais elevada de propionato no sistema de fermentação *in vitro* e a diminuição na população de protozoários quando houve a presença de taninos (Makkar et al., 1995 a, b), resultou na maior eficiência da síntese de proteína microbiana. Embora os efeitos dos taninos na população de protozoários do rúmen sejam inconsistentes (Benchaar et al., 2008).

Apesar de diversos estudos encontrarem aumento na síntese de proteína microbiana, esse fato ainda é muito controverso (Jetana et al., 2012, Ahnert et al., 2015). Pode ocorrer inibição ou não alterar a síntese de proteína microbiana como o uso do TC, devido à inibição específica de determinados grupos de microrganismos (Makkar et al., 2003; Vasta et al, 2010).

À exemplo disso, foi demonstrado que TC de *Leucaena leucocephala* em quantidades elevadas de 73 e 116 g.kg^{-1} MS nas rações de ovelhas não afetou o fluxo de proteína microbiana estimada pela excreção de derivados de purinas na urina (McNeill et al., 2000). Da mesma forma, não foi observado efeito com relação à produção de proteína microbiana de caprinos suplementados com TC de *Antidesma thwaitesianum* nas quantidades de 16 e 24 g.kg^{-1} do consumo total de MS (Gunun et al., 2015). Entretanto, houve aumento na produção de propionato e não afetou a digestibilidade do alimento (Gunun et al., 2015).

Em contrapartida, a utilização de 4 g.kg^{-1} da MS de TC de quebracho teve efeitos prejudiciais sobre a degradabilidade dos carboidratos no rúmen, o que diminuiu o fornecimento de energia para os microrganismos, reduzindo a produção de proteína microbiana (Ahnert, 2015). O efeito da inibição ou não do crescimento microbiano no rúmen pode variar de acordo com o tipo de tanino, a sua origem e quantidade na dieta, e a composição do microbiana no rúmen (McSweeney et al, 2001; Makkar et al., 2003). Os estudos se contradizem muito e há uma falta de padronização com relação aos métodos utilizados para analisar a síntese de proteína microbiana.

Efeito do Tanino nos Protozoários do Rumen

Os efeitos dos taninos sobre os protozoários do rúmen são muito variáveis. Primeiramente, Newbold et al., (1997) trabalharam com TC de Sesbania (*Sesbania sesban*) e descreveram que os taninos não afetaram na população de protozoários. Da mesma forma, Benchaar et al., (2008) não observaram qualquer efeito do tanino sobre o número de protozoários em vacas holandesas com peso de 730 ± 89 kg alimentadas com quebracho (70% de TC) na quantidade de 150 g.dia^{-1}. De acordo com esses autores, a quantidade fornecida aos animais foi baixa e por isso não houve efeito. Já Salem et al. (1997), trabalhando com ovinos alimentados com dietas a base de alfafa adicionando folhas de Acácia saligna (*Acacia cyanophylla*), as quais continham 45 g.kg^{-1} MS de TC, observaram o aumento da população de protozoários no fluido ruminal dos animais.

Apesar desses resultados, estudos sugerem que os TC podem reduzir até 79% da população de protozoários no rúmen (Piñeiro-Vázquez et al., 2015). Gunun et al., (2016) e Saminathan et al., (2016) relataram em estudos *in vitro* o efeito inibidor do tanino sobre os protozoários do rúmen, utilizando como fonte de TC a planta quebracho. De acordo com esses autores, o número dos protozoários das ordens Entodiniomorfos e, principalmente, Holotriquios, foram menores no fluído ruminal.

A única afirmação que pode ser feita por enquanto com relação aos efeitos do TC sobre a população de protozoários é que ela dependente do tipo e da fonte de tanino e dos níveis de suplementação (Patra e Saxena, 2011). A remoção de protozoários tem sido um alvo dos pesquisadores para manipulação do rúmen em benefício da diminuição da emissão de metano. Os efeitos anti-protozoários dos taninos podem inibir a metanogênese do rúmen (Belanche et al., 2014). No entanto, os mecanismos de inibição de protozoários no rúmen por taninos não são claramente conhecidos.

2. INTRODUCTION

Livestock farming in Brazil has great prominence worldwide. However, Brazilian performance indexes are still modest, and one of the reasons for this is the under-utilization of our pastures. The main factors for the decrease of pasture productivity are incorrect management and lack of maintenance of adequate nutrients for the plants, which lead to a decrease in animal production. This process has economic and ecological implications and it is therefore fundamental to find solutions.

Nitrogen (N) is a nutrient that contributes to the productivity of forage grasses (Voisin, 1988), and its main input into pasture systems is mineral fertilization. However, despite its benefits, nitrogen fertilization may cause negative impacts to the environment when it is incorrectly carried out, due to losses (Galloway et al., 2004). In this regard, the use of legumes arises as an alternative to introduce N into the system in a more sustainable way, reducing the agricultural supplies input.

Some legume species have the capacity to establish symbiosis with soil microorganisms, which induces the formation of root nodules where the biological fixation of atmospheric nitrogen occurs (Peix et al., 2015). Thus, with the introduction of legumes, there is less requirement for mineral nitrogen fertilizer input, resulting in lower environmental impact (Dejun Li et al., 2013).

The *Arachis pintoi* (forage peanut) is a legume that has had prominence in the use of mixed pastures pastures in tropical conditions. As it is a stoloniferous plant, the stem roots, when in contact with the soil, give rise to new plants, developing several growth points by vegetative ways (Pereira et al., 2009). This kind of growth is fundamental for this plant to resist grazing.

In addition to the environmental aspects aforementioned, there is another important point about forage legumes. These plants have promising characteristics with regard to ruminant nutrition. Firstly, legumes have better nutritive value compared to the grasses, which is due to the formation of their cell walls, anatomy of organs and tissues, affecting directly the digestibility, mainly of fiber (Minson, 2012). Another point is related to the secondary metabolites.

Secondary metabolites are commonly the products of complex biochemical pathways, which may be involved in the synthesis of pigments or other compounds that have a role in plant defense or pollination (Buchanan et al., 2000). One of these metabolites is tannin. There

are two types of tannin in plants, condensed and hydrolysable. Both tannin types may elicit different responses on rumen metabolism due to their distinct chemical structure.

Condensed tannins (CT), also known as proanthocyanidins, have several effects on the rumen, the main action being the interaction with proteins, as well as cellulose, hemicellulose, starch and minerals (Mueller-Harvey, 2006). The CT and protein form a stable complex, which is not degraded in the rumen, and may eventually degrade in the post rumen, increasing the rumen undegradable protein (RUP). However, the affinity of CT with protein will depend on its source, resulting in different degrees of complexation (Asquith E Butler, 1986; Zelter et al., 1970), which may be related to the molecular weight of CT (Mangan, 1988). There is nothing described in the literature concerning the possible interactions of CT from *Arachis pintoi* cv. Mandobi with the proteins in the rumen.

Protein-tannin complexes are stable in rumen; yet, when the pH falls below 3.5 (as in abomasum) or is greater than 8 (as in duodenum), the complex dissociates (Mangan, 1988). The reversible binding of protein and tannin protects them from ruminal degradation and increases the RUP. In this regard, several studies have demonstrated that tannin on ruminant diet reduces the ammonia ($N\text{-}NH_3$) concentrations in rumen fluid (Barry et al., 1986, Aguierre et al., 2016). This feature is advantageous because there are situations in which the amount of ammonia released in the rumen is greater than the capacity of use and incorporation as microbial protein.

Excessive $N\text{-}NH_3$ is removed from the rumen, metabolized in the liver and excreted through the urine; or recycled in the form of urea to go back into the rumen. Therefore, since part of the excessive nitrogen released in the rumen is transformed in RUP and absorbed by the intestine, the nitrogen balance in the metabolism of ruminants may become more positive. This might increase the efficiency of N use and decrease the losses of this nutrient, reducing its excretion in the environment (Van Soest, 1994).

In grazing animals, this degradation imbalance of carbohydrates and proteins occurs most of the time, because the majority of the carbohydrate available for ruminal degradation is fiber, which is slowly degraded. This imbalance is observed mainly in forage grasses fertilized with nitrogen, since the fertilization may alter the distribution and composition of nitrogenous fractions in plants. Nitrogen fertilization increases the non-protein nitrogen (NPN), from the increase of nitrate content in the leaves (Van Soest, 1994). The NPN is composed of peptides, nitrate and non-essential amino acids, is soluble in the rumen and rapidly converted to $N\text{-}NH_3$. The increase of NPN in the rumen with the slow degradation of fiber may result in a nitrogen

imbalance in the animal metabolism, leading to higher losses of this nutrient. Legumes have more digestible organic matter than grasses, which provides more substrate for the microbial protein production, and therefore, better use of N by microorganisms.

Besides the biological reasons for reducing the loss of nitrogen in excreta, there are also environmental reasons. Due to the national cattle herd size, excreta (urine and feces) represent the majority of nitrogen losses to the environment in livestock systems. These losses are directly related to emissions of greenhouse gases (GHG) from the emission of nitrous oxide (N_2O) directly or indirectly by ammonia volatilization (MCTI, 2013). Consequently, the bigger the loss of N, the more polluting the system, mainly through urine N (Powell et al., 2011).

Moreover, tannins are modulators of ruminal fermentation, because they change the population of microorganisms. The degradation of fibers and proteins may be modified, as tannin inhibits the activities of proteolytic and fibrolytic enzymes (Makkar et al, 2003; McSweeney et al., 2001), the activity of methanogenesis (Mao et al., 2010) and protozoa (Piñeiro-Vázquez et al., 2015), because of its antimicrobial action. However, the effects of the tannins on the protozoa in rumen are variable. The effects of CT on the protozoa are dependent on the type and source of tannin and its levels of supplementation (Patra and Saxena, 2011).

Removal of protozoa has been a target of researchers for manipulation of the rumen, since the protozoa population output is minimal, which increases the retention and decreases the turnover rate (Van Soest, 1994). The predation of the protozoa over the rumen bacteria is responsible for an apparent inefficiency in the production of microbial protein. Nevertheless, the inhibition mechanisms of protozoa in the rumen by tannins are not clearly known.

Within this context, studies relating condensed tannin and nitrogen balance in ruminant metabolism are fundamental to find the best way to reduce the loss of N through excreta, optimizing the use of this nutrient in the system. To determine the effect of condensed tannin from *Arachis pintoi* on the N balance of animals and the degradability of forage nutrients from grass and legume intercropping

Objective:

In this context, this study aims at determining the metabolism of nitrogen and carbohydrates of animals feeding on two types of pastures: brachiaria grass fertilized with nitrogen (monoculture) or intercropped with legume.

Hypothesis:

In the present study, we assessed the potential for the inclusion of tropical climate condensed tannin legume in pastures for beef cattle, by testing the following hypotheses:

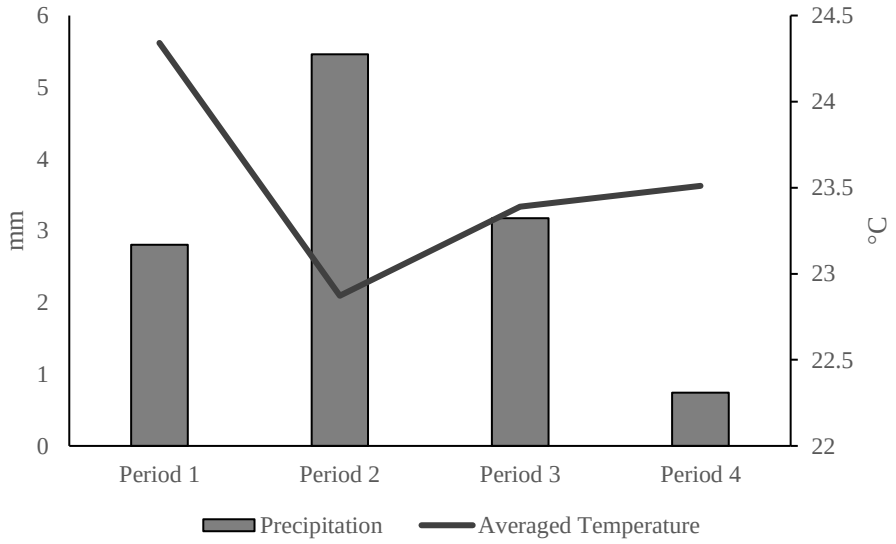
- ✓ *Arachis pintoi* decreases ruminal protein degradability
- ✓ *Arachis pintoi* increases digesta nitrogen flow and decreases nitrogen losses through excreta
- ✓ Diet containing legume increases the intake of digestible fiber, serving as substrate for greater production of microbial protein
- ✓ Animals consuming *Arachis pintoi* have better nitrogen balance, thus having greater efficiency in the use of this nutrient
- ✓ Condensed tannin intake acts as a manipulator of the ruminal microbiota, affecting the protozoa population.

3. MATERIALS AND METHODS

3.1 Experimental Site

The trial was carried out at the experimental farm of the Animal Science Department of University of Lavras (UFLA), Lavras – MG. That place is located at 21º14'06 " south latitude, 44º58'06 " west longitude and 918 meters altitude and has a subtropical humid mesothermal climate with dry winters (Koppen-Geiger climate classification: Cwa; Sa Junior et al., 2012). Meteorological data were obtained from a weather station located 1,000 m from the experimental area (Figure 1).

Figure 4. Monthly temperatures (°C) and rainfall (mm) in Lavras, Brazil, during the experimental period.



Two areas were used to conduct the experiment: 0.8 ha of *Brachiaria brizantha* (Hochst ex A. Rich) cv. Marandu (brachiaria grass) intercropped with *Arachis pintoi* cv. Mandobi (forage peanut) and 0.6 ha of *Brachiaria brizantha* cv. Marandu, which was fertilized with mineral nitrogen applied at of 150 kg $N.ha^{-1}$ per year. The utilized pastures were already in place. The intercropped pasture was established in December 2006 by seeding of brachiaria grass with forage peanut. The seeding rates were 7.6 and 7.2 $kg.ha^{-1}$ of pure live seed of brachiaria grass and forage peanut, respectively. In addition, when the experiment started, the intercropped pasture had a botanical composition about 35% of legume. The botanical composition was estimated by collecting forage mass at the soil level, subsequently, weighed the wet legume mass. The monoculture pasture was established in 2015 by seeding of brachiaria grass.

3.2 Animal management, experimental design and treatments

The management and care of animals were performed in accordance with the guidelines and recommendations of the Committee of Ethics on Animal Studies at the University of Lavras (UFLA), MG, Brazil, protocol number 042/18. There were three months of experimental period in the field, which were divided in four periods of 21 days. Each period was divided in two: 10 days for adaptation of animals to the diet and 11 days of sampling and evaluations.

Six Tabapuã heifers with an averaged body weight of 425 kg ± 43.88 performed grazing. Those animals had a fistula located in the rumen. Before starting the experiment, all animals were weighed, identified, and treated against ecto and endoparasites. Additional put-and-take heifers were used to manage forage to a target height of 25 cm, approximately. Those animals belonging to the Animal Science Department of UFLA were assigned randomly to treatment sequence in a double *Cross-over* with four experimental periods (Figure 2). Experimental treatments were grass-legume pasture of brachiaria grass with forage peanut (IP), and brachiaria grass pasture monoculture fertilized with mineral nitrogen, using urea as the source of N (FP). Details regarding the characteristics of canopy are described in Table 1 and to the animals' diet are described in Table 2.

Figure 5. Experimental design: brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP), on four experimental periods

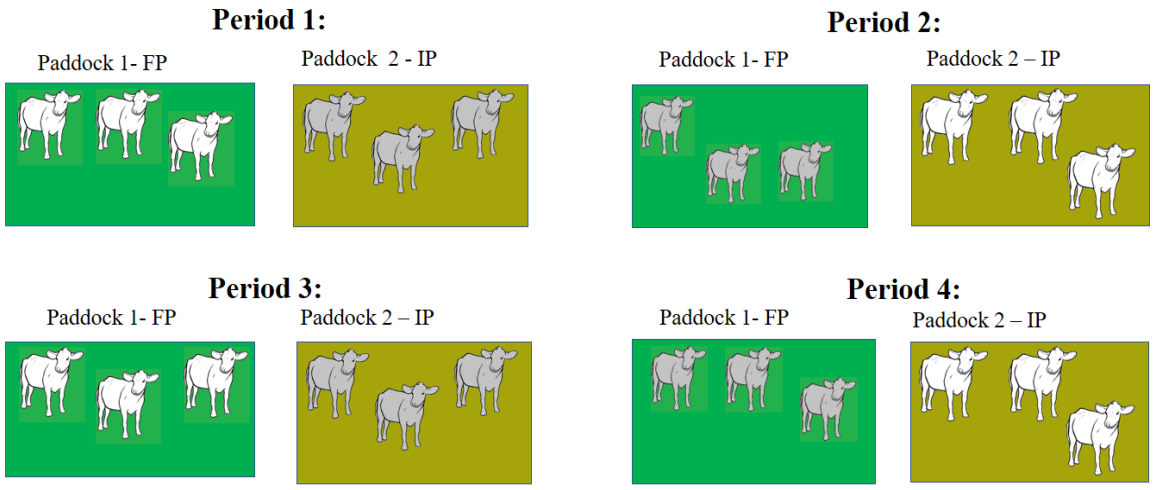


Table 1. Canopy characteristics of forage fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP) all experimental periods.

	FP Periods				**IP** Periods			
Item	1	2	3	4	1	2	3	4
Total mass, kg.ha^{-1}	11,337	10,996	12,053	10,622	10,819	10,808	11,413	9,817
Grass	11,337	10,996	12,053	10,622	8,916	8,374	8,326	8,266
Legume	0	0	0	0	1,903	2,434	3,087	1,551
Total leaves, kg.ha^{-1}	3,037	2,590	2,285	3,543	2,602	2,199	2,084	2,138
Grass	3,037	2,590	2,285	3,543	1,881	1,479	1,369	1,859
Legume	0	0	0	0	721	720	715	279
Total Stem, kg.ha^{-1}	2,994	2,930	3,665	2,600	3,076	3,578	4,120	2,605
Grass	2,994	2,930	3,665	2,600	1,919	1,911	1,866	1,480
Legume	0	0	0	0	1,157	1,667	2,254	1,125
Dead material, kg.ha^{-1}	5,281	5,363	5,881	3,987	5,090	4,967	4,867	4,818
Grass	5,281	5,363	5,881	3,987	5,065	4,921	4,749	4,670
Legume	0	0	0	0	25	46	118	148
Canopy height, cm	24.78	25.01	24.09	23.58	23.32	24.83	23.84	23.36

Table 2. Chemical composition of hand-plucked forage samples of brachiaria-grass fertilized with mineral nitrogen (BF) or intercropped with forage peanut (BI), and of forage peanut (LE) on four experimental periods

	Forage		
Item	BF	BI	LE
Ash, %DM	7.86	7.95	7.48
CP[1], %DM	11.82	10.96	23.43
A[2], %CP	31.85	42.75	26.51
B1 + B2[2], %CP	32.65	24.43	27.63
B3[2], %CP	26.59	22.61	38.84
C[2], %CP	8.91	12.21	7.02
NDF[3], %DM	64.60	68.41	41.87
ADF[4], %DM	27.15	26.09	17.88
Cellulose, %NDF	37.44	24.22	14.80
Hemicellulose, %NDF	24.54	42.32	23.98
Lignin, %NDF	2.61	1.87	3.08
Ether extract, %DM	1.82	1.51	1.67
IVDMD[5], %	66.71	70.55	86.97
Condensed tannin, %DM	ND	ND	2.56
NFC[6], %	12.55	11.17	25.54

[1]CP: crude protein; [2]Protein fractions (A, B1+B2, B3, C); [3]Neutral detergent fiber.

[4]Acid detergent fiber; [5]*In vitro* dry matter digestibility;

[6]NFC: Nonfibrous carbohydrate (NFC = 100 - %NDF - %IP - %Fat - %Ash)

3.1 Experimental evaluations

Sward measurements

In order to characterize the pastures, the sward heights, forage mass, pasture botanical composition were monitored. The sward height was recorded weekly in each treatment by taking at least 100 measurements randomly, using a sward stick (Barthram, 1985). Forage mass was sampled by using ten frames of 1 x 0.5m per paddock, once for each period. Harvested forage was collected, weighed, subsampled, and the botanical separations were performed. Forage samples were dried at 55 °C for 72 h and weighed to obtain forage dry matter (DM) content. Total forage mass (kg/ha) was the sum of grass and legume components in the canopy above the ground.

Nutritive value

Hand-plucked forage samples were collected (Langlands, 1974) on animal intake evaluation days. After being collected, the samples were dried at 55 °C for 72 h, weighed, and ground in a Cyclotec mill (Tecator, Herndon, VA) at 1 and 2 mm for further bromatological evaluations.

The DM of each sample was obtained by oven drying at 100 °C for 18 hours (method 934.01: AOAC, 2000). The ash concentration was determined by 2 hours of incineration process in a 600 °C muffle furnace (method 942.05; AOAC, 2000). The CP concentration was obtained based on the N concentration (CP = total N × 6.25), which was determined using the Kjeldahl procedure (method 920.87; AOAC, 2000).

From the constituents of the fibrous fraction, the ash-free NDF and ADF were analyzed by filtration in porous crucibles (Van Soest et al., 1991). The lignin was analyzed in ADF analysis sequence, according to the method 973.18D (AOAC, 2000). The hemicellulose and cellulose were determined according to the calculations (Eq. 1 and 2):

$$\text{Hemicellulose (\%)} = \%\text{FDN} - \%\text{FDA} \quad (1)$$

$$\text{Cellulose (\%)} = \%\text{FDA} - \%\text{Lignin} \quad (2)$$

As a means to obtain the protein fractions, residues of filtration of NDF and ADF were analyzed by the Kjeldahl procedure (method 920.87; AOAC, 2000).The aim was determining the neutral detergent insoluble protein (NDIP), protein insoluble in neutral detergent but extracted with acid detergent (Fraction B3), and acid-detergent insoluble protein (ADIP). The *non-protein nitrogen (*NPN), or A fraction, was estimated using the method described by Licitra et al. (1996), with the use of trichloroacetic acid. The calculations of protein fractions (A, B1 + B2, B3, and C) were estimated according to Tylutki et al. (2008). The fraction A was established by the difference between the quantity of insoluble N in 10% trichloroacetic acid solution and the total sample N. The fraction B3 was determined by the difference between NDIP and ADIP (fraction C). The fraction B1 + B2 was determined by the difference between the quantity of total N and the other fractions. Nonfibrous carbohydrate (NFC) as calculated by difference: NFC=100 - (%NDF_ %CP_ %Fat _%Ash).

To quantify the condensed tannin (CT), an extraction with acetone and methanol solution in an ultrasonic water bath at room temperature was performed. The contents were subjected to centrifugation for 10 min at 3,000 rpm and 4°C after the extraction. The supernatant was collected and kept in a freezer. Subsequently, CT was quantified using the butanol-HCl solution and ferric reagent (Porter et al., 1986).

Intake and apparent digestibility of forage

Forage intake was estimated from fecal excretion and indigestible neutral detergent fiber (iNDF). Fecal excretion was measured indirectly using the external titanium dioxide marker (MYERS and ROBBINS, 1991). The marker was given to the animals for 12 days, which were seven adaptation days to the marker and five days of fecal sampling. The titanium dioxide was dosed daily in the amount of 10 g/animal. From the 11th to the 15th days of each period, spot fecal samples were collected directly from the rectum once a day and a compound sample was performed for each animal per period. The fecal samples were dried at 55 °C for 72 h and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass 1 and 2 mm screen for further evaluations. The fecal samples were analyzed for titanium dioxide concentration according to Myers et al. (2004).

Fecal and hand-plucked forage samples were incubated in the rumen for 288 hours to determinate iNDF (Huhtanen et al., 1994). The fecal excretion was used to find the total amount of iNDF in feces; thus, the estimate of iNDF intake per day was obtained. After that, iNDF from the hand-plucked samples was acquired to estimate the forage intake.

The grass (C4 plants) and legume (C3 plants) proportion on forage intake was calculated using the ^{13}C technique (Ludlow et al., 1976). The forages that we used (brachiaria grass and forage peanut) have different photosynthetic cycles, resulting in different patterns of isotopic fractionation. Plants of C3 cycle assimilate the CO_2 with a low 13C/12C ratio and C4 plants have a high $^{13}C/^{12}C$ proportion. In this way, in order to calculate the proportion of legume in the diet, the ^{13}C isotopic abundance was determined on iNDF residual of the feces and hand-plucked samples (Lopes de Sá, 2017). A Thermo brand Delta V Advantage isotope ratio mass spectrometer coupled with a Costech ECS 4010 model C and total N automatic element analyzer from Costech, located at the John M. Day Laboratory of Stable Isotopes of Embrapa Agrobiologia.

The coefficients of apparent digestibility of the CP, NDF, ADF in the total digestive tract was determined through fecal excretion, by the external titanium dioxide indicator (Myers and Robbins, 1991), as cited above. Furthermore, DM, OM, NDF, ADF and CP contents of fecal samples were determined. The apparent digestibility coefficients were calculated from the mean amount consumed and excreted by feces of DM, OM, NDF, FDA and CP.

Ruminal kinects

Rumen fractional rates of passage (kp) and digestion (kd) were calculated from rumen flow and pool sizes, as described by Robinson et al. (1987). To this end, we collected the digesta passing the reticulo-omasal orifice through the ruminal fistula, as suggested by Huhtanen et al. (1997) and Ahvenjarvi et al., (2000). The aim was to estimate the ruminal digestion and the digesta flow. This sampling requires the insertion of a tube via rumen fistula that reaches the reticulo-omasal orifice. A vaccum pump connected to a kitassato was attached to a tube, which was used to draw omasal digesta into a collecting flask.

Consequently, the digesta flow was determined using two markers, cobalt ethylenediamine tetraacetic acid (CoEDTA) and indigestible neutral-detergent fiber (iNDF). The CoEDTA was dosed daily in the amount of 6 g/animal for 10 days, which were seven adaptation days. The omasal samples were collected after the animal's adaptation to Co-EDTA, every nine hours, over 3 days. Thus, the sampling times were 7am and 4pm on day one; 1am, 10am and 7pm on day two; 4am, 1pm and 10pm on day three, simulating 24 hours of collections at 3-hour intervals (ALLEN and LINTON, 2007).

Omasal samples were poured into containers and stored at −20°C, immediately after sampling. Posteriorly, those samples were thawed at room temperature, and they were filtered and separated in two phases: the filtrated liquid constituted the liquid phase and small particles (LP), and the residue retained the phase of large particles (SP) of digesta. Samples from different phases of the omasal digesta were oven-dried at 55 °C for 96 hours and ground in a ball mill for further analysis. Measurements of digesta flow using double-marker systems were based on Co as the LP and iNDF as SP markers. All samples were analyzed for DM, NDF, ADF, CP and iNDF content. In addition, cobalt (Co) was

assessed in an atomic absorption spectrophotometer digested with nitroperchloric acid. Digesta flow was calculated by Faichney (1975) method

The ruminal pool sizes were estimated by manual evacuation of the entire rumen contents on two different times, once at the minimum and once at maximum expected pool size (Allen And Linton, 2007). The first evacuation was at 9am, time before the peaks of eating activity, when the ruminal volume was lower. The next day, the same procedure of emptying was carried out, however, at 4pm, when the rumen was at its highest volume. This schedule was established through the ingestive behavior of the same animals in this experimental area. Animals were allowed to graze immediately after the removal of ruminal contents. The latter were weighed and a sample of each animal was taken. The samples were separated as solid and liquid fraction, and subsequently frozen for further analysis at -20 °C. We returned the digesta to the rumen of the animals after this sampling.

The rates were calculated using the daily mean pool size. Ruminal turnover rate was calculated by dividing the rate of intake by ruminal pool size (Equation 3):

$$\text{Ki (rate of intake; } h^{-1}) = \text{intake } (kg.h^{-1}) / \text{rumen pool (kg)} \quad (3)$$

The passage rate of a homogenous fraction was calculated dividing flux of the fraction passing from the rumen by ruminal pool size (Equation 4):

$$\text{Kp (rate of passage; } h^{-1}) = \text{digesta flow } (kg.h^{-1}) / \text{ruminal pool (kg)} \quad (4)$$

The digestion rate of the fraction was calculated by subtracting the passage rate from the rate of intake in the rumen, using the equation 5:

$$\text{Kd (rate of digestion)} = Ki - Kp \quad (5)$$

The ruminal coefficient of digestibility was determined using the average intake and the estimated amount of DM and diet components in the abomasum. Intestinal digestibility was calculated using the estimated amount of DM and diet components in the abomasum and the amount of DM in feces.

Ruminal parameters

The ammonia concentration (N-NH3), pH and volatile fatty acids (VFAs) in the rumen were determined through ruminal fluid samples. We collected the samples directly from the ruminal fistula of each animal in the ventral portion of the rumen on the 11th day of each experimental period.

Samplings were done at 6am, 12pm, 6pm and 12am. An approximately 80 ml sample was separated for pH reading using digital pHmeters, immediately after sampling. The samples for $N\text{-}NH_3$ analysis were acidified with 20% sulfuric acid solution in the ratio of 1 mL of acid per 5 mL of sample, and then frozen. The $N\text{-}NH_3$ concentration was obtained by the phenol-hypochlorite method, according to the technique described by Weatherburn, (1967).

Samples for analysis of VFAs were frozen immediately after being collected, using liquid nitrogen to paralyze the fermentative processes, and stored at -20 °C until preparation for analysis of VFA by GC with a HP-FFAP capillary column (Agilent Technologies, Palo Alto, CA).

Microbial protein synthesis

Microbial N synthesis (g of N/d) was estimated by using the technique of the purine derivatives (uric acid and allantoin) in urine, by the colorimetric method, according to Fujihara et al. (1987), described by Chen & Gomes (1992). Spot urine samples were obtained by vulval stimulation at 4am, 8am, 11am, 2pm, 5pm, 8pm and 12am, from the 11th to the 15th days of each period. The urine samples were poured in a collecting flask, diluted with 10% sulfuric acid solution in the ratio of 5 mL of acid per 45 mL of sample, and then frozen. Urine creatinine concentration was determined using a commercial kit (Creatinine K, Labtest, Lagoa Santa, Brazil).

Urine volume was estimated using creatinine concentration as a marker and assuming a daily creatinine output, according to Equation (Costa e Silva et al. 2012):

$$UV = (0.0345 \times SBW0.9491) \div UCc \quad (6)$$

Where, UV (L/d) is daily total urinary production, SBW (kg) is shrunk body weight, UCc (g/L) is urine creatinine concentration. Efficiency of microbial synthesis in the rumen (g microbial N/kg of digestible OM) was calculated by dividing the production of ruminal microbial N by the digestible OM intake (kg/d). Urinary N excretion (g of N/d)

was determined by its N concentration relative to urinary volume (method 920.87; AOAC, 2000).

Nitrogen balance

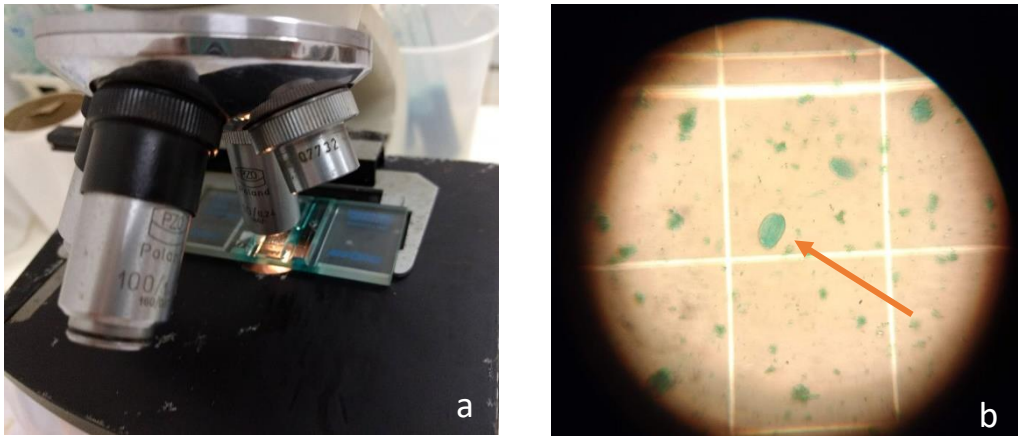
The N balance was obtained by subtracting the total excreted N in the feces and urine of the total N consumed, representing the total N that was effectively retained in the animal organism. For this purpose, the diet components, fecal and urine samples were analyzed for nitrogen content (N), according to AOAC (1990).

In order to determine the fecal N excretion, the fecal production values were multiplied by the total N content analyzed in the feces. Urinary excretion of N was calculated using urinary volume and total N content in these samples. The N consumed was obtained based on the value of forage consumed by the animals and the total N content found in them.

Protozoa

The samples used for rumen protozoal counting were collected at the end of each period, on the same days as ruminal emptying. Approximately 200 mL of ruminal fluid were collected per day in the ventral portion of the rumen. These samples were mixed with a 36% formaldehyde solution. The sample for protozoa enumeration was stained and evaluated with an optical microscope in a Neubauer chamber (Figure 3 a and b), according to Warner (1962) and Dehority (1984).

Figure 6. Protozoal counting (a) and, in detail, a protozoan (b).



Statistical analysis

A double *Cross-over* was used with three replicates (animals), evaluated in four periods and two treatments. Data were analyzed using the mixed models method (Littell et al., 2000), performed by the MIXED procedure of SAS (SAS Institute, Cary NC). The effects of treatments and periods were considered fixed and the effect of animal as random. The treatment averages were estimated using the LSMEANS statement and compared using Student's *t*-test with $P \leq 0.10$ The statistical model for data analysis was as follows:

$$Yijk = \mu + Ti + Pj + \gamma ij + Ak + \varepsilon ijk$$

Where:

Yijk = value observed in the *i*th treatment of the *j*th period of the *k*th animal

μ = overall average;

Ti = fixed effect associated with the *i*th treatment, $i = 1, 2$;

Pj = radom effect associated with the *j*th period, $j = 1, 2, 3, 4$;

γij = fixed error associated with the *i*th treatment in the *j*th period

Ak = random effect associated with the *k*th animal, $k = 1, 2, 3, 4, 5, 6$;

εijk = random error associated with the *i*th treatment, the *j*th period and the *k*th animal

4. RESULTS

4.1 Canopy characteristics

The canopy height was kept close to 25 cm in both treatments, as proposed in the methodology. In this way, canopy height did not differ between treatments (P = 0.19, Table 3), which averaged 24.11 % ± 0.339. Leave mass tend to be 26% greater at treatment FP in relation to IP (P = 0.11, Table 3). No effect were observed on forage mass, stem mass and dead material (P > 0.10, Table 3).

Table 3. Canopy characteristics of brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP)

	Treatment		SEM[1]	*P*– value
Item	FP	IP		
Canopy height, cm	24.37	23.84	0.339	0.19
Forage total mass, kg.ha-1	11,152	10,814	417.8	0.30
Leave mass, kg.ha-1	2,864	2,256	210.9	0.11
Stem mass, kg.ha-1	3,047	3,345	279.4	0.15
Dead material, kg.ha-1	5,128	4,935	288.0	0.65

Means in the same row was statistically different at P<0.10
[1]Standard error median

4.2 Ruminal metabolism

Diet

All fiber-related nutrients were greater at treatment FP compared to IP (P < 0.05, Table 4). The NDF and ADF were 10.8% and 15.3% greater at treatment FP in relation to IP, respectively (P < 0.01, Table 4). On the other hand, CP was significantly greater at treatment IP in relation to FP, with a difference of 26.2% between them (P < 0.01, Table 4). In addition, there were effects on A and C fractions of protein, in which were 10.2% and 19.3% greater, respectively, at treatment IP in relation to FP (P < 0.10, Table 4). Even though no effects on fractions B1+B2, FP tended to be 27% greater than treatment IP (P = 0.12, Table 4).

There was effect on NFC content, which was 24.8% greater at treatment IP in relation to FP (P < 0.01, Table 4). In addition, IVDMD was 13.3% greater at treatment IP in relation to FP (P < 0.01, Table 4).

Table 4. Diet of heifers grazing pastures of brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP)

Item	Treatments		SEM[1]	*P*– value
	FP	IP		
Ash, %DM	7.86	7.85	0.254	0.96
CP, %DM	11.82	14.92	0.999	<0.01
A[2], %CP	34.20	37.68	1.706	0.07
B1 + B2[2], %CP	30.31	23.84	3.492	0.12
B3[2], %CP	26.58	27.73	2.708	0.52
C[2], %CP	8.91	10.63	0.799	<0.01
NDF, %DM	64.59	58.26	0.856	<0.01
ADF, %DM	27.15	23.54	0.840	<0.01
Cellulose, %NDF	37.45	34.81	1.024	<0.01
Hemicellulose, %NDF	24.54	21.22	0.835	<0.01
Lignin, %NDF	2.61	2.31	0.398	0.05
Ether extract, %DM	1.82	1.52	0.111	<0.01
IVDMD[3], %DM	66.71	75.59	1.832	<0.01
NFC[4], %DM	12.55	15.45	0.139	<0.01
Condensed Tannin, %DM	ND	0.83	0.106	NA

Means in the same row was statistically different at P<0.10
ND: not detected
NA: not analyzed
[1]Standard error median.
[2]Protein fractionation (A, B1+B2, B3, C).
[3]IVDMD: In Vitro Dry Matter Digestibility.
[4]NFC: Nonfibrous carbohydrate (NFC = 100 - %NDF - %CP - %Fat - %Ash).

Intake and Digestibility of Nutrients

DM and OM intakes were, approximately, 11% greater at treatment FP in relation to IP (P = 0.06, Table 5). The animals consumed 2.41 $kg.d^{-1}$ ± 0.22, which was constituted about 31.4% of DM intake. N intake was 15.0% greater at treatment IP in relation to FP (P = 0.09, Table 5). In addition, Effects on Nitrogen fractionation of intake were observed, which the fraction greater 23.4%, 34.0% and 25.6% at treatment IP compare to FP (P = 0.01; P < 0.01 and P = 0.01, respectively, Figure 7).

NDF and ADF intake were 24.1% and 29.6% greater at treatment FP in relation to IP, respectively (P ≤ 0.01, Table 5). The forage intake of fibrous fractions, cellulose and hemicellulose, were 29.6% 20.5% and 16.2%, respectively, greater at treatment FP in relation to IP (P < 0.01, Table 5). Besides no effects on NFC intake, IP tended to be

11.2 % greater than treatment FP (P = 0.12, Table 5). Condensed tannin intake was about 63.4 $g.d^{-1}$ on treatment IP (Table 5).

DM, OM, N and NDF apparent ruminal digestibility as a percentage of apparent total-tract digestibility were 10.6%, 7.1%, 8.1% and 8.7% greater, respectively, at treatment FP in relation to IP ($P \leq 0.01$, Table 5). The apparent total-tract digestibility of DM, OM were greater at treatment FP in relation to IP ($P < 0.01$, Table 5). In addition, apparent total-tract digestibility of fiber-related nutrients (NDF, ADF, hemicellulose and cellulose) were greater at treatment FP compare to IP ($P < 0.01$, Table 5). However, apparent total-tract digestibility of N did not differ between treatments ($P > 0.10$, Table 5), which averaged 78.1 % ± 0.325. Moreover, there was effects between treatments on total digestible nutrients (P = 0.03, Table 5).

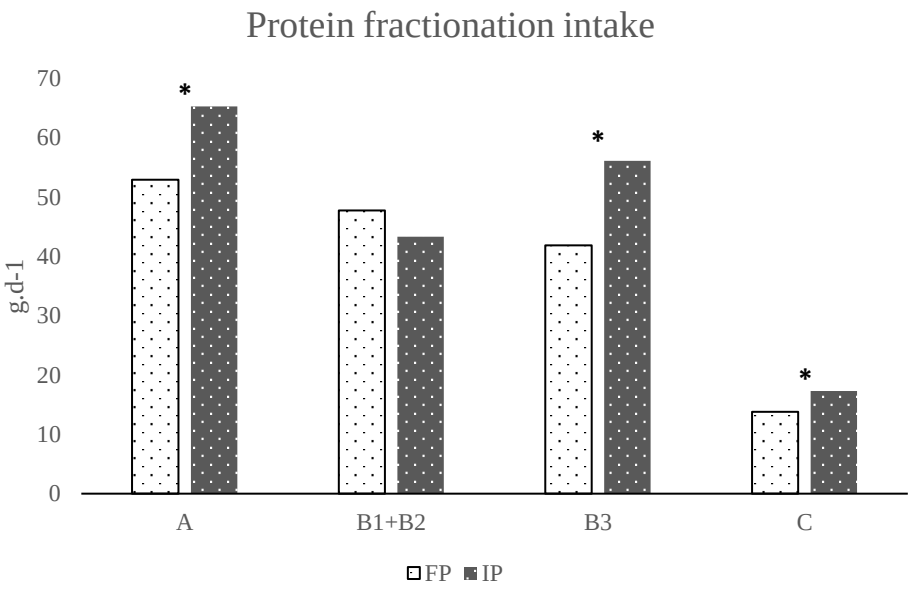


Figure 7. Nitrogen fractionation intake of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP). Means with * was statistically different ($P \leq 0.10$).

Table 5. Intake and digestibility of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

Item	Treatments		SEM[1]	*P*– value
	FP	IP		
Total Digestible Nutrients, kg.d^{-1}	6.06	5.34	0.337	0.03
DM				
Intake, kg.d^{-1}	8.44	7.55	0.343	0.06
Legume intake, kg.d^{-1}	ND	2.41	0.220	NA
Legume intake, % BW	ND	0.58	0.056	NA
Legume intake, % DMI	ND	31.4	2.560	NA
Total tract digestibility, %	75.2	71.8	0.975	<0.01
Ruminally digested, kg.d^{-1}	4.95	3.94	0.306	0.02
Apparent ruminal digestibility[1], %	77.8	70.3	1.636	0.01
OM				
Intake, kg.d^{-1}	7.77	6.96	0.359	0.06
Total tract digestibility, %	74.8	71.6	1.025	<0.01
Ruminally digested, kg.d^{-1}	4.79	3.84	0.283	0.01
Apparent ruminal digestibility[1], %	82.1	76.63	1.529	0.01
N				
Intake, kg.d^{-1}	0.158	0.182	14.27	0.09
Total tract digestibility, %	78.3	78.0	1.001	0.84
Ruminally digested, kg.d^{-1}	0.0918	0.0984	0.0132	0.50
Apparent ruminal digestibility, %	71.7	66.3	4.047	0.06
NDF				
Intake, g.d^{-1}	5.46	4.40	242.5	0.01
Total tract digestibility, %	78.6	73.6	1.327	<0.01
Ruminally digested, kg.d^{-1}	3.54	2.53	0.191	<0.01
Apparent ruminal digestibility, %	82.24	75.69	1.889	0.03
Cellulose				
Intake, g.d^{-1}	2.07	1.56	0.108	<0.01
Total tract digestibility, %	78.7	76.1	1.641	0.01
Hemicellulose				
Intake, g.d^{-1}	3.16	2.62	0.152	<0.01
Total tract digestibility, %	78.1	72.5	1.733	<0.01
Condensed tannin intake, g.d^{-1}	ND	63.4	3.531	NA

Means in the same row was statistically different at P<0.10

[1]Standard error median.

Ruminal Parameters and Microbial Supply

No effects on the treatment*time interaction were observed on all ruminal parameters data (P > 0.10, Table 6). There was effect between treatments on pH, in which IP was smaller than FP (P = 0.04, Table 6). The N-NH_3 on ruminal fluid was 28% greater at treatment FP in relation to IP (P < 0.01, Table 6).

Concerning the VFA (M*m*), there was effect between treatments on total VFA, in which IP was greater than FP (P = 0.03, Table 6). Similarly, acetate and valerate (M*m*) was greater at treatment IP compare to FP (P ≤ 0.02, Table 6). The A: P ratio was greater

at treatment IP in relation to FP (P = 0.02, Table 6). In addition, A: P ratio was smaller at time-point 18:00 compare to the others (P < 0.01, Figure 8). The molar ratio of acetate was greater at treatment IP compare to FP (P = 0.02, Table 6). Conversely, molar ratios of propionate and isobutyrate were smaller at treatment IP compare to FP (P ≤ 0.06, Table 6).

The molar ratio of butyrate, valerate and isovalerate did not differ between treatments (P ≥ 0.10, Table 6). Regarding to the difference among the time points (00:00; 06:00; 12:00 and 18:00), total VFA, acetate and propionate (M*m*) were smaller at time 12:00 (P ≤ 0.01, Figure 8).

Data of ruminal microbial N supply are presented in Figures 9, 10 and 11. The microbial CP was 22.9% greater at treatment IP in relation to FP (P = 0.06, Figure 9). Similarly, the efficiency of microbial protein supply (g $CP.kg^{-1}$/ApDMO) and CP intake: digestible OM ratio were 47.8% and 32.5% greater at treatment IP in relation to FP (P = 0.01, Figure 10 and 11).

Table 6. Ruminal parameters of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

	Treatment			P-value		
Item	FP	IP	SEM[1]	Treat	Time	Treat*Time
pH	6.61	6.54	0.025	0.04	<0.01	0.63
N-NH_3, (mg/dL)	0.959	0.749	0.106	<0.01	<0.01	0.86
VFA (M*m*)						
Acetate (A)	49.4	52.9	1.854	0.01	<0.01	0.99
Propionate (P)	17.5	18.0	0.907	0.40	0.01	0.58
Butyrate	10.4	10.9	0.441	0.22	0.43	0.93
Valerate	0.869	0.944	0.0477	0.02	0.07	0.98
Isobutyrate	1.05	1.02	0.0576	0.23	0.43	0.93
Isovalerate	1.40	1.45	0.0873	0.26	0.69	0.99
Total VFA	80.61	85.26	2.983	0.04	0.01	0.98
A:P	2.84	2.97	0.103	0.02	<0.01	0.16
VFA, mol/100 mol						
Acetate	61.2	62.0	0.406	0.02	<0.01	0.22
Propionate	21.7	21.2	0.681	0.06	<0.01	0.17
Butyrate	12.9	12.8	0.312	0.62	0.09	0.52
Valerate	1.08	1.10	0.0325	0.22	<0.01	0.97
Isobutyrate	1.31	1.19	0.0607	<0.01	<0.01	0.83
Isovalerate	1.75	1.70	0.0841	0.17	<0.01	0.93

Means in the same row was statistically different at P<0.10.
[1]Standard error median.

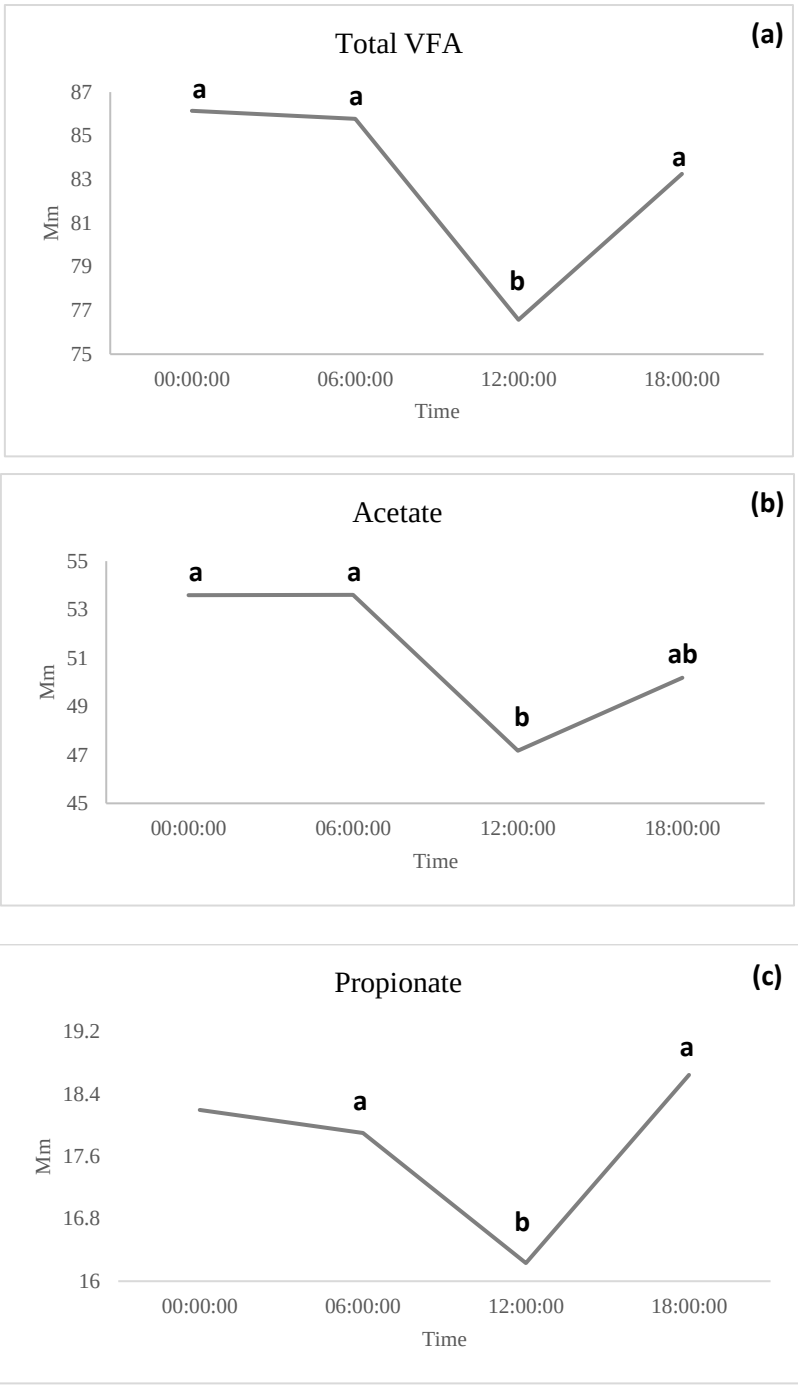


Figure 8. Total VFA (a), acetate (b) and propionate (c) in four times-points. Letters in the same figure with different letters means significant difference at P<0.10.

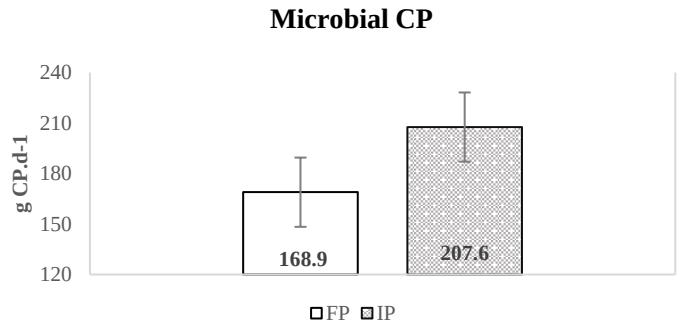


Figure 9. Microbial CP production (g $CP.d^{-1}$) of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP). SEM: 20.57. P = 0.06.

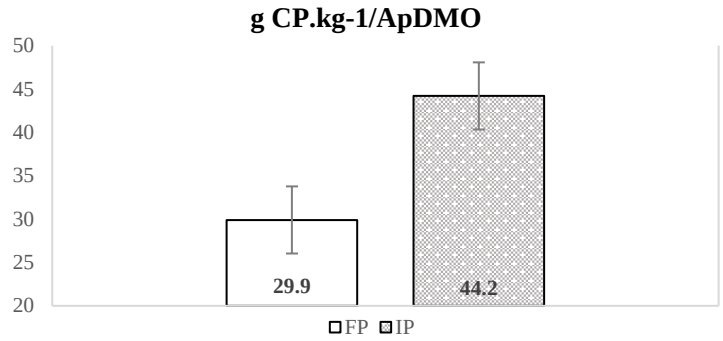


Figure 10. Efficiency of microbial protein supply (g $CP.kg^{-1}$/ApDMO) of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP). SEM: 3.869. P = 0.01.

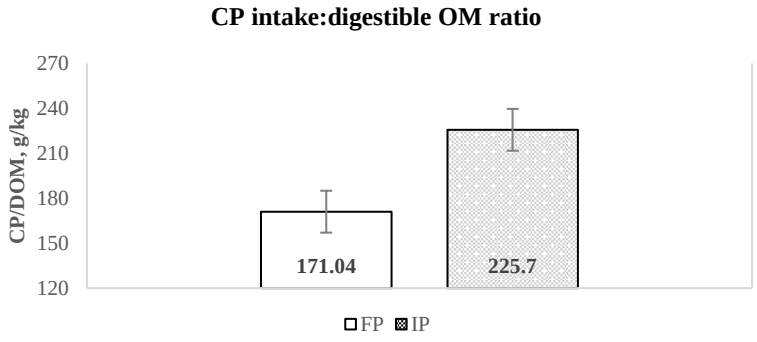


Figure 11. CP intake:digestible OM ratio of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP). SEM: 3.869. P = 0.01.

Rumen kinetics

No effects of treatments were detected on DM and N ruminal turnover rate, which averaged 17.7 %.h^{-1} ± 1.02 and 15.8 %.h^{-1} ± 1.85, respectively (P > 0.10, Table 7). Nevertheless, NDF ruminal turnover rate was 25.6% greater at treatment FP in relation to IP (P = 0.02, Table 7). The DM and NDF ruminal passage rates did not differ between treatments (P > 0.10, Table 7). However, N ruminal passage rate was 12.3% greater at treatment IP in relation to FP (P > 0.10, Table 7). The DM and NDF ruminal digestion rates were 21.3% and 39.2% greater at treatment FP in relation to IP, respectively (P = 0.07 and P = 0.01, respectively, Table 7). No effects between treatments were observed on the CP ruminal digestion rate (P = 46, Table 7).

Table 7. Rumen kinetics of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

Item	Treatment		SEM	P-value
	FP	IP		
Ruminal turnover rate (ki), %.h^{-1}				
DM	18.4	16.9	1.02	0.22
NDF	19.8	15.7	1.19	0.02
N	15.0	16.6	18.5	0.25
Ruminal passage rate (kp), %.h^{-1}				
DM	7.5	8.1	0.480	0.31
NDF	6.9	6.9	0.467	0.97
N	6.5	7.3	0.387	0.09
Ruminal digestion rate (kd), %.h^{-1}				
DM	10.8	8.9	0.649	0.04
NDF	12.7	9.1	0.788	0.01
N	8.42	9.24	1.482	0.46

Means in the same row was statistically different at P<0.10.
[1]Standard error median.

Post-ruminal metabolism

Intestinal Digestibility

N Apparent intestinal digestibility as a percentage of apparent total-tract digestibility and N Intestinally digested were 23.3% and 19.1% greater at treatment IP compare to FP (P = 0.06 and P < 0.01, Table 8). Likewise, there was effect between treatments on NDF Apparent intestinal digestibility, which was greater 36.5% on treatment IP than FP (P = 0.03, Table 8). The DM and OM were 33.8% and 41.9% greater in IP in relation to FP (P = 0.01, Table 8). No effects between treatments were observed on DM, OM and NDF Intestinally digested (P > 0.10, Table 8).

Table 8. Intestinal digestibility of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

Item	Treatments		SEM[1]	*P*– value
	FP	IP		
DM				
Total tract digestibility, %	75.2	71.8	0.975	<0.01
Intestinally digested, kg.d^{-1}	1.39	1.50	0.086	0.37
Apparent intestinal digestibility[1], %	22.2	29.7	1.636	0.01
OM				
Total tract digestibility, %	74.8	71.6	1.025	<0.01
Intestinally digested, kg.d^{-1}	1.03	1.17	0.083	0.24
Apparent intestinal digestibility[1], %	17.9	25.4	1.569	0.01
N				
Total tract digestibility, %	78.3	78.0	1.001	0.84
Intestinally digested, kg.d^{-1}	35.6	43.9	2.313	<0.01
Apparent intestinal digestibility[1], %	28.3	33.7	4.047	0.06
NDF				
Total tract digestibility, %	78.6	73.6	1.327	<0.01
Intestinally digested, kg.d^{-1}	756.9	715.7	85.71	0.62
Apparent intestinal digestibility[1], %	17.8	24.3	1.889	0.03

Means in the same row was differ significantly at P<0.10.
[1]Standard error median.

Digesta Flows

Effects on N and non-ammonia nitrogen (NAN) flows only were observed between treatments, which were greater 14.1% and 15.7% on treatment IP in relation to FP ($P < 0.01$ and $P = 0.02$, Table 9). The DM, OM and NDF flows did not differ between treatments ($P > 0.10$, Table 9).

Table 9. Digesta flows of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

	Treatments		SEM[1]	*P*– value
Item	FP	IP		
DM flow, kg.d^{-1}	3.48	3.61	0.967	0.36
OM flow, kg.d^{-1}	2.84	3.13	0.938	0.28
N flow, g.d^{-1}	69.4	79.2	3.32	<0.01
NAN flow, g.d^{-1}	66.2	76.6	3.32	0.02
NDF flow, g.d^{-1}	1920	1865	87.4	0.55

Means in the same row was differ significantly at P<0.10.
[1]Standard error median. NAN = non-ammonia nitrogen.

Nitrogen Balance

Nitrogen intake and fecal nitrogen output were 15.0% and 15.9% greater at treatment IP in relation to FP ($P = 0.09$ and $P = 0.03$, respectively, Table 10). However, urine nitrogen output did not differ between treatments ($P = 0.80$, Table 10). The nitrogen balance was 49.6% greater at treatment IP in relation to FP ($P = 0.10$, Table 10).

Table 10. Nitrogen balance of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP).

	Treatments		SEM[1]	*P*– value
Item	FP	IP		
Intake, g N.d^{-1}	158.3	182.1	14.28	0.09
Urine, g N.d^{-1}	90.5	91.9	5.18	0.80
Feces, g N.d^{-1}	33.9	39.3	1.904	0.03
Nitrogen balance, g N.d^{-1}	33.5	50.1	8.90	0.10

Means in the same row was differ significantly at P<0.10.
[1]Standard error median.

Protozoa

The ruminal protozoal population was demonstrated on Figure 12. The protozoa population on FP treatment was greater in relation to IP, which averaged 7.05 (x104/ml) and 3.40 (x104/ml), respectively (P = 0.01).

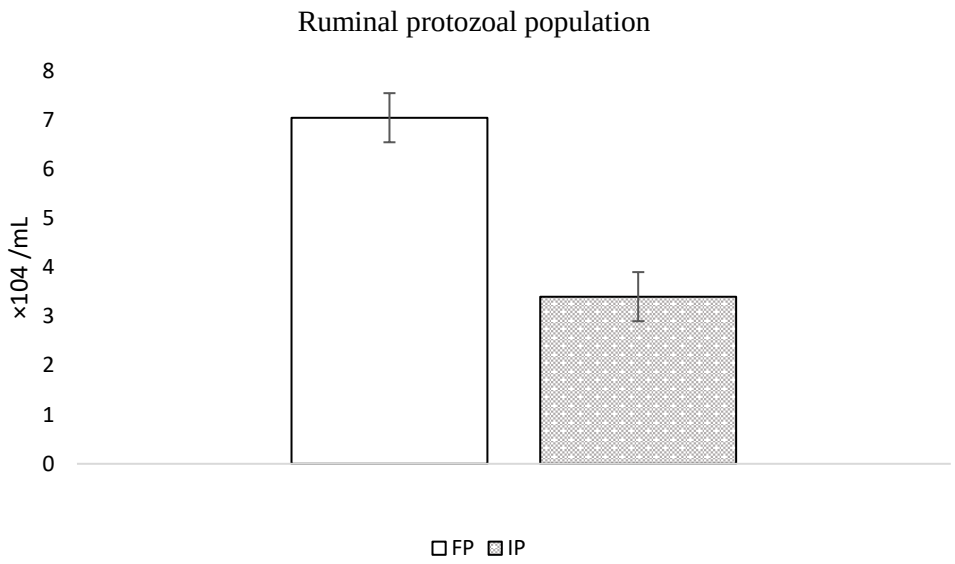


Figure 12. Ruminal protozoal population of heifers grazing brachiaria-grass fertilized with mineral nitrogen (FP) or intercropped with forage peanut (IP). SEM: 3.869. P = 0.01.

5. DISCUSSION

5.1 Ruminal metabolism

Forage Intake, Digestibility and Ruminal Kinects

The animal yields products from grazing system depends on a number of associated factors. One of the most important of these components is the forage intake by grazing livestock, which is responsible to the nutrients input, determining animal performance. Several factors may affect the voluntary intake as related to animal, environment, forage crop and pasture management.

The canopy structure that we have provided to animals is fundamental, because it determines how easily animals eats the forage. Such morphological characteristics as canopy height and density is directly related to the forage intake on tropical forage grasses (HODGSON, 1990). By the way, in our study we kept same canopy height on both pastures (P=0.30, Table 3). In addition, the forage mass did not differ between treatments (P = 0.19, Table3). Therefore, canopy structure did not affected forage intake.

Voluntary forage intake also is directly related to forage digestibility, which is dependent of cell wall and its availability to digestion that is determined by some factors. Digestibility is related to fill gut capacity and, intake may to be limited by the maximal volume that the digestive tract can extend (Allison, 1985; Allen, 1996).

It is believed that feed low in digestibility impose restrictions on intake due to their slow release of rumen and passage through the digestive tract. The reticulorumen and abomasum has stretch and touch receptors in its walls, that negatively impact the intake as the weight and the volume of digesta accumulates (Allen, 1996). Owing to low digestion rates, neutral detergent fiber (NDF) it is considered the primary diet constituent associated with the filling effect. The dry matter and organic matter apparent digestibility in our study were greater on FP ($P \leq 0.01$, Table 5). By the same way, was observed increase on apparent digestibility of all fiber-fractions at treatment FP compare to IP ($P \leq 0.01$, Table 5). This fact may explain the greater dry matter intake on animals consumed FP (Table 5).

Another plausible explanation for increase on DMI in FP treatments is the interaction between forage and rates of passage and digestion. This represent a route of flow from the digesting organ. There faster is the digesta clearance; forage intake is greater. While the passage rate is considerably important accounting for the animal intake, also the digestion rate has been directly related (Gill et al., 1969). Even though the passage rate was statistically equal between

treatments, digestion rate was greater in FP (Table 7). Again, this may justify greater dry matter intake in FP.

Several factors may affect the forage digestibility, especially upon the NDF. The maturity is the main factor that decreased the digestibility. When cell and stem diameters increase, increases the thickness and lignification of their cells, and, NDF digestibility decreases rapidly. The maturity mechanism that reduce NDF digestibility are similar in grasses and legumes. However, in our study digestibility was not affected by this mechanism. Other factors is not less important the plant growth environment, ruminal pH and compounds that have any mechanism preventing fiber fermentation as a lipids, additives or compounds of secondary metabolism. In our study, the latter factor may affected digestibility (Table 8), especially of fiber, considering that others factors were similar between treatments or were not important in our case.

The digestibility of nutrients (proteins, carbohydrates, and fats), may be depressed by the condensed tannin (CT) presence (Mueller-Harvey, 2006; Waghorn, 2008). However, the nutritional effect of CT in the ruminant diet depends on its concentration, molecular weight and type of CT present in forage plants, and these factors influence intake and animal performance (Vastra et al., 2009). However, several studies suggest that amounts of CT above 50 $g.d^{-1}$ DM reduce the intake significantly (Aerts et al., 1999; Barry and Manley, 1984; Waghorn, 1994; Barry and Duncan, 1984). Modest amounts, ranging from 20 to 50 $g.kg^{-1}$ DM, have less effect on DMI (Aguierre et al., 2016).

Condensed tannin intake of heifers feeding on IP treatment was about 63.4 $g.d^{-1}$ DM (Figure 12), which is considered sufficient to handle ruminal conditions. In this way, the ruminal digestibility was negatively affected by the intake of forage peanut, which contains condensed tannin. CT intake can decrease carbohydrate fermentation because of its binding with cellulose, hemicellulose and pectin (MCSWEENEY et al., 2001). Thus, decreases in fiber digestibility are likely to occur when animals feed on legumes containing CT (Scharenberg et al., 2007) or are supplemented with CT extracts (Carulla et al., 2005).

The nutritive value of IP treatment was better than FP, with higher level of CP and lower levels of NDF and ADF (Table 4). The quantity of cellulose, hemicellulose and lignin was greater in treatment FP. In addition, the IVDMD of IP diet was 75.8%, while FP treatment averaged 66.7%. According to the fiber contents and IVDMD, higher digestibility was expected in legume grass, but this did not occur. In our experiment, NDF and ADF apparent total-tract digestibility was greater without the inclusion of forage peanut (Table 4). In addition, DM, OM, NDF and apparent ruminal digestibility was smaller in IP treatment, which would be attributed

to the reactivity of tannins with fibers compounds, inhibiting the activities of fibrolytic enzymes (Makkar et al., 1989; Mcsweeney et al., 2001).

It has been reported that positive effects of ruminal kinects are associated with higher digestibility of rumen, such as faster digestion (Andrighetto et al., 1993) and higher passage rate (Moseley and Jones, 1979). Although no effect on DM, NDF passage rates were observed between the treatments, DM and NDF ruminal digestion rates were greater at treatment FP (Table 7), probably due to the effects caused by tannin, as previously mentioned.

It is a fact that the general idea of the effect of tannins on rumen degradation is more evident for CP than for other components, a feature attributed to the strong affinity of the tannin molecule with carbonyl oxygen of the peptide group (MCLeod, 1974). Nevertheless, several studies have demonstrated the reduction of ruminal fiber digestion of animals consuming feeds containing condensed tannin (MCSweeney et al., 2001). Tannins may reduce fiber digestion by complexation with lignocellulose, preventing microbial degradation, or by directly inhibiting cellulolytic microorganisms, or both acting together (MCSweeney et al., 2001).

Nitrogen compounds

Several benefits are assigning to legume upon ruminant nutrition. One of them is the higher protein content of legume in relation to grasses. We witnessed higher N intake in IP, more specifically N coming from the fraction A, which is rapidly degradable and represented of total nitrogen. The highest CP intake in IP treatment was due to the higher nitrogen content of the legume, with a 23.43% CP (Table 2). Between the grasses, the difference on CP contents was small, about 7.8%.

Even though the higher N intake on IP than FP treatment, the nitrogen apparent digestibility was similar between them. In addition, even the highest N intake, ruminal N digestibility was smaller in heifers feeding on IP treatment. This effect on nitrogen digestibility may be explained by the CT mechanism on the proteins. A large number of studies have reported CT-protein complexation and precipitation on ruminal pH (Jones and Mangan, 1977; Hagerman, 2012; Zeller et. al., 2015). The use of CT is one efficient way to reduce ruminal protein degradation by binding with protein under ruminal pH conditions (Hagerman and Klucher, 1986). Therefore, this can act against an excess of ruminal digestible protein, as the microorganisms are not able to degrade CT-protein complexes (MCSweeney et al., 2001). This event is due to the inhibitory effects of tannins on proteolytic bacteria and the activity of their enzymes (MCSweeney et al., 2001).

Within this context, it is a fact that lower ruminal ammonia has been observed when feeding CT (Scharenberg et al., 2007; Arrigo and Dohme, 2009; Azuhnwi et al., 2013; Ineichen et. al., 2019). In the present study, we observed that IP treatment fulfilled this purpose, evident from the changes determined in ruminal ammonia (Table 6). Despite the higher N intake, N-NH_3 in ruminal fluid was lower at IP treatment ($P < 0.01$). This demonstrates that CT decreased rumen proteolysis. A decline in ruminal ammonia content in IP treatment was also associated with efficient N use by microorganisms, especially because the N consumed was higher. A small reduction on the ruminal protein degradation rate may increase the microbial protein synthesis efficiency, due to the release of nutrient synchronization (Makkar, 2003), as well as the inhibition of microbial species responsible for protein degradation (Jones et al., 1994; Min et al., 2002; Vasta et al., 2010).

Another reason to increase of microbial protein production on IP treatment is upon NFC contents. The legume utilized in this study has a greater amount of NFC, which provides more energy for ruminal microorganisms (Niwińska, 2012). NFC is considered a source of readily available energy for microbial growth (Ariza et al., 2001), which was demonstrated by the higher production of microbial CP in heifers feeding on IP (Table 6). In order to optimize microbial production, ruminal digestible protein may be balanced with available energy (Brooks et al., 2012). Microbial protein is the most important source of metabolized protein for ruminants; moreover, it is an economical and very efficient way to provide high quality protein (Van Soest, 1994). Therefore, increase on ruminal degradable protein is compensated in case of microbial protein production is not affected, and, our study achieved this goal.

Ruminal parameters

Microbial fermentation of carbohydrates in rumen is determined by formation of volatile fatty acids (VFA) and gas (CH_4, CO_2, and H_2). Although numerous intermediates may be formed, the final fermentation products that accumulate within the rumen are acetate, propionate, butyrate, carbon dioxide, and methane. In addition, proteins also are degraded in rumen, and ammonia, carbon dioxide, and either short straight, branched-chain, or aromatic fatty acids are formed. The VFA's are utilized as the major energy source by ruminants; therefore, the VFA production is important for evaluating the energy supply for ruminants. The amount of VFA produced is depended upon feed intake and chemical composition, particularly the nature and degradation rate of carbohydrates. In our study, the carbohydrate comes only from forage. Proteins also are degraded in the rumen, and ammonia, carbon dioxide, and either short straight, branched-chain, or aromatic fatty acids are formed.

A smaller acetate production was expected on animals that consumed IP, as a consequence of lower NDF ruminal degradation (Table 5), since this process produced more acetate than propionate. Yet, ruminal VFA in the present study was different from the expected pattern (Table 6). Other plants compounds may affect ruminal fermentation, as nonfibrous carbohydrates (NFC): simple sugar, sucrose, starch, organic acids, fructans and pectin. Those carbohydrates are highly digestible, increasing the digestible organic matter. During the pectin breakdown, 84 to 95% of the VFA were produced in form of acetate (Marounek et al., 1985). The NFC intake on IP treatment trend to be greater about 11.2% than FP (Table 5), owing to the legume presence. In present study, forage peanut had NFC contents of 25.5%, which was almost double that we found on Brachiaria of FP and IP treatment (Table 2). Moreover, the pectin content of legumes is much higher than grasses, whose level is low that it is often ignored in analysis (Van Soest, 1994). Grasses contain from 3% to 4% of pectin in the dry matter, legumes plants from 5 to 12% (Niwińska, 2012). In addition, it was reported that the main pectin fermentation product is acetate (Marounek, et. al., 1985). That was evident from the changes in acetate molar proportion in ruminal fluid of heifers feeding on IP (Table6).

In addition to changes on VFA patterns, was observed that total VFA (mM) was greater at IP treatment (Table 6). As noted above, the legume utilized in this study has a greater amount of NFC, which provides more energy for ruminal microorganisms (Niwińska, 2012). This would explain the higher total VFA and molar proportion of acetate (Russel et. al., 1992), whereas NDF digestibility was smaller on IP treatment. NFC is considered a source of readily available energy for microbial growth (Ariza et al., 2001), which was demonstrated by the higher production of microbial CP in heifers feeding on IP (Table 6).

Whereas, the legume utilized in this study has a greater amount of NFC, which provides more energy for ruminal microorganisms (Niwińska, 2012). This would explain the higher total VFA and molar proportion of acetate (Russel et. al., 1992), whereas NDF digestibility was smaller on IP treatment. NFC is considered a source of readily available energy for microbial growth (Ariza et al., 2001), which was demonstrated by the higher production of microbial CP in heifers feeding on IP (Table 6). In order to optimize microbial production, RDP may be balanced with the energy available (Brooks et al., 2012). Microbial protein is the most important source of metabolized protein for ruminants; moreover, it is an economical and very efficient way to provide high quality protein (Van Soest, 1994).

5.1 Post-ruminal metabolism

Hindgut fiber fermentation

The lower part of ruminant gastrointestinal tract receive much less attention that the reticulorumen. It is obvious that the rumen has an efficient fermentation system. However, it is evident that considerable matter disappears in the lower tract (Vidal et al., 1969; Sherry et al., 1985). Mineral, nitrogen, water and VFA is absorbed (Van Soest, 1994). Occur an escape of potentially fermentable carbohydrate from the rumen, including structural carbohydrates, which may be fermentable on hindgut. Post ruminal digestion of cellulose was studied in sheep, steers, and goats, and estimates ranging from 5 to 30% cellulose digestion in large intestines have been reported (Sherry, 1985). In this study, DM and NDF intestinal apparent digestibility averaged 26,0% and 21.0%, respectively (P = 0.01 and P = 0.03, respectively, Table 8).

Moreover, cellulose and hemicellulose escape are not similar; the last tend to be greater, leading to more hemicellulolytic digestion in the lower tract (Beever et al., 1972). This fact give rise to the question of hemicellulose alteration during peptic digestion. That greater fermentation may be occurred because of hemicellulose is sensitive to acid. If gastric acid cleave binds that allow hemicellulose to be unavailable on ruminal conditions, these fractions would be become available to lower tract fermentation. In this connection, hemicellulose apparent digestibility was greater than cellulose, especially in treatment IP (Table 8). The apparent digestibility of cellulose was almost 10% smaller than hemicellulose in IP treatment (P = 0.01, Table 5).

The ruminal digestibility was negatively affected by the intake of forage peanut, which contains condensed tannin. CT intake can decrease carbohydrate fermentation because of its binding with cellulose, hemicellulose and pectin (MCSWEENEY et al., 2001). The DM, OM and NDF apparent intestinal digestibility were greater at IP treatment, which suggested a shift of fiber fermentation to the hindgut.

Nitrogen

The nitrogen digestibility in rumen was smaller at IP treatment. Because of increase on ruminal proteolysis, N-NH_3 also was smaller at treatment PC. Although the similarity in

apparent total nitrogen digestibility between treatments, we observed a shift in the pattern of ruminal and intestinal nitrogen digestibility. If the ruminal N digestibility was smaller in heifers feeding on IP treatment, the opposite occurred with intestinal digestibility (Table 5). In other words, the ruminal undigestible N in rumen was absorbed on post rumen, since the total digestibility was similar.

It has been suggested that CT can increase the nitrogen flow to the intestine (MCSweeney et al, 2001), as observed in intestinal N digestibility data, which consequently allowed a greater amount of CP to reach the small intestine. Avoiding ruminal protein degradation to increase N flow to the intestine is advantageous, when the microbial protein production efficiency is not affected, as in the present study.

Even with the N intake increase in heifers feeding on IP, the total N excreted through urine did not differ between treatments (Table 10). In contrast, the N excreted through feces was 15.9% greater at IP treatment, when compared to FP. Yet, the pattern of N fractionation excreted by feces is worth mentioning. Fraction B1+B2 of total N excreted was 10.6 $g.d^{-1}$ at FP treatment, which was greater than IP (5.58 $g.d^{-1}$). Nevertheless, fraction C excreted in feces was greater at IP treatment, representing 73% of total N released through feces, when compared to FP (Table 10). This can be considered a beneficial effect concerning N losses to the environment, as the degradation is quite slower than urine (LESSA et. al., 2014). This is of great relevance to an environmentally friendly animal husbandry, as less gaseous (nitrous oxide and ammonia emissions) and liquid (nitrate leaching) N is emitted (Dijkstra et al., 2007).

As mentioned before, the protein/condensed tannin binding is released below pH 4 (Mangan, 1988), as is the case in the abomasum, so that the protein protected from ruminal degradation can ideally be digested in the intestine (Waghorn, 2008), thereby contributing to the metabolic protein supply. Despite the fact that complexation of protein with CT seldom results in a better N utilization, probably due to an incomplete separation of the CT-protein complexes in the intestine (Beauchemin et al., 2007, Brinkhaus et al., 2016), CP intestinal digestibility was greater in IP, as previously stated (Table 5). The higher excretion of fecal N, especially fraction C, of animals feeding on IP may be the result of tannin-protein complexes that possibly were not completely dissociated in the intestine.

Nitrogen retention was higher in heifers feeding on IP treatment ($P = 0.10$, Table 10). This fact contradicts the results in the majority of studies on condensed tannin in ruminant diet, which observed no effect (Komolong et al., 2001; Anhert et. al., 2015; Ebert et. al., 2017) or a

negative one (Al-Dobaib, 2009) of CT on N retention. The higher N retention with, rather than without, CT in the present study may be explained by the following facts:

- There was greater CP intake in IP treatment;
- The degradation of ruminal nitrogen was smaller, increasing N-NH_3 in ruminal fluid, which prevented losses through urine excretion;
- There was no difference in N urine excretion;
- There was ruminal N optimization across the efficiency of microbial protein production, which was greater on IP treatment;
- There was larger use of CP in post-rumen, since the intestinal N digestibility was greater in IP treatment.

Protozoa

It is a fact that protozoa are the most conspicuous organisms in the ruminal biomass. They make up a large proportion of the rumen biomass, about 20-40% of net microbial nitrogen (Van Soest, 1994). However, their role in ruminal fermentation, contribution to ruminant metabolism and nutrition are still controversial (Newbold et. al., 2015). Anti-protozoa activities of condensed tannin are dependent on type, source and level of tannin consumed (Patra and Saxena, 2011). In the present study, the average intake of condensed tannin was about 62.4 $g.d^{-1}$. Nevertheless, the protozoa populations in ruminal samples decreased 19% in IP (Figure 12).

The decrease in protozoa population may be desirable, since the output of protozoa from the rumen is minimal, because of the slow turnovers (Van Soest, 1994). Furthermore, the protozoa predation on ruminal bacteria is responsible for an apparent inefficiency over the microbial protein production. Indeed, the efficiency of microbial protein supply (g $N.kg^{-1}$/ApDOM) was 20.8% greater at treatment IP in relation to FP.

6. CONCLUSION

The forage peanuts used in our study, which contain condensed tannin, altered the ruminal environment. Ruminal fiber degradation was lower and there was a decrease in apparent dry matter digestibility. The protein had lower ruminal degradation, as a result of binding with condensed tannin, generating an increase in the N flow to the post-rumen. There was higher production of microbial protein with the use of legumes. As a result of these facts, N balance was higher in the animals that fed on forage peanut, indicating that this plant provided more retained nitrogen. Lastly, CT was able to manipulate the ruminal environment in a way that decreased the protozoa population.

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