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Claudio A. Pozo

AVALIAÇÃO DA INCLUSÃO DE TANINOS NA DIETA OU DO TURNO DE PASTEJO COMO ESTRATÉGIAS PARA MELHORAR O USO DO NITROGÊNIO ALIMENTAR EM VACAS LEITEIRAS

Santa Maria, RS
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Tese apresentada ao Curso de Pós-Graduação em Zootecnia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Zootecnia.**

Orientador: Prof. Dr. Gilberto Vilmar Kozloski

Co-orientador: Prof. Dr. José Luis Repetto

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Aprovado em 23 de fevereiro de 2018:

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RESUMO

AVALIAÇÃO DA INCLUSÃO DE TANINOS NA DIETA OU DO TURNO DE PASTEJO COMO ESTRATÉGIAS PARA MELHORAR O USO DO NITROGÊNIO ALIMENTAR EM VACAS LEITEIRAS

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O objetivo deste estudo foi avaliar o efeito da inclusão de extrato tanífero de *Acacia mearnsii* (TA) na dieta ou do manejo do horário de pastejo na resposta ingestiva, digestiva e produtiva de vacas leiteiras pastejando azevém combinadas com ração totalmente misturada (RTM). Foram utilizadas nove vacas de raça holandesa em lactação, bloqueadas em 3 quadrados latinos 3 x 3, com três períodos experimentais de 22 d. Os três tratamentos consistiram em: pastejo de manhã e RTM de tarde sem TA (AM), pastejo de manhã e RTM da tarde adicionado com 15 g TA/kg de matéria seca (MS) (AMt) e RTM de manhã sem TA e pastejo de tarde (PM). Os tratamentos não afetaram o consumo de MS, mas PM aumentou a porcentagem de pastagem na dieta. A proporção de vacas comendo, ruminando ou fazendo outras atividades através de 16 h de observaçao não foi afetada pelos tratamentos, porém AM aumentou a taxa de ingestão de MS (TCMS) de RTM comparado com PM. Além disso, AM e PM aumentaram a proporção de tempo consumindo RTM 2 h após início da alimentação. Ao pastejo, a TCMS da pastagem foi semelhante para todos os tratamentos. As vacas em AM e AMt apresentaram um padrão de comportamento semelhante e mostraram dois picos de pastejo, enquanto a proporção de vacas que pastejam em PM foi mais estável do que AM e AMt. Os tratamentos não afetaram a produção de leite nem a produção de constituintes do leite. A inclusão de TA na dieta tende a reduzir a porcentagem de proteína e caseína no leite, porém não teve impacto na produção de proteína e caseína do leite. Embora, AMt produz algumas mudanças no perfil de ácidos graxos (AG) do leite, a proporção de AG mais relevantes do leite permanece inalterado. Da mesma forma, AM e PM apresentam um perfil de AG leite similar. Os tratamentos não afetaram os parâmetros de fermentação ruminal. Da mesma forma, o fluxo de proteína microbiana para o duodeno, nem a maioria das variáveis de digestibilidade foram afetadas pelos tratamentos. A ingestão das diferentes frações de N e a excreção de N no leite e nas fezes foram semelhantes entre os tratamentos. No entanto, AMt e PM reduziram as quantidades de N e N ureico excretada na urina. Embora a soma da excreção de N urinário e fecal não foi afetada pelos tratamentos, a relação entre o N urinário e N fecal foi menor em AMt e PM em comparação com AM. Conclui-se que a inclusão de uma baixa quantidade de TA na TMR e o pastejo de tarde foram estratégias eficazes para reduzir a excreção de N urinário sem impactar negativamente a digestão de nutrientes nem o desempenho produtivo de vacas leiteiras em lactação.

ABSTRACT

EVALUATION OF INCLUDING TANINS IN THE DIET OR MANAGING THE GRAZING SCHEDULE TO IMPROVE THE DIETARY NITROGEN USE IN DAIRY COWS

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The aim of this study was evaluate if including *Acacia mearnsii* tannin extract (TA) in the diet or managing the grazing schedule impacts on ingestive, digestive and productive response of dairy cows grazing ryegrass combined with total mixed ration (TMR). Nine lactating Holstein cows were arranged on a triplicate 3 x 3 Latin square design, conducted through three experimental periods of 22 d. The three treatments consisted of: morning grazing and afternoon TMR without TA (AM), morning grazing and afternoon TMR added with 15 g TA/kg of dry matter (DM) (AMt), and morning TMR without TA and afternoon grazing (PM). Treatments did not affect the DMI, but PM increased the percentage of pasture in the diet, and induced little changes on nutrients intake. The proportion of cows eating, ruminating or doing other activities through 16 h-observations was not affected by the treatments, however AM achieved high dry matter intake rate (DMIR) of TMR than PM. Also, AMt and PM increased the proportion of time spent eating TMR in 2 h relative to start of feeding. At grazing, the DMIR of pasture was similar for all treatments. In addition, cows in AM and AMt displayed a similar behavior pattern and showed two peaks of grazing, while the proportion of cows grazing in PM seems to be more stable than AM and AMt. Treatments did not affect the milk production or milk constituents yield. Dietary tannin tended to reduce the percentage of protein and casein in the milk, however did not impact on milk protein and casein production. Although, AMt induced little changes on milk FA, the most relevant FA in milk remain unchanged. In the same way, AM and PM present a similar profile of FA in the milk. Treatments did not affect the rumen fermentation parameters. No differences among treatments were detected in microbial protein flow to the duodenum, nor most of digestibility variables. The intake of N fractions and the excretion of N in milk and feces were similar between treatments. However, AMt and PM lowered the amount of N and N-urea excreted in urine. Although the excretion of total manure N was not affected by treatments, the urinary N to fecal N ratio were lower in both AMt and PM in comparison with AM. It is concluded that the inclusion of low amounts of TA in the TMR or grazing in the afternoon were both effective strategies to reduce the excretion of urinary N without impacting negatively on nutrient digestion nor performance of lactating dairy cows.

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INTRODUÇÃO

Um dos objetivos da nutrição dos ruminantes é fornecer a quantidade de nutrientes que o animal precisa para alcançar um certo nível de produção (KEIM & ANRIQUE, 2011). Qualquer deficiência ou desequilibrio de nutrientes na dieta resultará em menor produção, enquanto que um excesso de nutrientes implica perdas que podem afetar os custos de produção, aos animais e ao meio ambiente (HRISTOV & JOUANY, 2005; PACHECO et al., 2008).

Nos sistemas leiteiros a pasto o fornecimento de nitrogênio (N) é geralmente desbalanceado, e consequentemente a eficiência do uso do N alimentar para a produção de leite (EUN) é usualmente inferior a 30% (POWELL et al., 2010), o que representa perdas significativas de N através das excretas. O N excretado em urina e fezes do gado leiteiro contribui para a poluição ambiental, como amônia (NH_3) e óxido nitroso (NO_2) no ar, ou como nitrato no solo e nas águas subterrâneas (TAMMINGA, 1992). Além disso, dentro do N excretado, o N urinário é muito mais volátil e suceptivel a perdas em forma de gás do que o N fecal (DIJKSTRA et al., 2013b).

Devido aos efeitos negativos da baixa EUN, o setor leiteiro está sob uma crescente pressão para ajustar a alimentação no gado, a fim de reduzir as perdas de nutrientes no meio ambiente (WATTIAUX & KARG, 2004). Em consequência, nos últimos anos tem havido crescente interesse em estudar diferentes estratégias nutricionais e/ou de manejo que permitam melhorar a EUN e reduzir as excreçoes de N na pecuária leiteira.

Ajustar o teor de proteína às necessidades dos animais permite melhorar a EUN (HOEKSTRA et al. 2007), contudo, em sistemas leiteiros a pasto o ajuste do teor de proteína da forragem torna-se difícil, pois implica, com aumento da maturidade, consequentes perdas na qualidade da pastagem. Neste sentido, a incorporação de ração totalmente mixturada (RTM) em sistemas pastoris tem se mostrado como uma estratégia de alimentação com potencial para melhorar a EUN aumentando o consumo de nutrientes e a produção individual (BARGO et al., 2002ab). Além disso, a incorporação de pastagem na dieta baseada em RTM permite níveis de ingestão e produção semelhantes às dietas RTM sem afetar as respostas digestivas nem a excreção de N

(SANTANA et al., 2016; MENDOZA et al., 2016ab; DALL-ORSOLLETA et al., 2016; PEREZ-RUCHEL et al., 2017). Contudo, em vacas leiteiras alimentadas com dietas que combinam pasto com RTM, a conversão do N alimentar em N do leite varia de 19 a 27 % (BARGO et al., 2002a; MENDOZA et al., 2016b; PASTORINI, 2016), valores que se encontram abaixo do limite superior teórico para a utilização de N em vacas leiteiras (43%; DIJKSTRA et al., 2013b). Isto sugere uma margem significativa para melhorar a EUN e reduzir as perdas urinárias de N. Neste sentido, resultados de estudos previos indicam que existem duas alternativas de fácil aplicação em dietas que combinem pastejo com RTM, que são promissóras para melhorar a EUN dos animais e que não implicarian uma redução no aporte de N da dieta: 1) o uso de taninos para reduzir a degradabilidade da proteína no rumen e aumentar o fluxo de proteína alimentar ao duodeno; 2) o manejo do horário de pastejo para aumentar a oferta de carboidratos solúveis (CS) na pastagem e favorecer a síntese e o fluxo de proteína microbiana ao duodeno. Contudo, a informação sobre os efeitos destas estratégias sobre o comportamento ingestivo, o consumo de nutrientes e o seu aproveitamento digestivo, a produção animal e a qualidade do produto final é escassa. Alem disto, a maioria dos experimentos que reportam o uso destas estratégias foram conduzidos com vacas leiteiras sob dietas RTM ou a pasto com ou sem suplementação. No entanto, nao existem estudos em vacas leiteiras alimentadas com dietas mistas que combinanam pastagem com RTM. Sendo assim, o foco de estudo desta tese será a avaliação nutricional e produtiva destas duas estratégias com potencial para melhorar a EUN e reduzir o impacto ambiental negativo da pecuária leiteira.

JUSTIFICATIVA

Em sistemas de produção de leite em base a pastagems temperadas, usualmente os animais consumen uma dieta com altos teores de proteína bruta de alta solubilidade e degradabilidade no rúmen (REPETTO et al., 2005). Nesta situação nutricional, a proteína que atinge o rúmen (Figura 1) é rapidamente hidrolisada por proteases extracelulares a peptídeos e aminoácidos passiveis de ingressar na célula bacteriana (1). Dentro da célula, os peptídeos são degradados por peptidases a aminoácidos (AA). Tanto os AA que ingressaram na célula quanto aqueles resultantes da hidrólise intracelular de peptídeos podem ser incorporados em proteínas (2), ou desaminados e metabolizados a ácidos graxos voláteis e NH_3, que são liberados ao meio extracelular (3). Contudo, as bactérias ruminais sintetizam a maior parte de suas proteínas a partir de AA sintetizados “de novo” a partir de NH_3 e α-cetoácidos (AGV de cadeia ramificada resultantes da desaminação de AA de cadeia ramificada) (4).

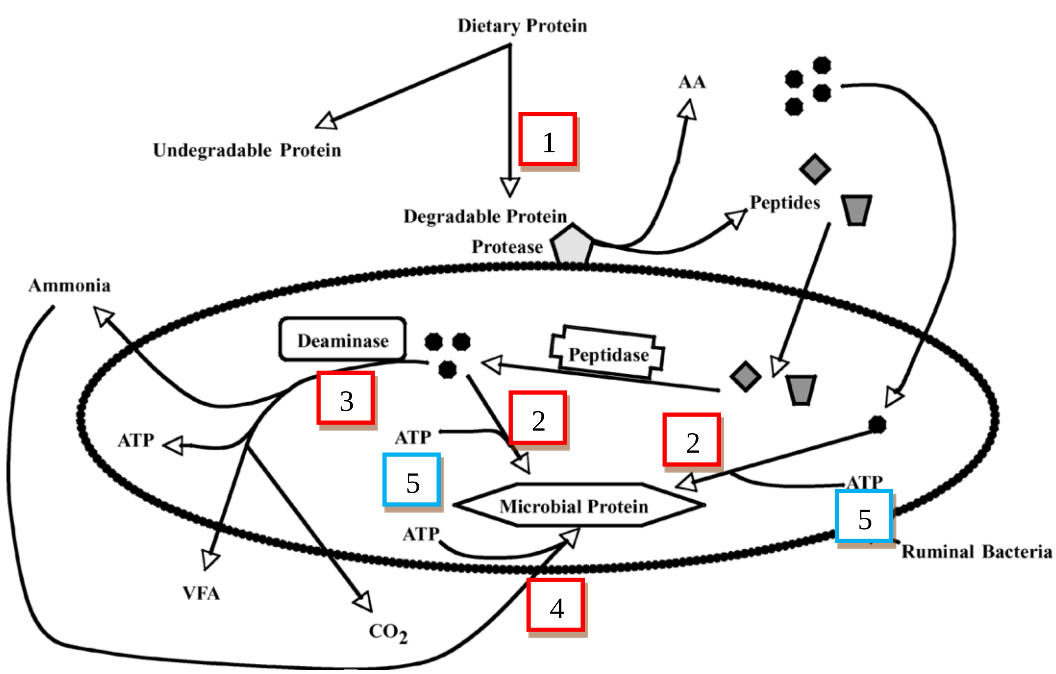

Figura 1. Representação esquemática da degradação proteica no rúmen.

Na situação mencionada inicialmente, a produção de NH_3 supera a capacidade de captação por parte das bactérias, o que resulta num aumento da concentração de NH_3

no conteúdo ruminal. A NH_3 é absorvido por difusão passiva, portanto este processo é altamente dependente de sua concentração no rúmen. A NH_3 absorvida passa a sangue portal e é conduzida ao fígado onde é captada e metabolizada. A NH_3 ingressa na mitocôndria do hepatócito, onde entra via carbamoil-fosfato no ciclo da ureia (KOZLOSKI, 2011). Em sistemas a pasto, entorno dum 30 % do N ingerido é excretado em forma de ureia na urina (LAZZARINI, 2010), onde por sua vez representa o principal composto nitrogenado (BRISTOW et al., 1992). Então, quando o N é consumido acima das necessidades dos animais há um aumento nas excreções de N principalmente na forma de ureia na urina (HOESKTRA et al., 2007). Num experimento com vacas leiteiras em pastoreio (TAAS et al., 2006) foram reportadas excreções de N urinário que corresponderam ao 57% do N ingerido. Já, em estudos com vacas leiteiras alimentadas com dietas que combinam pastagem com RTM, as excreções de N urinário corresponderam entre 28 e 46 % do N ingerido (MENDOZA at al., 2016b; PASTORINI, 2016), valores que põem de manifesto a baixa eficiência do uso do N alimentar.

Portanto, melhorar o uso da proteína alimentar, reduzindo suas perdas em forma de N e ureia na urina, é muito importante já que proteína é um nutriente com um custo econômico comparativamente mais elevado que a energia. Além disso, a transformação de NH_3 para ureia envolve processos metabólicos que geram um custo de energia para o animal (REED et al., 2017). Os excessos de N na dieta também estão associados a problemas reprodutivos (BUTLER, 1998; MCCORMICK et al., 2001; GEHMAN et al., 2006), a efeitos negativos sobre a qualidade do leite e seus derivados (MARTIN et al., 1997; BENDALL, 2001), e a poluição do meio ambiente, principalmente através das excreções do N urinário (PACHECO et al., 2008).

O objetivo deste experimento é aumentar a eficiência do uso do N atuando então sobre a produção e utilização de NH_3 no rúmen por duas estratégias:

1) Adicionando taninos na RTM:

Os taninos são compostos vegetais polifenólicos com capacidade de se complexar com proteínas, reduzindo assim sua degradabilidade ruminal (MAKKAR, 2003). Estes compostos tem um ou mais de um anel aromático e grupos carboxilos e oxidrilos livres, com os quais reagem entre eles e com outros compostos químicos como as proteínas (CASTILLO et al., 2013). Os principais mecanismos de interação com as proteínas são:

1) mediante pontes de hidrogênio entre os radicais hidroxilos dos grupos fenólicos dos taninos (Figura 2) (1) e os oxigênios das uniões peptídicas das proteínas; 2) por interações hidrofóbicas entre os anéis aromáticos dos compostos fenólicos e as regiões hidrofóbicas das proteínas (FRUTOS et al., 2004). Estes complexos taninos-proteínas alteram a estrutura secundaria da proteína dificultando o acesso das bactérias para a degradação. Além disto, são citados outros mecanismos para reduzir a degradabilidade da proteína: interagem com enzimas e afetam o processo de adesão das bactérias. Como ambas interações são reversíveis a pH maiores que 8 ou menores que 3.5, no abomaso (pH≈2.5) e no duodeno (pH≈8) estes complexos taninos-proteinas se dissociariam (FRUTOS et al., 2004), permitindo a liberação de aminoácidos. Portanto, com esta estratégia buscamos reduzir a produção de NH_3 no rumen e aumentar a o fluxo de proteína alimentar para o duodeno.

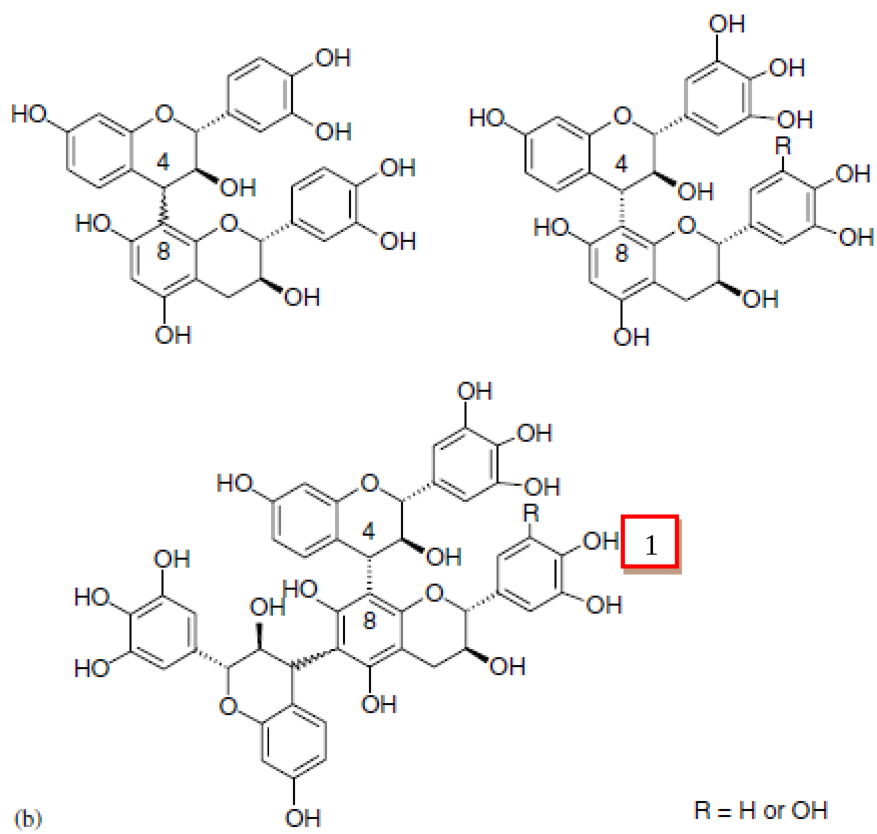

Figura 2. Taninos de *Acacia mearnsii*.

2) Manejando o horário de pastejo:

A o longo do dia e como resultado do processo de fotossíntese, as pastagems acumulan CS. Como a taxa de acumulação dos CS é maior do que proteína e fibra, suas concentrações são diluídas à medida que o dia avança (GREGORINI, 2012). Como resultado, aumenta tanto a relação CS/N na pastagem como a digestibilidade da matéria orgânica. Como a utilização da NH_3 e AA para síntese de proteína microbiana irá depender da disponibilidade de energia (Figura 1.) (5). Neste caso, um aumento da oferta de CS pelo pastejo da tarde, iria a disponibilizar mais substratos energéticos para as bactérias e assim maximizar a captação da NH_3, e como consequência disto aumentar a produção de proteína microbiana. Com esta estratégia buscamos então, favorecer a captação de NH_3 pelas bactérias e a passagem de proteína microbiana ao duodeno.

ESTUDO BIBLIOGRÁFICO

UTILIZAÇÃO DE TANINOS NA NUTRIÇÃO DE RUMINANTES

Numerosos estudos científicos avaliaram os efeitos de diferentes tipos de taninos na alimentação de ruminantes com resultados muito variáveis, às vezes positivos e negativos em outros. A variabilidade de resposta a estes aditivos dependem principalmente do nível de incorporação na dieta, e do tipo de tanino (MUELLER-HARVEY, 2006; WAGHORN, 2008), mas também dos outros componentes da dieta, da espécie e fisiologia do animal consumidor (HAGERMAN & BUTLER, 1991). De maneira geral, entre os principais efeitos negativos encontramos a diminuição do consumo e da digestibilidade do alimento (SILANIKOVE, 2001), que usualmente afetan negativamente a produção (GRAINGER et al., 2009; GERLACH et al., 2018). Entre os efeitos positivos destes aditivos alimentares são destacadas a redução da degradabilidade ruminal da proteína (CARULLA et al., 2005), o aumento do fluxo de aminoácidos disponíveis para o duodeno (WAGHORN, 1996; ORLANDI et al., 2015), a redução da excressao urinaria de N e ureia (AGUERRE et al. 2016; HENKE et al., 2017), e em alguns casos a melhoria de índices produtivos dos animais (DSCHAAK et al., 2011). Devido as fontes de variação dos resultados em relação à utilização de diferentes tipos de taninos na dieta de ruminantes, esta secção será focada sob trabalhos realizados com TA.

As publicações que reportam o efeito da suplementação com TA na dieta de vacas leiteiras sobre o consumo, a produção e composição do leite são apresentados na Tabela 1. Nestes estudos, independentemente da composição da dieta, e excluindo o estudo de Grainger et al. (2009), inclusões de TA entorno 0,3 a 1,2 % da dieta não afetam o consumo nem a performance das vacas leiteiras. Contudo, com inclusões de TA na ordem do 1,9 (GRAINGER et al., 2009) e 2,8% da dieta (GRIFFITHS et al., 2013) a produção de leite foi reduzida de maneira significativa em 30 e 11 % respectivamente, principalmente devido a uma queda do consumo. Nos estudos de Aprianita et al. (2014) e Gerlach et al. (2018), a incorporação de TA entre 1,6 e 3 % da dieta não afetou o consumo nem a produção de leite, no entanto, a produção total de proteína do leite foi diminuída.

Tabela 1. Efeito de diferentes níveis de inclusão de taninos de *Acacia mearnsii* na dieta de vacas leiteiras sobre o consumo, a produção e composição de leite.

Referência	Dias em lactação	Alimento	PB (% MS)	TA (% MS)	Efeitos
Grainger et al., 2009	32	Azevém + triticale	22,0	0,9 e 1,5	↓ produção e solidos de leite
	75	Azevém + triticale	16,0	1,1 e 1,9	↓ consumo, produção de leite e solidos
Griffiths et al., 2013	82	Azevém e trevo branco	19,8	0,6, 1,4 e 2,9	Sem efeitos em doses baixas; 2,9 % de TA na dieta ↓ produção e sólidos de leite
	77	Azevém e trevo branco + concentrado	23,1	1,2	Sem efeitos
Aprianita et al., 2014	39	Feno de alfafa + concentrado	20,5	1,6	Sem efeitos no consumo, produção de leite, nem perfil de ácidos graxos no leite; ↓ produção de proteina no leite
Maciel Dias, 2016	-	Festuca e trevo branco + RTM		1,5	Sem efeitos na produção e solidos de leite, nem no perfil de ácidos graxos no leite
Alves et al., 2017a	150	Aveia e azevém + concentrado	16,5	1,4	↑ consumo; Sem efeitos na produção e solidos de leite
Alves et al., 2017b	143	Milheto + concentrado	17,7	0,6	Sem efeitos no consumo nem a produção de leite e solidos;
Gerlach et al., 2018	49	RTM	15,7	1,0	Sem efeitos no consumo nem a produção de leite e solidos;
	70	RTM	16,0	3,0	Sem efeitos no consumo nem a produção de leite e gordura; ↓ produção de proteina no leite
Orlandi et al., 2018	45	Tifton 85 + silagem de milho + concentrado	12.7	0,3	Sem efeitos no consumo nem a produção de leite e solidos
Orlandi, 2016	35	Azevem + concentrado	22.6	0,6	Sem efeitos na produção de leite e sólidos

A ação dos taninos sob a digestão de compostos nitrogenados no rúmen e intestino tem consequências importantes sob a forma e via de excreção de N pelo animal. A redução da taxa de produção de NH_3 no rúmen induzida pela presença de taninos e leva a uma diminuição de N excretado na urina (SCHARENBERG et al., 2007). Adicionado a isto, uma eventual queda da digestibilidade intestinal dos compostos nitrogenados conduz a um aumento da excreção de N nas fezes (DEAVILLE et al., 2010; AUFRERE et al., 2012.). Para avaliar o efeito dos extratos taníferos na digestibilidade da MO e nas excresoes de N, foram recopilados resultados de experimentos com ruminantes não lactantes (Anexo 1). O efeito dos taninos foi avaliado utilizando regressões lineares entre os níveis inclusão de taninos na dieta (%), e as excresoes urinarias e fecais de N (g de N/N ingerido). Os resultados deste analise de dados, mostram que à medida que aumentam os níveis de TE na dieta, a excreção urinária de N diminui linearmente, enquanto que a excreção de N fecal aumenta linearmente (P <0,01; Figura 3).

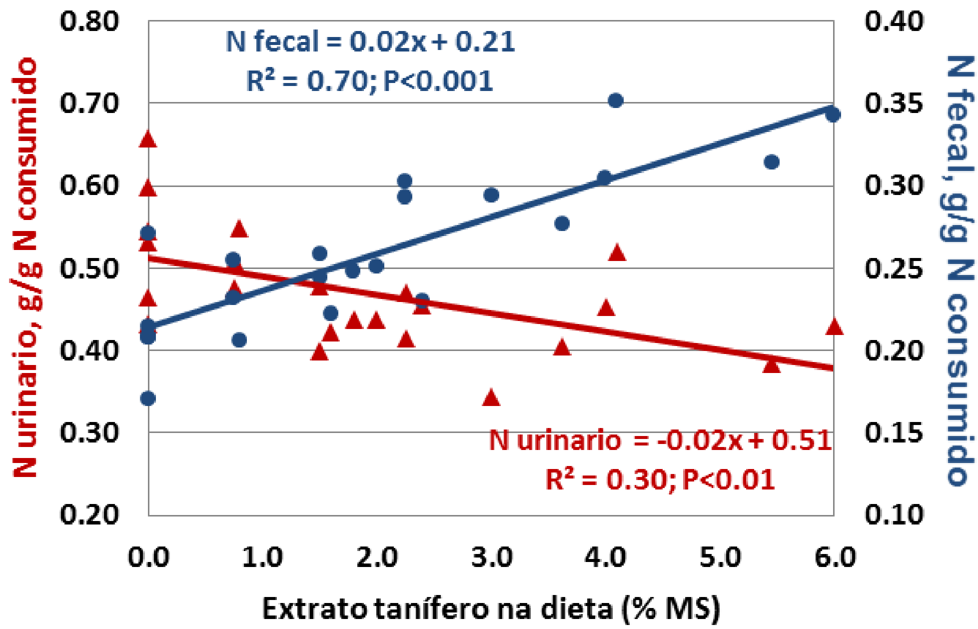

Figura 3. Relaçao entre níveis de extrato tanífero na dieta de ruminantes e as excreções urinarias e fecais de N.

Além disso, os resultados dos experimentos foram agrupados em 3 tratamentos: sem ET (controle), e inclusões de ET a níveis inferiores e superiores a 2% da dieta (T1 e T2 respectivamente). As medias foram comparadas por um análise de variância (ANOVA) com teste de Tukey. Através desta abordagem apenas níveis de taninos superiores a 2 % da dieta reduzirom a digestibilidade da MO (DMO), e afetarom tanto as excreções de N urinário como de N fecal, embora as excreções totais de N não tenham sido afetadas (Tabela 2). Esta sistematização de resultados da literatura permite imferir que os extratos taníferos mudam o site de excreção do N da urina para fezes sem afetar a excreção total de N. Contudo, so níveis de inclusão de taninos superiores ao 2 % da dieta presentam efeitos mais consistentes.

Tabela 2. Efeito da inclusão de extratos taníferos sobre a digestibilidade da matéria orgânica (DMO) e as excreções de N em ruminantes.

Items	Controle (n= 6)	T1 (n= 8)	T2 (n= 9)	*P*-valor
DMO (%)	$75^{a} \pm 6,1$	$74^{a} \pm 5,8$	$67^{b} \pm 6,5$	0.02
Excreeções de N, % do N ingerido				
Urinario	$54^{a} \pm 8,5$	$47^{ab} \pm 5,0$	$43^{b} \pm 5,3$	0.01
Fecal	$21^{b} \pm 3,2$	$24^{b} \pm 1,9$	$30^{a} \pm 3,5$	<0.01
Total	$75 \pm 8,7$	$70 \pm 4,0$	$73 \pm 6,8$	0.36

Controle= sem tanino; T1= inclusão de extrato tanífero < 2 % da dieta; T2 = inclusão de extrato tanífero > 2 % da dieta.

Estes padrões das excreções do N são largamente reportados em experimentos com vacas leiteiras suplementadas com extratos taníferos de quebracho (AGUERRE et al., 2016; HENKE et al., 2017). Tambem, os mesmos padrões das excreções do N foram observados em dois estudos onde foram adicionados TA na dieta de vacas leiteiras. Observou-se uma redução na concentração de N ureico no leite (GRIFFITHS et al., 2013), uma redução do N urinário e um aumento do N fecal (ambos expressos em g/kg de N consumido) sem afetar o balanço de N (GRAINGER et al., 2009).

De maneira geral, podemos dizer então, que as excreções totais de N permanecem inalteradas e há uma partição do N consumido em direção das fezes. No entanto, é preciso ser cautelosos no momento de afirmar este último, uma vez que entre as consequências da ingestão de taninos incluem-se o aumento da secreção de proteínas endógenas (glicoproteínas salivares, mucus, enzimas digestivas) e o aumento da descamação das células intestinais (MEHANSHO et al., 1987; WAGHORN, 1996). Portanto, este aumento em N fecal poderia estar associado ao aumento da excreção de

N endógeno, que não implicaria uma diminuição na quantidade de proteína absorvida dos alimentos (FRUTOS et al., 2004).

A troca do sítio de excreção do N, da urina para as fezes tem uma importância desde o ponto de vista ambiental. Estudos demostraram que a inclusão de taninos na dieta de bovinos permite reduzir as emissões totais de NH_3 e óxido nitroso (N_2O) provenientes das excretas (SLIWINSKI et al., 2004; MISSELBROOK et al. 2005; POWELL et al. 2011). Isto poderia ser explicado devido ao N fecal ser essencialmente formado por moléculas orgânicas, por isso é menos volátil, enquanto que a maior parte do N urinário encontra-se formado em parte por ureia que é mais susceptível a volatilização como NH_3 e N_2O (VAREL et al., 1999; GRABBER et al., 2001). Além disso, o complexo tanino-proteína das fezes teria uma degradação mais lenta que iria retardar a mineralização da matéria orgânica (POWELL et al. 2011).

MANEJO DO PASTOREIO PARA AUMENTAR O TEOR DE CS NA PASTAGEM

Os CS presentes nos pastagens temperadas como o azevém, incluem fructanos e açúcares (glicose, frutose, sacarose, etc.). Estes compostos encontram-se em proporções muito variáveis dependendo da época do ano, da hora do dia (Tabela 3) e sob certas condições pode atingir valores perto de 30% da matéria seca (MC DONALD, 2010). Com relação à variação diária do teor de CS em pastagens temperadas, vários estudos demostraram que a concentração destes compostos é mais elevada no fim da tarde do que durante a manhã. Este aumento no teor de CS ao longo do dia é dado quando a taxa de fotossíntese excede a taxa de respiração e da fixação de carbono (SMITH, 1973; CIAVARELLA et al., 2000). Somado a isso, e inversamente ao que é observado com o conteúdo de CS, os níveis de PB das pastagens são mais elevados de manhã (Tabela 3).

O conceito de sincronização de nutrientes refere-se ao fato de fornecer ao sistema ruminal, fontes de proteína e energia em forma simultânea e nas quantidades necessárias para otimizar seu uso pela microbiota associada (HALL & HUNTINGTON, 2008). Considerando que os CS tem uma alta velocidade de fermentação, em pastagens com alto teor de proteina solúvel, beneficiaria um rápido aumento da biomassa microbiana e uma maior utilização do N, e em consequência um maior fornecimento de nutrientes para o animal (BERCHIELLI et al., 2011). Diante disso, um aporte de forragens nas horas da tarde poderia ser considerada como uma estratégia para alcançar um aumento na relação CS/PB em pastagens com altos teores de PB, o que permitiria melhorar a EUN (KEIM & ANRIQUE, 2011). Neste sentido, alguns autores demostraram que o pastejo de tarde, melhora a relação CS/PB e aumenta ou tende a aumentar a produção de leite (Tabela 4).

Nestas publicações, o manejo da hora de fornecimento de pasto permitiu melhorias de produção de leite que foram de 0,8 ate 2,6 kg/dia. Estes autores argumentam que a melhoria da produtividade observada estaria ligada principalmente a um aumento no consumo de matéria seca (CMS) (BRITO et al., 2009) e da digestibilidade da pastagem (TREVASKIS et al., 2004), e por consequência a um aumento na disponibilidade e utilização de energia. Em relação a este último, Brito et al. (2009) estimam que o aumento do consumo e da digestibilidade quando a pastagem de alfafa foi fornecida de tarde teve um aporte extra de 3,1 Mcal de EM para o animal. Estes resultados são consistentes com os encontrados em outros estudos onde foram testados cultivares de pastagens com altos teores de CS na alimentação de vacas leiteiras. Em comparação com cultivares de pastagens com menores teores de CS, quando as vacas foram alimentadas com estas pastagens é geralmente observado, um aumento do consumo e da produção de leite (MILLER et al., 2001; MOORBY et al., 2006; TAWEEL et al., 2006).

Em outros estudos, apesar de que o fornecimento de pasto na tarde não melhorou o consumo nem a produção de leite, foram observadas melhorias no conteúdo de sólidos do leite (g/dia), especialmente em gordura (ORR et al., 2001; ABRAHAMSE et al., 2009; VIBART et al., 2017). Isto coincide com os resultados de Brito et al. (2009), que reportaram um aumento no rendimento de gordura e lactose (g/dia) em vacas alimentadas com pasto na tarde.

Tabela 3. Efeito do momento do dia sobre o teor de CS, carboidratos não estruturais (CNES) e PB em azevém.

Referencia	Pastagem	Mudanças de amanhã à tarde (g/kg MS)				
		CS (g/kg MS)	CNES (g/kg MS)	PB (g/kg MS)	FDN (g/kg MS)	Digestibilidade (%)
Trevaskis et al. 2004	*Lolium multiflorum*	74 a 124	-	328 a 268	390 a 390	74 a 82
Gregorini et al. 2006	*Lolium multiflorum* (inverno)	-	136 a 203	152 a 143	500 a 460	79 a 83
	Lolium multiflorum (primaveira)	-	103 a 163	144 a 129	460 a 400	72 a 75
Vasta et al., 2012	*Lolium perenne*	72 a 106	-	260 a 254	412 a 380	-
Bryant et al., 2013	*Lolium perenne*	164 a 174	-	187 a 171	360 a 376	84,7 a 85,4
Pulido et al., 2015	*Lolium perenne*	60 a 88	-	272 a 243	557 a 560	
Ueda et al., 2016	*Lolium perenne*	123 a 163	-	251 a 235	358 a348	67,1 a 60,4
Vibart et al., 2017	*Lolium perenne*	76 a 109	-	222 a 205	504 a 488	77.9 a 78.1
Chen et al., 2017	*Lolium perenne*	154 a 179	-	158 a 151	466 a 453	72,1 a 73,5

Tabela 4. Efeito do momento de acesso a pastagem sobre a produção e composição do leite.

Referencia	Dieta	Efeitos do pastejo de tarde
Orr et al., 2001	*Lolium perenne* + *Trifolium repens*	Tende a ↑ a produção de leite
Trevaskis et al., 2004	*Lolium multiflorum* + concentrado	↑ producao de leite e proteina
Abrahamse et al., 2009	*Lolium perenne* + concentrado	↑ producao de leite corrigida por gordura e proteína; ↑ produção de gordura do leite
Hernández-Ortega et al., 2014	Azevém + TMR e concentrado	↑ consumo de pasto; ↑ concentração de AG poli-insaturados, ruménico e linoleico no leite
Pulido et al., 2015	*Lolium perenne* + concentrado	Tende a ↑ a produção de leite; ↓ ureia em sangue
Vibart et al., 2017	*Lolium perenne*	↑ gordura no leite; tende a ↑ produção de proteína e sólidos do leite; ↓ concentração de AG poli-insaturados no leite
Chen et al., 2017	*Lolium perenne*	Tendencia a ↑ sólidos no leite

A partir dos resultados de publicações que avaliaram o efeito de pastagens com diferentes teores de CS sobre a EUN em vacas leiteiras, Keim & Anrique (2011) colocam em evidência que a EUN tende a aumentar linearmente quando a relação CS/PB aumenta (Figura 4). A relação previamente mencionada coincide com os resultados de estudos onde demostraram que o pastejo ou corte e fornecimento de pasto de tarde aumentou a EUN (TREVASKYS et al., 2004; BRITO et al., 2009; ABRAHAMSE et al., 2009; VIBART et al., 2017). Estes autores argumentam que essa melhoria na EUN com relação ao fornecimento de pasto na manhã estaria ligada basicamente à melhoria da relação CS/PB. Por consequência, o aumento da ingestão de energia rapidamente disponível nestes casos, pode ter aumentado o uso de N-NH_3 pela microbiota do rúmen. Isto seria apoiado pela redução das concentrações de N-NH_3 encontrados no rúmen (TREVASKYS et al., 2004; BRITO et al., 2009; UEDA et al., 2016), pelo aumento do fluxo omasal de N bacteriano e pela redução dos níveis de N ureico no plasma e no leite (BRITO et al., 2009). Estes fatores explicariam também as reduções de concentrações urinárias de N (g/L) e a melhoria em 15 % da EUN para produção de leite reportadas por Vibart et al. (2017). O conjunto de resultados obtidos sugere que a pastagem fornecida de tarde produz uma melhoria geral na utilização de N pelos animais.

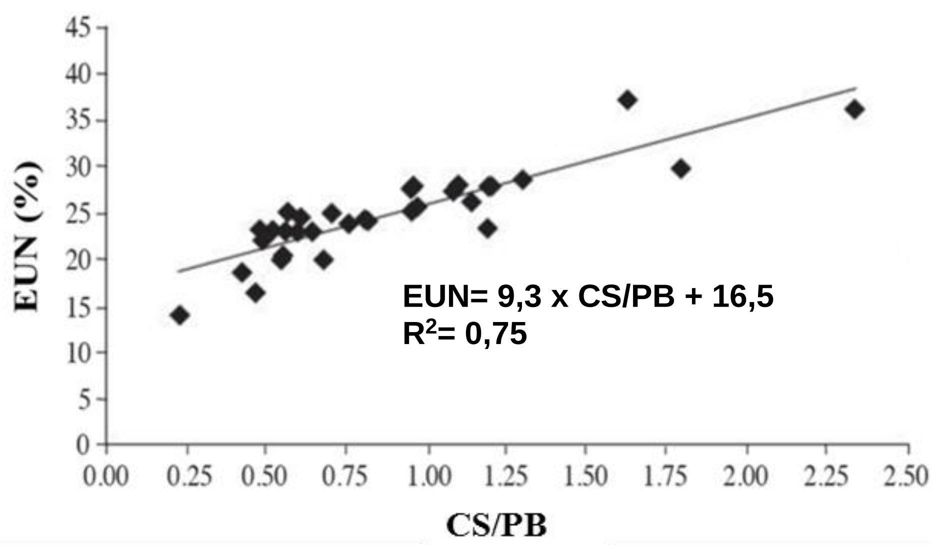

Figura 4. Relação entre CS/PB da dieta e a EUN em vacas leiteiras. Adaptado de Keim & Anrique (2011).

HIPÓTESE

A inclusão de extrato tanífero na dieta ou o pastoreio no período da tarde em vacas leiteiras alimentadas com uma dieta combinando pastagem com TMR, permitirão melhorar o uso do N alimentar.

OBJETIVO

Avaliar os efeitos da inclusão de extrato tanífero de Acacia mearnsii na TMR ou do horário de pastoreio sobre parâmetros nutricionais e produtivos de vacas leiteiras alimentadas com uma dieta combinando pastagem com TMR..

CAPÍTULO I

Impact of tannins or grazing schedule on intake, performance and milk fatty acids profile in dairy cows fed a diet combining pasture with total mixed ration

Capítulo baseado nas normas para submissão de artigo científico da revista Journal of Dairy Science

Impact of tannins or grazing schedule on behavior patterns, intake, performance and milk fatty acids profile in dairy cows fed a diet combining pasture with total mixed ration.

ABSTRACT

The aim of this study was evaluate if including *Acacia mearnsii* tannin extract (TA) in the diet or managing the grazing schedule impacts on the ingestive behavior, intake, performance and milk fatty acids (FA) profile of dairy cows grazing ryegrass combined with total mixed ration (TMR). Nine Holstein cows were arranged on a triplicate 3 x 3 Latin square design, conducted through three experimental periods of 22 d. The three treatments consisted of: morning grazing and afternoon TMR without TA (AM), morning grazing and afternoon TMR added with 15 g TA/kg of dry matter (DM) (AMt), and morning TMR without TA and afternoon grazing (PM). Treatments did not affect the DMI, but PM increased the percentage of pasture in the diet, and induced little changes on nutrients intake. The proportion of cows eating, ruminating or doing other activities through 16 h-observations was not affected by the treatments, however AM achieved high DM intake rate (DMIR) of TMR than PM. Also, AMt and PM increased the proportion of time spent eating TMR in 2 h relative to start of feeding. At grazing, the DMIR of pasture was similar for all treatments. Also, cows in AM and AMt displayed a similar behavior pattern and showed two peaks of grazing, while the proportion of cows grazing in PM seems to be more stable than AM and AMt. Treatments did not affect the milk production or milk solids yield. Although AMt tended to reduce the percentage of protein and casein in the milk, did not impact on milk protein and casein production. Although, AMt induced little changes on milk FA, the most relevant FA in milk remain unchanged.

In the same way, AM and PM present a similar profile of FA in the milk. It is concluded that the incorporation of low levels of TA in the TMR and managing the grazing schedule had not significant effect on intake, performance variables or milk FA profile. However, grazing in the afternoon is a tool with potential to increase the proportion of pasture in the diet in dairy cows fed mixed diets.

Key Words: tannin, grazing schedule, behavior, nutrient intake, performance

INTRODUCTION

In dairy systems, pasture provides a low-cost feed and confers nutraceutical characteristics to the final products. However, grazing systems provide an unbalanced diet and can limit the DMI, compromising the performance of dairy cows (Kolver and Muller, 1998). In contrast, TMR systems which provide a balanced ration, reach higher levels of DMI, production and nutritional efficiency (Kolver and Muller, 1998; Bargo et al., 2002). The use of mixed diets combining pasture with TMR, could be a nutritional alternative with potential to retain the advantages of both feeding systems. In this way, recent studies shown that the use of mixed diets allows similar levels of intake and production than TMR diets (Dall-Orsoletta et al., 2016; Mendoza et al., 2016). Additionally, as demonstrated by Mendoza et al. (2016) and Barca et al. (2017), the inclusion of pasture on TMR-based diet is an efficient way for increasing the content of beneficial fatty acids (FA) in milk, such as linolenic acid (LNA) and rumenic acid (RA). However, in studies with mixed diet the conversion of ingested N to milk N remain lower than 27 % (Bargo et al., 2002; Mendoza et al., 2016b; Pastorini, 2016), which is reflect of an unbalanced diet usually as consequence of pasture incorporation in the diet. In diets combining TMR with pasture, there are two easily applicable strategies with potential to improve the utilization of dietary N and performance by 2 pathways: reducing ruminal degradability of dietary N through tannin supplementation, and increasing the energy to N ratio into the rumen through grazing management.

Tannins are plant polyphenol compounds with the capacity to form complexes mainly with proteins and carbohydrates reducing their degradation in the rumen (Patra and Saxena, 2011). The tannin extract of *Acacia mearnsii* (TA) is an industrial tannin extract (TE) with in relatively low doses (≤ 15 g/kg of DM) presented potential to increase the amino acids supply without affecting the organic matter (OM) digestibility (Ávila et al., 2015; Orlandi et al., 2015). Also, TA shown potential in reducing urinary N losses and methane emissions without affecting the performance of dairy cows (Griffiths et al. 2013; Alves et al., 2017a; Alves et al., 2017b). At ruminal level, TE may also inhibit growth and activity of rumen microbes responsible for biohydrogenation (Min et al., 2003; Vasta et al., 2010). In this way, an *in vitro* trial showed that the TA was effective in increasing the vaccenic acid in detriment of stearic acid (Khiaosa-Ard et al. 2009). However, in *in vivo* experiments the information about the effects of TE on milk FA profile are scarce and inconsistent. In some cases, TE induced positives modifications on milk FA profile (Dschaak et al., 2010; Henke et al., 2017), while no effect were reported in others (Toral et al. 2013; Aprianita et al. 2014).

On another hand, pasture chemical composition varies throughout the day as consequence of diurnal processes of plant photosynthesis. Concentration of DM and water soluble carbohydrates (WSC) increases during the day, while concentration of nitrogen and fibrous fractions of herbage are diluted and digestibility improved (Bryant et al., 2013;

Cajarville et al., 2015). These changes in chemical composition present potential to increase the energy and N balance into the rumen (Ueda et al., 2016) improving the microbial uptake of ammonia-N, and increasing the flux of microbial N from the rumen to the small intestine, which in some cases can be reflected in improved intake and performance of dairy cows (Orr et al., 2001; Brito et al, 2009). In this way, a recent study shown that the allocation of a fresh strip pasture in the afternoon increased the milk solids yields (Vivart et al., 2017). In contrast, the FA profile of pasture grazed in the afternoon affected negatively the milk FA profile (Vivart et al. 2017). However, in dairy cows fed a mixed diet, the negative impact of grazing in the afternoon on milk FA profile could be compensate with a higher pasture intake and performance.

While the use of TA as dietary additive or managing the grazing schedule were tested in grazing or confined systems, it is necessary to elucidate the impact of these strategies on performance of dairy cows fed a diet combining pasture with TMR. Therefore, the aim of this study was to evaluate the impacts of dietary TA and managing the grazing schedule on intake behavior, nutrient intake, performance and milk FA profile of dairy cows fed a diet combining pasture with TMR.

MATERIALS AND METHODS

The experiment was carried out from September to November of 2015 at the Experimental Station of the Veterinary Faculty (Universidad de la República), in San José, Uruguay (S 34º40´, W56º32´). All procedures carried out during this experiment were previously approved by the Bioethics Committee of the Veterinary Faculty (Universidad de la República, Uruguay).

Animals, Experimental Design, Treatments

Nine multiparous Holstein dairy cows averaging [mean ± standard deviation (SD)] 546 ± 34 kg of BW, 197 ± 12 DIM, and 23.8 ± 3.4 kg of initial milk were used in this experiment. Cows were blocked into tree squares balanced for body weight, DIM, milk yield and previous lactation 305-d milk yield (7,320 ± 1044 kg). Each square was conducted simultaneously according to a 3 x 3 Latin square design. The cows in each Latin square received successively the 3 experimental treatments on 3 periods of 22 days, with 14 days of treatment adaptation and followed by 8 days of data and sample collection. Within each square, the three treatments were randomly distributed and consisted of: morning grazing and afternoon TMR without TA (AM), morning grazing and afternoon TMR added with 15 g/kg of DM of TA (AMt), and morning TMR without TA and afternoon grazing (PM). The TA (Weibull Black, Tanac S.A., Montenegro, Brazil) was the same previously used and described by Kozloski et al. (2012). Cows were milked twice a day at 0600 and 1500 h, and after milking, TMR or pasture (*Lolium multiflorum*) were offered at 0700 and 1600 h during 5 h. The TMR was formulated to meet the requirements of a cow with 550 kg of BW and producing 28 kg/d of milk (NRC, 2001). The DMI of TMR as a sole diet was predicted for each cow according to NRC (2011), and then, the supply of TMR was fixed for each cow at the start of experiment as 60 % of its predicted DMI. Experimental paddocks were managed with the aid of electric fences and targeting a daily pasture allowance of 14 kg DM/cow (calculated from clippings to a 5-cm height). After 5-h of TMR feeding or grazing the cows were kept together in an area without fed.

Nutrients Intake

The daily intake of TMR was measured on 5 days (between day 15 and 19) in individual feeders by weighing the amount of TMR offered and refused. On the same days of TMR intake measurements, the daily pasture DMI was estimated in individual plots as difference between the pre and post-grazing forage mass. The pre-grazing forage mass allowance, was estimated daily using an electronic rising plate meter (Farmworks Electronic Plate Meter, Farmworks Ltd, Feilding, New Zealand). The meter was calibrated in each period, 2-days previous intake measurements from three duplicated 0.1 m^2 quadrats, cuts at 5 cm to the ground and oven-dried at 100 ˚C for 24 h. The post-grazing forage mass was measured daily cutting with a mower (Toro CNB94, The Toro Company, MN, USA) a representative area corresponding to ≈ 14% of each paddock at 5 cm to the ground, weighing them and oven-dried a sample at 100 ˚C for 24 h.

Both TMR and pasture samples were collected on 8 days of each experimental period (from day 15 to 22). The TMR samples were immediately stored at −20°C, while pasture samples were taken through the hand clipping procedure, simulating the grazing behavior of cows, immediately placed in liquid N, and then stored at −20 °C. At the end of the experiments, TMR samples were partially dried at 60 °C for 48h, while pasture samples were freeze-dried. All samples were ground to 1-mm sieve, and pooled by period and treatment for chemical analyses.

Total dry matter content was determined by oven drying at 110 °C for 24h. Ash was determined by combustion at 600 °C for 3 h and OM by mass difference. Total N was determined by the Kjeldahl method (Method 984.13; AOAC 1997). The neutral detergent fibre (NDF) analysis was based on the procedures described by Mertens (2002) with use of heat-stable α-amylase and without sulphite. The concentration of acid detergent fibre (ADF) was analysed according to Method 973.18 of AOAC (1997) except that the samples were weighed in polyester filter bags (porosity of 16 μm) and treated with neutral and them with acid detergent in an autoclave at 110 °C for 40 min (Senger et al., 2008). For sulphuric-acid lignin (ADL) analysis, the bags containing residual ADF were treated with 12M H_2SO_4 for 3 h (Method 973.18 of AOAC 1997). Ether extract (EE) was determined using a fat/oil extractor (Ankom XT15; Ankom Technology, USA) based on petroleum ether solvent extraction. Pasture samples were additionally analyzed for WSC according to Dubois et al. (1956). The concentration of non-fibrous carbohydrates (NFC) was estimated as 100 – (% NDF + % crude protein (CP) + % ether extract + % ash) (NRC, 2001). The concentration of net energy for lactation (NE_L) of each feed was calculated according to NRC (2001) from feed analyses and individual DMI during each period.

Behavioral Recordings

On days 15 and 18 of each period, individual behaviors patterns were recorded by trained observers through visual observations every 5 min during 16 h beginning after morning milking (0700 h). The interval between the visual records was chosen from Hirata et al. (2002), and the following behaviors were observed: eating (i.e., grasping and chewing TMR or pasture), ruminating (i.e., chewing regurgitated boluses of feed), and others (i.e., not showing any of the other activities). The dry matter intake rate (DMIR) was calculated from TMR or pasture DMI divided theirs respective time eating. The proportion of eating or ruminating events were calculated as a fraction of the total observations. In addition, the proportions of each behavioral event in the first 7 h relative to start of meal were calculated hourly, and the values were expressed as a proportion of the total observations in each hour.

Milk Yield, Composition and Fatty Acid Profile

Milk yield was recorded on 5 days (from day 15 to 19) of each experimental period. A yield of 3.5% FCM was calculated according to Tyrrell and Reid (1965), and feed efficiency was calculated as 3.5% FCM yield/DMI. In days 15 and 17 of each period, individual milk samples were taken from two consecutive milkings and stored at 4ºC with bronopol preservative. The samples were then used to determine fat, protein, total casein and lactose by infrared analysis (model 2000, Bentley Instruments Inc., Chaska, MN).

In day 17 of each period, two individual milk samples were taken from each milking and stored at -20ºC without using preservatives. Then, the frozen milk samples were thawed at room temperature, and milk lipids were separated according to Feng et al. (2004). The FA composition was analyzed by gas chromatography as described by Mendoza et al. (2016). The Δ9-desaturase index was calculated as described by Kelsey et al. (2003), while the atherogenicity index was calculated as described by Ulbricht and Southgate (1991).

Statistical Analysis

Data were averaged per period and analyzed using the SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Data from traits with only one measurement during each period were analyzed using PROC MIXED with the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(}S_{i)} + P_k + T_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable; S_i is the random effect of square (i = 1 to 3); $C_{j(}S_{i)}$ is the random effect of cow nested in square (j = 1 to 3); P_k is the random effect of period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, AMt or PM); and e_{ijkl} is the residual error.

Behavioral records with repeated measurements over time during each period were analyzed using PROC MIXED with the following model:

$$Y_{ijklm} = \mu + S_i + C_{j(}S_{i)} + P_k + T_l + H_m + T_l \text{ x } H_m + e_{ijklm},$$

where Y_{ijklm} is the dependent variable; S_i is the random effect of square (i = 1 to 3); $C_{j(}S_{i)}$ is the random effect of cow nested in square (j = 1 to 3); P_k is the random effect of period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, AMt or PM); H_m is the fixed effect of hour of measurement (m = 1 to 7); T_l x H_m is the fixed effect of the interaction of treatment and hour of measurement; and e_{ijklm} is the residual error. The AR(1) covariance structure was used (Littell et al. 1998).

Differences among treatments were declared significant at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$ using the PDIFF option.

RESULTS

For health problems one cow was removed for statistical analyses of all evaluations during the second period.

The average pasture mass allowance for the 3 periods was 1,494 ± 755 kg DM/ha. Chemical composition values of TMR and pasture are presented in Table 1. The TMR of all treatments was prepared with the same ingredients and under the same conditions, therefore its chemical composition was similar. In contrast, pasture sampled in the afternoon presented a numerically higher concentration of DM, OM, WSC and NFC, but lower concentration of CP than pasture sampled in the morning.

The intake of pasture, TMR and nutrients are presented in Table 2. Total DMI was no affected by treatments, however PM tended to increase by 6 % the intake of pasture, while decreased by 2 % the intake of TMR compared to AM and AMt (P < 0.05; Table 2). As consequence, the percentage of pasture in the diet was higher in PM than AM and AMt (44.1 vs. 41.2; P < 0.05). Although, the intake of OM, NDF, CP or NEL balance remain unchanged by treatments, PM presented higher intake of NFC than AM and AMt (P < 0.01), and tended to present higher intake of NE_L than AMt (P = 0.08), but not differ from AM.

The time spent eating, ruminating or doing other activities by the cows through 16 h-observations as well as the time spent grazing were similar for all treatments (Table 3). In contrast, the time spent eating TMR in PM tended to be higher than AM, but not than AMt. The DMIR of TMR was higher in AM than PM (P < 0.05), however, were similar among AM and AMt or AMt and PM (Table 3). Although, cows presented similar behavioral patterns in all treatments, AMt and PM increased the proportion of time spent eating TMR in 2 h relative to start of feeding (*P* < 0.05; Figure 1). At grazing, the DMIR of pasture was similar for all treatments (Table 3). Also, cows in AM and AMt displayed a similar behavior pattern and showed two peaks of grazing in 1 and 5 h relative to start of grazing, while the proportion of cows grazing in PM seems to be more stable than AM and AMt (Figure 1).

Treatments did not affect any milk or milk constituent yield, feed efficiency to milk production, total casein to protein ratio, as well as milk fat and lactose percentages (Table 4). Likewise, the percentages of milk constituents were similar among AM and AMt. However, PM presented higher milk protein content (*P* < 0.05), and tended to present higher percentage of casein and total solids compared to AMt (*P* = 0.10; Table 4).

Total concentration of SFA, MUFA, PUFA, linoleic acid, LA, RA and vaccenic acid were not affected by treatments. Likewise, the milk FA profile was similar between AM and PM, however AMt presented lower concentration of palmitoleic acid (POA) than AM and PM (*P* < 0.05) and tended to reduce the concentration of palmitic acid (PA) compared to AM (*P* < 0.10; Table 5). In consequence, AMt presented a tendency to reduce the concentration of FA from mixed origin when compared with AM. Similarly, compared to AM and PM, AMt presented lower Δ9-desaturase ratio for 14C and 16C FA. However, Δ9-desaturase index and atherogenicity index remain unchanged by treatments (Table 5).

DISCUSSION

The diurnal changes in chemical composition of pasture were largely reported for ryegrass (Bryant et al., 2013; Chen et al., 2017; Vivart et al., 2017). Increased accumulation of photosynthates and water loss through transpiration as the day progresses can explain the higher WSC and DM content in pasture sampled in the afternoon. As the accumulation of WSC through the day are higher than protein and fiber, their concentrations are diluted as the day progresses (Gregorini, 2012).

In our experiment, the addition of 15 g TA/kg of DM in the TMR, which represent a dietary concentration of 8.7 ± 4 g TA/kg of DM did not impact on feed intake. The lack of effect of tannins on feed intake were reported in several studies. For example, the administration via ruminal fistula of an amount equivalent to 16 g TA/kg of DMI in dairy cows feeding concentrate plus alfalfa hay (Aprianita et al., 2014), as well as, the dietary inclusion of 6.4 g TA/kg of DM through the concentrate in dairy cows grazing a tropical pasture (Alves et al., 2017b) did not impact on DMI. In the same way, the supplementation with 6.4 to 30.0 g/kg of DM of quebracho TE (Beenchar et al., 2008; Henke et al., 2016) did not affect the DMI of dairy cows feeding TMR. On the other hand, the inclusion of increasing levels (from 4.5 to 18.0 g/kg of DM) of a blend containing quebracho and chestnut TE decreased linearly the DMI in dairy cows feeding TMR (Aguerre et al., 2016). Also, the addition of 36 and 54 g TA/kg of DM in the concentrate did not impact on concentrate intake, but reduced by 13.2% and 26.4% the total DMI in dairy cows fed ryegrass (Grainger et al., 2009). In contrast, the addition of 20 g TA/kg of DM in the concentrate, reduced the intake of concentrate, but increased the total DMI in dairy cows grazing a temperate pasture (Alves et al., 2017a). The reduction on feed intake induced by tannins could be due to a reduction on palatability of feeds and by decreasing the rate of digestion in the rumen (Patra and Saxena, 2011). However, the effect of tannins on DMI are mainly dependent of the incorporation level in the diet, but also, to the source of tannins, the way of supplementation, the components of the diet and the animal physiological state (Mueller-Harvey, 2006; Waghorn, 2008). Although, those factors could contribute to inconsistent results presented in the literature, there are no studies with dairy cows reporting a reduction in DMI when supplemented with TE at level used in our experiment. The similar intake reported between treatments are consistent with the lack of effects of AMt on most of behavioral variables. However, when compared with AM, cows in AMt increased the time spent eating TMR in 2 h relative to beginning of meala. Considering that AM and AMt were exposed to similar feed management, this result could be attributed to a reduction of palatability by a short-term astringency effect of tannins.

In the present experiment, the allocation of pasture in the afternoon increased the percentage of pasture in the diet without affecting the total DM. The increased NFC intake observed in PM is consistent with a higher NFC content observed in pasture grazed in the afternoon, however the additional NFC intake did not represent a significant supply of NE_L. The information available about the impact of different grazing schedule on intake in similar conditions with the present study are scarce. Hernández-Ortega et al. (2014) reported higher pasture intake in dairy cows feeding TMR with pasture allocated following the afternoon milking. In grazing experiments, the longest grazing events were reported in the afternoon (Abrahamse et al., 2009; Ueda et al., 2016), and these events were associated with a greater intake rate at this time of day (Orr et al., 2001) or a high disappearance rate of herbage mass (Vivart et al., 2017). Also, when cows have access to TMR throughout the day, the eating activity is highest after evening fed allocation than morning (De Vries et al. 2003). Although the proportion of cows grazing in PM seems to be more stable than AM and AMt, the increased pasture intake observed in PM was not consistent with changes on most of behavioral variables at grazing. In contrast, the lower

DMIR of TMR observed in PM, could suggest a preference for eating TMR in the afternoon, which was reflected in the low proportion of time spent eating and higher intakes of TMR observed in AM and AMt.

Milk production were similar between treatments. In contrast, AMt presented less percentage of milk protein and casein than PM. Also, PM tended to increase the percentage of milk solids. However, these little changes on milk composition did not represented significant changes on milk constituent's yield. The lack of effects of TA in milk production was reported in several studies with grazing cows supplemented with 3.4 to 12.0 g TA/kg DM (Griffiths et al., 2013; Alves et al., 2017a; Alves et al., 2017b). In contrast, when grazing dairy cows were supplemented with TA at levels ranging between 11.0 to 29.0 g/kg of DM, the reduction in milk and milk solids yields observed were mainly associated to a decreased feed intake (Grainger et al., 2009; Griffiths et al., 2013). In the present experiment was observed that AMt did not impact on DMI nor digestive variables (Pozo et al., submitted), which could suggest that inclusion of low quantities of TA are not enough to significantly impact on nutrient availability for milk production.

On another hand, the lack of effect of PM on milk responses was consistent with those were reported by Hernández-Ortega et al. (2014), which feed lactating cows with TMR plus grazing pasture at different times of day. In grazing experiments, the increase in milk yield or milk solids yield when pasture are allocated in the afternoon are usually related to higher intakes or improved pasture digestibility (Orr et al., 2001; Abrahamse et al., 2009; Vivart et al., 2017). In contrast, similar intakes in the morning and afternoon pasture allocation led similar performances (Pulido et al., 2015; Chen et al., 2017). In the present experiment, the changes in nutrient intakes of cows in PM were minimal, and did not cause any change on digestive variables (Pozo et al., Submitted). Therefore, these results could suggest that nutrient availability for milk production was not affected. Also, in the present experiment the percentage of pasture in the diet represented 43 %, therefore the effects of different composition of pasture grazed in the morning or afternoon was likely diluted by the inclusion of TMR.

The most relevant FA in milk remain unchanged by treatments. Only a little reduction of FA from mixed origin was induced by AMt. This indicate that tannins could have an effect at both rumen and mammary gland level, however, the existing data of the present study cannot explain these results.

CONCLUSIONS

Results of this experiment shown that the inclusion of low quantity of TA in the TMR did not affect the performance or the profile of most relevant FA in milk. The grazing in the afternoon is a tool with potential to increase the proportion of pasture in the total DMI without affecting on performance nor FA profile of dairy cows fed diet combining pasture with total mixed ration.

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Table 1. Ingredients and chemical composition of TMR and pasture (with SD in parentheses) grazed in the morning (a.m.) or in the afternoon (p.m.)

		Pasture	
Item	TMR	a.m	p.m.
Ingredients of TMR, % DM			
Corn silage	60.0	-	-
Solvent-extracted soybean meal	19.5	-	-
High moisture corn grain silage	18.5	-	-
Urea	0.70	-	-
Sodium bicarbonate	0.40	-	-
Dicalcium phosphate	0.20	-	-
Calcium carbonate	0.20	-	-
Salt	0.20	-	-
Vitamin and mineral premix[1]	0.30	-	-
Chemical composition			
DM, % of the amount fed	41.0	18.6 (2.1)	19.6 (2.5)
OM	94.0	87.9 (1.1)	88.7 (1.2)
WSC	-	6.9 (1.2)	8.9 (1.4)
NDF	27.1	45.9 (5.2)	43.4 (7.9)
ADF	16.6	27.1 (2.7)	26.3 (3.8)
ADL	1.3	3.7 (1.1)	3.6 (1.6)
NFC	46.7	28.7 (5.3)	33.0 (7.7)
EE	3.75	2.1 (0.04)	1.8 (0.16)
CP	16.5	13.3 (1.4)	12.3 (1.0)
NE_L, Mcal/kg of DM	1.87	1.33 (0.08)	1.36 (0.13)

WSC to CP ratio	.	0.53 (0.15)	0.73 (0.15)

[1]Provided (per kg DM): 125 g of Procreatin 7®, 125 g of Precisión Mix Vacas Lecheras®, 38 g of Rumensin® 200, and 713 g of excipients.

Table. 2. Effect of Tannin and grazing schedule on nutrient intake

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
DM, kg/d					
Pasture	8.1[y]	8.1[y]	8.6[x]	0.50	0.06
TMR	11.1[a]	11.1[a]	10.9[b]	0.47	0.03
Total	19.3	19.2	19.6	0.74	0.33
Pasture in total DM, %	42.1[b]	42.3[b]	44.1[a]	1.66	0.01
TMR in total DM, %	57.9[a]	57.7[a]	55.9[b]	1.66	0.01
OM, kg/d	17.6	17.6	17.9	0.65	0.21
NDF, kg/d	6.7	6.7	6.7	0.34	0.87
NFC, kg/d	7.5[b]	7.5[b]	8.0[a]	0.45	<0.01
CP, kg/d	2.92	2.92	2.87	0.14	0.17
NE_L, Mcal/d	31.7[xy]	31.6[y]	32.2[x]	1.21	0.08
NE_L balance, Mcal/d	4.9	4.8	5.3	0.58	0.58

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

Table 3. Effect of tannin and grazing schedule on behavioral events (min) and DMI rate (DMIR) during the first 16 h after the initial feeding (0700 h)

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
Eating	273	290	291	14.3	0.30
Pasture	189	196	189	12.7	0.60
TMR	84^{y}	94^{xy}	101^{x}	5.0	0.07
Ruminating	327	332	329	19.5	0.95
Others	360	338	341	26.4	0.47
DMIR, kg DM/h					
Pasture	2.6	2.5	2.7	0.20	0.35
TMR	8.2^{a}	7.2^{ab}	6.7^{b}	0.45	0.03

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = and morning TMR without TA and afternoon grazing.

Table 4. Effect of tannin and grazing schedule on milk yield and composition

	Treatment				
Item	AM	AMt	PM	SEM	*P*-value
Milk, kg/day	21.7	21.6	21.4	2.12	0.86
3.5% FCM, kg/day	23.6	23.8	24.2	1.89	0.63
Fat, kg	0.88	0.89	0.92	0.062	0.40
Fat, %	4.11	4.13	4.35	0.196	0.21
Protein, kg	0.76	0.74	0.75	0.062	0.55
Protein, %	3.51[ab]	3.43[b]	3.54[a]	0.095	0.03
Total casein, kg	0.57	0.55	0.56	0.044	0.41
Total casein, %	2.65xy	2.56y	2.66x	0.095	0.07
Casein: protein ratio	0.75	0.75	0.75	0.008	0.54
Lactose, kg	1.02	1.02	1.02	0.107	0.99
Lactose, %	4.70	4.72	4.75	0.089	0.41
Total solids, kg	2.65	2.65	2.69	0.226	0.72
Total solids, %	12.3[y]	12.3[y]	12.6[x]	0.279	0.09
Feed efficiency[2]	1.23	1.23	1.24	0.071	0.98

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

[2]3.5% FCM yield (kg/d)/DMI (kg/d).

Table 5. Effect of tannin and grazing schedule on milk fatty acid (FA) profile and components

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
FA content (g / 100 g of total FA)					
Selected individual FA					
4:0	1.41	1.15	1.54	0.322	0.66
6:0	1.38	1.13	1.41	0.249	0.66
8:0	0.98	0.88	1.02	0.157	0.80
10:0	2.33	2.45	2.70	0.418	0.81
12:0	3.15	3.06	3.51	0.341	0.60
14:0	12.4	12.4	12.8	0.79	0.91
14:1 *cis*-9	1.26	1.09	1.30	0.147	0.42
15:0	0.94	0.93	1.01	0.065	0.50
16:0	38.2[x]	35.0[y]	36.3[xy]	1.79	0.08
16:1 *cis*-9	2.35[a]	1.86[b]	2.34[a]	0.177	<0.01
18:0	8.86	10.69	8.83	1.373	0.19
18:1 *cis*-9[2]	21.4	23.8	21.9	1.14	0.31
18:1 *trans*-9	0.24	0.27	0.21	0.054	0.31
18:1 *trans*-11[3]	1.60	1.85	1.63	0.506	0.75
18:2 *cis*-9, *cis*-12	1.26	1.20	1.38	0.163	0.66
18:2 *cis*-9, *trans*-11[4]	0.70	0.65	0.78	0.181	0.67
18:3 *cis*-9, *cis*-12, *cis*-15[5]	0.32	0.36	0.30	0.066	0.54
Summation by origin					
De novo (4:0-15:0)	22.7	22.1	24.1	2.15	0.78

Mixed origin (16:0 + 16:1)	40.5^{x}	36.9^{y}	38.6^{xy}	1.96	0.06
Preformed (> 17:0)	35.1	39.5	35.7	2.92	0.28
Summation by saturation					
SFA	70.3	68.4	69.7	1.53	0.65
MUFA	27.3	29.2	27.8	1.27	0.52
PUFA	2.25	2.20	2.46	0.308	0.72
Saturated: unsaturated ratio	2.43	2.21	2.37	0.159	0.55
n-6 : n-3 ratio	4.55	4.42	4.16	0.328	0.35
Δ^9 – desaturase ratio					
14:1 / 14:0	0.103^{a}	0.086^{b}	0.101^{a}	0.0068	0.01
16:1 / 16:0	0.062^{a}	0.053^{b}	0.064^{a}	0.0042	0.01
18:1 / 18:0	2.92	2.59	2.83	0.244	0.33
18:2 *cis*-9, *trans*-11 / 18:1 *trans*-11	0.39	0.47	0.42	0.104	0.82
Δ^9 – desaturase index[6]	0.30	0.31	0.31	0.016	0.66
Atherogenicity index[7]	3.16	2.85	3.11	0.262	0.59

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = and morning TMR without TA and afternoon grazing.

[2]Oleic acid

[3]Vaccenic acid

[4]Rumenic acid

[5]Linolenic acid

[6]Calculated as: (14:1 *cis*-9 + 16:1 *cis*-9 + 18:1 *cis*-9 + 18:2 *cis*-9, *trans*-11)/(14:0 + 16:0 + 18:0 + 18:1 *trans*-11 + 14:1 *cis*-9 + 16:1 *cis*-9 + 18:1 *cis*-9 + 18:2 *cis*-9, *trans*-11).

[7]Calculated as: (12:0 + 4 x 14:0 + 16:0)/(MUFA + PUFA).

Pozo et al. Figure 1.

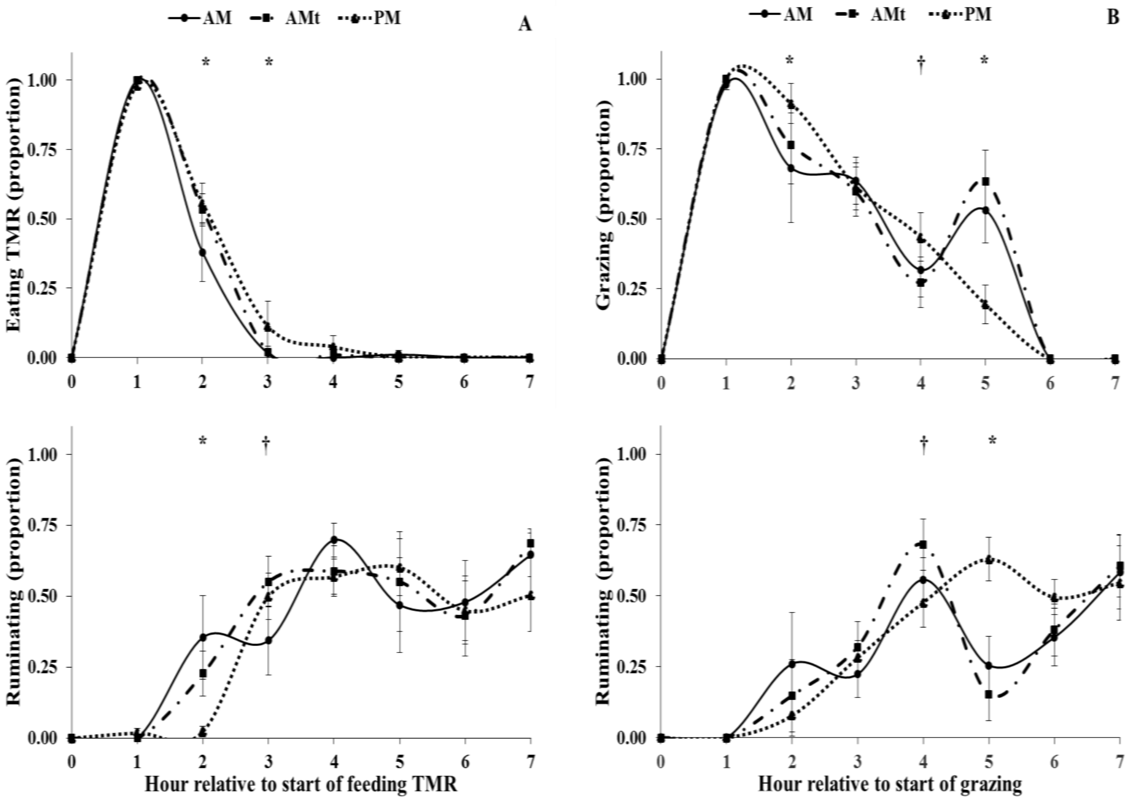

Figure 1. Behavioral events in the first 7 h relative to start of meal: feeding TMR (A) or grazing (B).

The values are expressed as a proportion of the total observations in each hour. Symbols indicate significance (*$P \leq 0.05$) or tendency (†$P \leq 0.10 > 0.05$). Treatments consisted of: AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

CAPÍTULO II

Impact of tannins or grazing schedule on digestion and N partitioning in dairy cows fed a diet combining pasture with total mixed ration

Capítulo baseado nas normas para submissão de artigo científico da revista Journal of Dairy Science

Impact of tannins or grazing schedule on digestion and N partitioning in dairy cows fed a diet combining pasture with total mixed ration

ABSTRACT

The aim of this study was to evaluate if including *Acacia mearnsii* tannin extract (TA) in the diet or managing the grazing schedule impacts on digestibility, rumen fermentation, microbial protein synthesis and nitrogen (N) partitioning of dairy cows grazing ryegrass combined with total mixed ration (TMR). Nine lactating Holstein cows were arranged on a triplicate 3 x 3 Latin square design, conducted through three experimental periods of 22 d. The three treatments consisted of: morning grazing and afternoon TMR without TA (AM), morning grazing and afternoon TMR added with 15 g TA/kg of dry matter (DM) (AMt), and morning TMR without TA and afternoon grazing (PM). Treatments did not affect the pH, nor concentrations of NH_3-N, sugars of VFA in the rumen. No differences among treatments were detected in microbial protein flow to the duodenum, nor most of digestibility variables. The intake of N fractions and the excretion of N in milk and feces were similar between treatments. However, AMt and PM lowered the amount of N and N-urea excreted in urine. Although the excretion of total manure N was not affected by treatments, the urinary N to fecal N ratio were lower in both AMt and PM in comparison with AM. It is concluded that the inclusion of low amounts of TA in the TMR or grazing in the afternoon were both effective strategies to reduce the excretion of urinary N without impacting negatively on nutrient digestion of lactating dairy cows.

Key Words: tannin, grazing schedule, digestibility, rumen fermentation, nitrogen metabolism

INTRODUCTION

Ruminants have a low efficiency of nitrogen (N) utilization compared with non-ruminants, and this inefficiency has implications not only for productive or economic point of view, but also for the emission of contaminants to the environment (Calsamiglia et al., 2010). In pasture-based dairy system, the N supply is usually unbalanced, and this can affect negatively on performance and N utilization. In consequence, the N use efficiency for milk N production (NUE) are usually lower than 30 % (Powell et al., 2010), representing significant N losses through manure. Nitrogen excreted in manure from dairy cattle contributes to environmental N pollution either as ammonia (NH_3) and nitrous oxide (NO_2) in air, or as nitrate in soil and ground water (Tamminga, 1992). Furthermore, the urinary N in manure is much more susceptible to gaseous losses than fecal N (Dijkstra et al., 2013a). Therefore, improving N utilization by the animal to reduce manure N output and changing the N partitioning from urine to feces are suitable. In this way, a recent study reported that the inclusion of a total mixed ration (TMR) in a pasture-based diet shown potential to improve N utilization compared to a pasture diet (Santana et al., 2016). In addition, the incorporation of pasture in a TMR based diet allows similar levels of intake and production than TMR diets (Dall-Orsoletta et al., 2016; Mendoza et al., 2016a) without affecting digestive responses nor N excretion (Santana et al., 2016; Mendoza et al., 2016b; Perez-Ruchel et al., 2017). However, in those mixed diets the NUE remain around 19 to 27% (Bargo et al., 2002; Mendoza et al., 2016b; Pastorini, 2016), which is below the theoretical upper limit for N utilization in dairy cows (43 %; Dijkstra et al., 2013b). This could suggest a significant margin to improve the NUE and reduce the urinary N losses. For achieve this challenge, there are two easily applicable strategies with potential to improve the NUE of dairy cows feeding mixed diets: reducing ruminal degradability of dietary N through tannin supplementation, and increasing the energy to N ratio into the rumen through grazing management.

Tannins are plant polyphenol compounds with the ability to form complexes mainly with proteins and to reducing their ruminal degradation (Patra and Saxena, 2011). The tannin extract of *Acacia mearnsii* (TA) is an industrial tannin extract (TE) with potential to be used as a feed additive for ruminants. Previous studies using this source of tannins, shown that low doses of TA (9 to 18 g/kg of dry matter (DM)) are capable to improve the amino acids supply and to reduce the urinary N excretion without affecting the organic matter digestibility in non-lactating ruminants (Ávila et al., 2015; Orlandi et al., 2015). In contrast, studies with dairy cows shown inconsistent results. For example, Grainger et al., 2009 reported that offering 19 g TA/kg of DM in grazing dairy cows supplemented with triticale can reduce urinary N excretion, but also reduced the organic matter digestibility. While, the inclusion of 185 g/day of TA as an oral drench in supplemented dairy cows grazing a ryegrass pasture, reported a positive potential in reducing urinary N losses (Griffiths et al., 2013). Despite these studies, to our knowledge no information are available about the use of TA in mixed diets combining pasture with TMR. In addition, little information is available about the impact of dietary TA on digestive responses or N partitioning in dairy cows.

In another hand, it is well known that diurnal processes of plant photosynthesis can increase the concentration of water-soluble carbohydrates (WSC) as the day progresses (Gregorini, 2012), which is accompanied with a dilution of crude protein (CP) content. Thus, the intake of a pasture with higher WSC to CP ratio could potentially improve the

N utilization in grazing dairy cows. Previous results shown that increasing the WSC to CP ratio of forages have potential to improve the utilization of ruminal NH_3-N (Trevaskys et al., 2004; Ueda et al., 2016), to increase the microbial protein synthesis (MPS) (Brito et al., 2008; Gregorini et al., 2008) and to reduce the plasma urea-N (PUN) (Pulido et al., 2015). However, the information about the effects of pasture allocation time on digestives responses or N partitioning are scarce. In addition, most of experiments were carried out with dairy cows fed a graze based diet, supplemented or not with concentrate, while, no study in cows fed a mixed diet combining TMR with pasture was reported.

Therefore, the aim of the present study was to evaluate the impacts of dietary TA and managing the grazing schedule on digestibility, rumen fermentation, microbial protein synthesis and N partitioning of dairy cows fed a diet combining pasture with TMR. It was hypothesized that the inclusion of TA in the TMR or the grazing in the afternoon are capable to reduce the excretion of urinary N without affecting negatively on digestives responses of dairy cows fed a diet combining pasture with TMR.

MATERIALS AND METHODS

Site, Animals and Experimental Design

All procedures carried out during this experiment were previously approved by the Bioethics Committee of the Veterinary Faculty (Universidad de la República, Uruguay). The study was conducted from September to November of 2015 at the Experimental Station of the Veterinary Faculty (Universidad de la República), in San José, Uruguay (S 34º40´, W56º32´).

Nine multiparous Holstein cows, selected by previous lactation 305-d milk yield (7,320 ± 1044 kg) and fitted with permanent rumen catheters were used in the present study. Cows were blocked into three squares balanced for body weight (BW), days in milk (DIM), milk yield and previous lactation 305-d milk yield. The experiment consisted of a replicated 3x3 Latin Square, when cows received successively the 3 experimental treatments on 3 periods of 22 days (14 days of treatment adaptation followed by 8 days of data and sample collection). The three treatments evaluated were: morning grazing and afternoon TMR without TA (AM), morning grazing and afternoon TMR added with 15 g/kg of DM of TA, and morning TMR without TA and afternoon grazing (AMt). At the start of experiment the cows averaged 546 ± 34 kg of BW, 197 ± 12 DIM, and 23.8 ± 3.4 kg of milk yield. The TA (Weibull Black, Tanac S.A., Montenegro, Brazil) was the same previously used and described by Kozloski et al. (2012).

Cows were milked twice a day at 0600 and 1500 h, and after milking they acceded to individual feeders with TMR or to individual grazing plots with a ryegrass pasture (*Lolium multiflorum*). After 5-h of TMR feeding or grazing the cows were kept together in an area with fresh water and without fed. The TMR was formulated to meet the requirements of a cow with 600 kg of BW and producing 30 kg/d of milk (NRC, 2001). The dry matter intake (DMI) of TMR as a sole diet was predicted for each cow according to NRC (2011), and then, the supply of TMR was fixed individually at the start of experiment as 60 % of its predicted DMI. Experimental plots were managed with the aid of electric fences and targeting a daily pasture allowance of 14 kg DM/cow (calculated from clippings to a 5-cm height).

Sample Collection and Measurements

Feed samples were collected on 8 days of each experimental period (from day 15 to 22). The TMR samples were immediately stored at −20°C. Pasture samples were taken through the hand clipping procedure, simulating the grazing behavior of cows 1 h after beginning of morning or afternoon grazing (0800 and 1700 h). Pasture samples were, immediately placed in liquid N, and then stored at −20 °C. On d 16, 17 and 19 of each period, spot fecal samples were collected from all cows twice a day at 1200 and 2100 h. Fecal grab samples (≈ 200 g of fresh matter) were collected directly from the rectum and stored at −20 °C. On the same days and hours that fecal collection, 2 spot urine samples were collected from all cows. A 10-mL sample of fresh urine was acidified with 1 mL of sulphuric acid (H_2SO_4, 20 % v/v), diluted with 49 mL of distillated water and stored at −20°C. On day 20 of each period, rumen fluid samples were collected at 0, 2, 4, 6, 9, 11, 13, 15 and 18 h relative to beginning of fist meal (0700 h). The pH was immediately measured using a digital pH meter (EW-05991–36, Cole Parmer, Vernon Hills, IL) and an aliquots (9 mL) of ruminal fluid were removed and conserved with 1-ml of 3.6 M H_2SO_4. Additionally, another 1-mL sample was preserved with 1-mL of 0.1 M perchloric acid. All samples of ruminal fluid were stored at −20°C until analysis. On d 22 of each period, individual blood samples were collected from the coccygeal vein at h 0, 3, 9 and 12 h relative to beginning of fist meal (0700 h) into heparinized tubes. After centrifugation (3,000 × *g* for 20 min at 20°C), the plasma was separated and stored at −20°C. In days 15 and 17 of each period, individual milk samples were taken from two consecutive milkings and stored at 4ºC with bronopol preservative.

Chemical Analyses, Feed Evaluations and Calculations

At the end of the experiments, TMR and fecal samples were partially dried at 60 °C for 48h, while pasture samples were freeze-dried. Feed and fecal samples were ground to 2 and 1-mm sieve. For chemical analyses, feed samples were pooled by period and treatment while fecal samples were pooled by cow, period and treatment. Total DM content was determined by oven drying at 110 °C for 24h. Ash was determined by combustion at 600 °C for 3 h and organic matter (OM) by mass difference. Total N was determined by the Kjeldahl method (Method 984.13; AOAC 1997) and analysis of N fractions was performed according to Licitra et al. (1996). The CP content was classified into five fractions (A, B1, B2, B3 and C) based on degradability characteristics according to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992). The neutral detergent fiber (NDF) analysis was based on the procedures described by Mertens (2002), with use of heat-stable α-amylase, except that the samples were weighed into polyester filter bags (porosity of 16 µm) and treated with neutral detergent in an autoclave at 110˚C for 40 min (Senger et al., 2008). Concentrations of acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Method 973.18 of AOAC (1997). Ether extract (EE) was determined using a fat/oil extractor (Ankom XT15; Ankom Technology, USA) based on petroleum ether solvent extraction. Pasture samples were additionally analyzed for WSC according to Dubois et al. (1956). The concentration of non-fibrous carbohydrates (NFC) of feeds was estimated as 100 – (% NDF + % CP + % ether extract + % ash) (NRC, 2001). The concentration of net energy for lactation (NE_L) of each feed was calculated according to NRC (2001) from feed analyses and individual DMI during each period. In addition, kinetics of fermentation of feeds samples were evaluated using an *in vitro* gas system as described by Mauricio et al. (1999).

Total fecal production was estimated using indigestible NDF (iNDF) as an internal marker as described by Huhtanen et al. (1994). Briefly, fecal and feed samples grounded at 2-mm screen were weighed (2.5 g) in a polyester filter bag (10 x 5 cm; 16 µm porosity) and incubated in the rumen of a canulated steer for 288 h. After rumen incubation, the bags were rinsed with tap water for 15 min and dried in a forced-air oven at 60°C for 48 h, the residues were analyzed for NDF as described above and was considered as iNDF. Fecal output was estimated for each animal by dividing iNDF daily intake by the iNDF concentration in feces. Ingestion of DM was recorded on 5 days (between d 15 to 20) of each period as described by Pozo et al. (Submitted). Briefly, the daily intake of TMR was measured in individual feeders by weighing the amount of TMR offered and refused, while the daily intake of pasture was estimated in individual plots as difference between the pre and post-grazing forage mass. Apparent total-tract digestibility coefficients for DM, OM, NDF, ADF, and total N were calculated as {[ingestion (g/d) – fecal output (g/d)]/ingestion (g/d)} × 100. For estimation of OM true digestibility was assumed that neutral detergent soluble fractions of the feces are from endogenous origin and only the NDF fraction of feces originated from feed (Van Soest, 1994) as follows: [OM intake (g/day) - Fecal NDF (g/day)]/OM intake (g/day). The true digestibility of N compounds was estimated considering that neutral detergent soluble N of the Feces are from endogenous origin and only the neutral detergent insoluble N (NDIN) fraction of Feces originated from feed (Van Soest 1994) as follows: [N intake (g/day) - fecal NDIN (g/day)]/ N intake (g/day).

Samples of ruminal fluid acidified with H_2SO_4 were thawed at room temperature and centrifuged at 4,000 x *g* 20 min, and thus analyzed for NH_3-N (Weatherburn 1967) and reducing sugars (Dubois et al. 1956). For volatile fatty acids (VFA) determination, only samples taken at h 0, 4, 9 and 13 selected for analyze. Samples were thawed at room temperature, centrifuged (15,000 rpm for 15 min at 4°C) and VFA were analyzed using HPLC (Dionex Ultimate 3000) as described by Adams et al. (1984).

Urine samples were thawed at room temperature, pooled by cow and period, filtered at 7.5 µm and analyzed for N (as described earlier), urea-N and creatinine with commercial kits (Bioclin MG, Brazil; Labtest, MG, Brazil). In addition, urine samples were centrifuged (15,000 rpm for 15 min at 4°C) and analyzed for purine derivatives (PD; i.e., allantoin and uric acid) using a HPLC (Dionex Ultimate 3000, Sunnyvale, CA) as described by Balcells et al. (1992). Assuming that the creatinine excretion is constant in function of body weight (Chizzotti et al., 2008) the urine production was calculated using a creatinine excretion factor of 21.9 mg/kg of BW (Pacheco et al. 2007) as creatinine excretion (mg/d) / creatinine concentration (mg/L). The quantity of absorbed purines (mmol/day) was calculated from the equation described by Chen and Gomes (1995): absorbed purines = (PD excreted - 0.385 x BW 0.75)/0.85. Flow of microbial N (MNF) to the duodenum was calculated assuming a purine N content of 70 mg N/mmol, a ratio of purines N/total N of 0.116 and a microbial purine digestibility of 0.83 (Chen and Gomes, 1995) as: MNF (g/day) = absorbed purines x 70/(0.116 x 0.83 x 1000). Efficiency of N use for microbial N synthesis (EUN) was calculated as: (MNF/total N intake) × 100. Milk N secretion (milk protein/6.38) was calculated from milk yield and milk protein concentration. Milk urea-N (MUN) was determined by infrared analysis (model 2000, Bentley Instruments Inc., Chaska, MN). The plasma urea-N (PUN) was determined by colorimetry using a commercial kit (Bioclin, MG, Brazil).

Statistical Analysis

Data were averaged per period and analyzed using the SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Data from traits with only one measurement during each period were analyzed using PROC MIXED with the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(}S_{i)} + P_k + T_l + e_{ijkl,}$$

where Y_{ijkl} is the dependent variable; S_i is the random effect of square (i = 1 to 3); $C_{j(}S_{i)}$ is the random effect of cow nested in square (j = 1 to 3); P_k is the random effect of period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, AMt or PM); and e_{ijkl} is the residual error.

Data from traits with repeated measurements over time during each period were analyzed using PROC MIXED with the following model:

$$Y_{ijklm} = \mu + S_i + C_{j(}S_{i)} + P_k + T_l + H_m + T_l \text{ x } H_m + e_{ijklm,}$$

where Y_{ijklm} is the dependent variable; S_i is the random effect of square (i = 1 to 3); $C_{j(}S_{i)}$ is the random effect of cow nested in square (j = 1 to 3); P_k is the random effect of period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, AMt or PM); H_m is the fixed effect of hour of measurement (m = 0, 2, 4, 6, 9, 11, 13, 15 and 17); T_l x H_m is the fixed effect of the interaction of treatment and hour of measurement; and e_{ijklm} is the residual error.

Differences among treatments were declared significant at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$ using the PDIFF option.

RESULTS AND DISUSSION

One cow was removed for statistical analyses of rumen variables. All evaluations made on the previous mentioned cow were removed for statistical analyses during the second period. Because only four measurements through the day were carried out for VFAs and PUN, the effect of hour as well as the interaction between treatment and hour were not presented, and therefore results will be presented as main effects of treatments. For AMt the intake of TA averaged 166 ± 11 g/d, which represent a dietary concentration of 8.7 ± 4 g/kg of DM. Data of nutrient intakes, behavioral events and performance of this experiment were presented in Pozo et al. (submitted).

Rumen Fermentation

Treatments did not affect the VFA concentrations, however AMt presented a smaller proportion of acetate than AM and PM (P <0.01), and higher proportion of propionate than PM (P <0.05; Table 3). The impact of tannins on ruminal concentration and proportion of VFA depends on level used and sources (Bhatta et al., 2009). For example, Beauchemin et al. (2007) shown a linear tendency to reduce the ruminal concentrations of total VFA in beef cattle fed a forage-based diet supplemented with 10 to 20 g quebracho TE/kg of DMI. In the same way, the inclusion of 30 g/kg of quebracho TE in a TMR reduced the ruminal concentration of VFA (Dschaak et al., 2011). In contrast, no effects were reported with level of quebracho TE supplementation lower than 20 g/kg of DM (Benchaar et al., 2008; Aguerre et al. 2016). Similar pattern of VFA proportions to those found in our study were reported in forage-fed sheep supplemented with 41 g/kg of DM of TA (Carulla et al., 2005). Thus, based on literature, at level inclusion of TA used in our study (8.7 g/kg of DMI) was not expected any change on VFA concentration or proportions. In our experiment the concentrations of individually VFA were not affected by treatments, so the reduction of acetate proportion in AMt was due to a numerically higher concentrations of propionate and butyrate which would not imply in significant changes on VFA profile. On the other hand, PM presented similar ruminal concentrations or molar proportions of VFA than AM and AMt, but increased the acetate-to-propionate ratio and the acetate+butyrate-to-propionate ratio. This results are consistent with a higher percentage of pasture in the diet observed in PM (Pozo et al., Submitted).

Average ruminal pH, concentrations of NH_3-N and reducing sugars were not affected by treatments (Table 3), but an interaction between treatment and hour was detected for those variables (P <0.01; Figure 1). This interaction was strongly influenced by the different feeding management through the day in PM compared to AM and AMt. Independently of treatments, the minimal values of pH and higher concentration of ruminal NH_3-N and reducing sugars were observed at 4, 2 and 6 h after TMR supply. Those ruminal kinetics patterns can be explained because the intake of TMR as well as its DMI rate were higher than pasture (Pozo et al. Submitted). The peak of NH_3-N concentration after beginning of TMR ingestion was 45 % higher in PM than AM and AMt (Figure 1). It was an unexpected result considering that TMR was the same in all treatments and its intake was lower in PM. However, this result could be consequence of a prolonged fasting period between two consecutive days, since concentration of metabolites in ruminal fluid can be affected by osmolarity and water volume in rumen, which are not constant throughout the day (Van Soest 1994; Van Thang et al. 2012). Ruminal concentration of NH_3-N and reducing sugars suggested no effects of treatments on protein or carbohydrate degradation. In a study with steers fed grass forage plus concentrate, the inclusion of 9 to

27 g TA/kg of DM reduced the concentration of ruminal NH_3-N and reducing sugars, which is consistent with a reduction on ruminal degradability of N and OM (Orlandi et al., 2015). In contrast, the intraruminally infusion of 20 to 60 g/kg of DMI of TA in wethers fed ryegrass (Kozloski et al., 2012) as well as the supplementation with 15 g/kg of DM of TA in steers fed maize silage plus concentrate (Avila et al., 2015) did not impact on concentration of ruminal NH_3-N and reducing sugars. However, this authors reported that TA reduced the ruminal degradability of N and OM, suggesting that concentration of metabolites in ruminal fluid could be a limited approach to infer on the quantitative digestion into the rumen (Avila et al., 2015). To our knowledge, there are no studies reporting the effect of grazing allocation on ruminal metabolites of cows feeding mixed diets. In an experiment with grazing dairy cows, the allocation of pasture in the evening did not impact on ruminal NH_3-N concentration, however the higher CP intake observed suggests an improvement on NH_3-N uptake by microbes (Ueda et al., 2016). In contrast, no effect on ruminal NH_3-N concentration were reported in supplemented dairy cows grazing in the afternoon (Abrahamnse et al., 2009; Pulido et al., 2015). In our experiment, the lack of effects of PM on fermentation parameters could be explained by a limited inclusion of pasture in total DM (< 45 %).

Microbial Protein Synthesis

In the present study AMt tended to present less purine derivatives to creatinine ratio than AM and PM (P <0.10; Table 4). This result are in concordance with Henke et al. (2016), and could suggest that TA reduces the flow of microbial protein to the duodenum. However, although urinary concentrations and excretion of purine derivatives, intestinal flow of microbial N, and efficiency of N use for microbial N synthesis were numerically lower in AMt, there are not a significant effect of treatments (Table 4). Microbial protein synthesis (MPS), depends on the availability of N compounds and carbohydrates in the rumen (Clark et al., 1992). So, a reduction of degradability of N compounds and fibrous carbohydrates induced by tannin inclusion could reduce the MPS. In this way, Orlandi et al. (2015) shown that the inclusion of 9 to 27 g TA/kg of DM tended to reduce linearly the duodenal flow of microbial N, which is consistent with a reduction of ruminal concentrations of NH_3-N and reducing sugars. However, only higher doses of TA (27 g/kg of DM) presented a duodenal flow of microbial N numerically lower than control treatment. In the same way, a study with dairy cows shown that only an inclusion of quebracho TE of 30 g/kg of DM was enough to reduce the excretion of purine derivatives in urine (Henke et al., 2016). Also, Avila et al. (2015) shown that the addition of 15 g TA/kg of DM did not affect the duodenal flow of microbial N nor ruminal concentrations of NH_3-N and reducing sugars. Thus, the results of literature and those presented in our study suggest that the level of TA used was not enough to reduce significantly the substrates for MPS. On the other hand, PM increased the NFC intake without affecting the N intake (Pozo et al., Submitted), which suggest an additionally energetic substrate for MPS. However, the low CP content of pasture reported in our experiment (12.8 %) suggest that MPS could be limited by N compounds instead of carbohydrates availability in all treatments. In consequence, when predicted from NRC (2001), the microbial N synthesis (MPS/6.25) were similar between AM or AMt and PM (254 vs. 260 g/d), suggesting that the changes on nutrient intakes induced by PM were minimal to impact on MPS.

Digestibility

Treatments did not impact on apparent digestibility of DM, OM, FDN nor true digestibility of OM, however PM tended to reduce the apparent digestibility of N, while AMt tended to present 2.2 % less N true digestibility than AM (P <0.10; Table 5). Previous studies shown that the dietary inclusion or ruminal infusion of increased level of TA induced a linear reduction on digestibility variables (Orlandi et al., 2015; Kozloski et al., 2012). Similarly, a reduction of digestibility variables were observed with addition of 15 and 40 g TA/kg of DM (Ávila et al., 2015; Carulla et al., 2005). However, in those trials the level of TA inclusion (≥ 9 g TA/kg of DM) were higher than the level used in our experiment. So, the low dietary inclusion of TA could explain the lack of effects of TA on most of digestibility variables. On another hand, a slight reduction on true digestibility of N was found in AMt compared to AM (*P* <0.10). True digestibility of N was estimated assuming that fecal NDIN was originated from indigested feed. This result could suggest that TA reduced the ruminal degradability of N compounds increasing the passage of dietary N to intestine, and the tannin-protein complex are not dissociate completely after rumen passage. In addition, to estimate N true digestibility we assumed that the excretion of fecal N from endogenous origin was reduced. However, is necessary to be careful about this result because the ingestion of tannins are associated with increased secretion of endogenous proteins, and increased desquamation of intestinal cells (Waghorn, 1996). In addition, the complex formed by tannin could not be soluble in neutral detergent (Makkar et al., 1995), which could underestimate the endogenous fecal N losses.

The accumulation of soluble carbohydrates through the day diluted the fibrous carbohydrates and CP of pastures (Gregorini, 2012), and this change on chemical composition could impact positively on digestibility (Gregorini et al., 2006) and *in vitro* gas production (Cajarville et al., 2015). In the current study, pasture sampled in the afternoon presented a numerically higher concentration of WSC and NFC, but lower concentration of CP than pasture sampled in the morning (Table 1). Also, the volume of gas measured *in vitro* was higher for pasture sampled in the afternoon (Table 2), which is consistent with the high WSC content. However, despite the changes on chemical composition of pasture, most of digestibility variables were not affected by PM, probably because the inclusion of pasture in total DM was less than 45 %. Furthermore, although little changes on DMI of pasture and TMR were reported PM compared to AM and AMt, the nutrients intake were similar for all treatments (Pozo et al., Submitted). In contrast, PM tended to reduce the apparent digestibility of N, which is consistent with a higher excretion of fecal DM (Table 6). However, the lack of effect of PM on true digestibility indicate a higher fecal excretion of endogenous N.

Nitrogen Partition

The variables related to N partitioning are presented in Table 6. Treatments did not affect N intake, milk N, MUN, BUN nor N use efficiency for milk production. Similarly, the excretion of fecal N was not affected by treatments, but AMt increased the excretion of fecal ADIN (P <0.01). Both AMt and PM reduced the excretion of urinary N and urinary urea-N compared to AM (P <0.05). Although treatments did not alter the quantity of N excreted in manure, AMt and PM presented lower urinary N to fecal N ratio than AM (P <0.01). Tannins were expected to reduce the ruminal CP degradation, reducing N loses via urine, and increasing the N flux from the rumen to the small intestine. These patterns of N partitioning were largely reported in studies where TA was included in the diet of non-lactating ruminants (Carulla et al., 2005; Ávila et al., 2015; Orlandi et al., 2015) or dairy cows (Grainger et al., 2009; Griffiths et al., 2013). However, in several of those experiment, the reduced excretion of urinary N was accompanied by an increased excretion of fecal N. In our experiment, ruminal concentration of NH_3-N or BUN and MUN did not allow to infer about any change on ruminal CP degradation induced by TA. However, the reduction of urinary N and urinary urea-N excretions without changes on excretions of fecal N in AMt could be consequence of a reduced ruminal degradability of N compounds. Considering that the intake of ADIN (fraction C of N) was similar between treatments, the increased fecal ADIN observed in AMt could suggest that tannin-protein complex was formed into the rumen and is not completely dissociate through the gastrointestinal tract. Also, if tannin-protein complex is dissociated in abomasum, tannin can rebind with feed or even with endogenous protein (Mueller-Harvey, 2006), which can increase the fecal ADIN (Makkar et al., 1995). On the other hand, PM was expected to increase the energy and N balance into the rumen, improving the microbial uptake of NH_3-N, reducing N loses via urine, and increasing the microbial N flux from the rumen to the small intestine. However, this hypothesis was not supported by the observed concentrations of ruminal NH_3-N, BUN or MUN. In addition, the lack of effect of PM on microbial N flow (Table 4) are not consistent with a higher intake of energetic substrate (NFC) observed in PM (Pozo et al., submitted). Therefore, we suppose that reduction of excretions of urinary N and urea-N observed in PM could be due to a combined effect of a reduced digestibility of N joined with an improved energy and N balance into the rumen.

At the environmental level the change of N partitioning from urine to feces observed in AMt and PM are very important, because fecal N is less volatile than urinary N, reducing the potential of NH_3 (Powell et al., 2011) and N_2O emissions from manure (Dijkstra et al., 2013). In addition, the fecal N bounded to tannins is expected to be released more slowly in the soil, which may be beneficial for long duration pastures and crops (Makkar, 2003).

CONCLUSION

Results of this experiment shown that the inclusion of low quantity of TA in the TMR, as well as the grazing in the afternoon were two easily applicable tools with potential to reduce the negative environmental impact from manure without significant effect on nutrient digestion of lactating dairy cows fed a diet combining pasture with total mixed ration.

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Table 1. Ingredients and chemical composition of TMR and pasture (with SD in parentheses) grazed in the morning (a.m.) or in the afternoon (p.m.)

		Pasture	
Item	TMR	a.m.	p.m.
Ingredients of TMR, % of DM			
Corn silage	60.0	-	-
Solvent-extracted soybean meal	19.5	-	-
High moisture corn grain silage	18.5	-	-
Urea	0.70	-	-
Sodium bicarbonate	0.40	-	-
Dicalcium phosphate	0.20	-	-
Calcium carbonate	0.20	-	-
Salt	0.20	-	-
Vitamin and mineral premix[1]	0.30	-	-
Nutrient composition			
DM, %	41.0	18.6 (2.1)	19.6 (2.5)
OM, % of DM	94.0	87.9 (1.1)	88.7 (1.2)
WSC, % of DM	-	6.9 (1.2)	8.9 (1.4)
NDF, % of DM	27.1	45.9 (5.2)	43.4 (7.9)
ADF, % of DM	16.6	27.1 (2.7)	26.3 (3.8)
ADL, % of DM	1.3	3.7 (1.1)	3.6 (1.6)
NFC, % of DM	46.7	28.7 (5.3)	33.0 (7.7)
EE, % of DM	3.7	2.1 (0.04)	1.8 (0.16)
CP, % of DM	16.5	13.3 (1.4)	12.3 (1.0)
CP fractions, % of CP[2]			

$A+B_1$	48.6	21.5 (1.5)	21.3 (4.8)
B_2+B_3	50.0	75.6 (2.5)	75.7 (5.8)
C	1.4	2.9 (1.0)	3.0 (1.4)
NE_L, Mcal/kg of DM	1.87	1.33 (0.08)	1.36 (0.13)
WSC to CP ratio	-	0.53 (0.15)	0.73 (0.15)

[1]Provided (per kg DM): 125 g of Procreatin 7®, 125 g of Precisión Mix Vacas Lecheras®, 38 g of Rumensin® 200, and 713 g of excipients.

[2]CP fractions, $A+B_1$ = soluble protein, B_2+B_3 = potencially degradable protein, and C = indegradable protein.

Table 2. *In vitro* evaluation of TMR and pasture (with SD in parentheses) grazed in the morning (a.m.) or in the afternoon (p.m.)

	TMR		Pasture	
Item	without TA	with TA	a.m.	p.m.
In vitro gas production, mL/g DM	203	191	186 (2.4)	192 (4.7)
Kd, %/h[1]	4.80	4.38	5.66 (0.80)	5.84 (0.97)
Lag time, h	1.97	2.24	1.13 (0.25)	1.00 (0.34)

[1]Fractional rate of gas production.

Table 3. Effect of tannin and managing the grazing schedule on ruminal metabolites

Item	Treatment[1]			SEM	*P*-value
	AM	AMt	PM		
pH	6.33	6.29	6.34	0.04	0.69
NH_3-N, mg/ dL	10.6	11.4	10.8	2.27	0.63
Sugars, mg/ dL	61.0	59.6	58.9	2.68	0.74
VFA, mmol/L					
Acetate	64.5	65.3	66.9	8.53	0.83
Propionate	20.1	24.4	19.8	3.78	0.27
Isobutyrate	0.72	0.71	0.77	0.15	0.74
Butyrate	15.6	18.4	19.1	3.79	0.18
Isovalerate	1.07	1.09	1.03	0.13	0.87
Total	102.0	109.9	107.7	15.1	0.55
VFA, mol/100 mol					
Acetate	64.1^{a}	61.0^{b}	63.3^{a}	1.84	<0.01
Propionate	19.6^{ab}	20.9^{a}	18.3^{b}	0.74	0.02
Isobutyrate	0.71	0.67	0.72	0.06	0.65
Butyrate	14.5	16.5	16.5	1.56	0.11
Isovalerate	1.03	1.02	1.06	0.13	0.94
Acetate/propionate	3.28^{b}	3.02^{b}	3.60^{a}	0.14	<0.01
(Acetate+Butyrate)/propionate	4.02^{b}	3.87^{b}	4.54^{a}	0.15	<0.01

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

Table 4. Effect of tannin and managing the grazing schedule on purine derivatives and microbial N flow

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
Creatinine, mmol/L	5.1	5.2	5.3	0.62	0.67
PD,[2] mmol/L					
Alantoin	12.5	11.3	13.4	2.19	0.15
Uric acid	1.07	0.99	1.17	0.17	0.27
Total	13.6	12.3	14.6	2.34	0.15
PD excretion, mmol/d					
Alantoin	265	232	267	19.4	0.12
Uric acid	22.9	20.0	23.3	1.93	0.16
Total	288	252	290	20.3	0.12
PD/creatinine[3]	2.7^{x}	2.3^{y}	2.7^{x}	0.17	0.07
Microbial N flow, g/d	208	177	211	17.3	0.11
EUN,[4] %	44.4	38.7	46.0	4.10	0.13

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

[2]PD = purine derivatives.

[3]PD and creatinine concentration in mmol/L.

[4] EUN = use efficiency of ingested N for microbial protein synthesis.

Table 5. Effect of tannin and managing the grazing schedule on digestibility of nutrients

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
Apparent digestibility, %					
DM	62.2	63.2	58.8	5.13	0.12
OM	65.3	66.0	62.1	4.88	0.13
NDF	51.6	52.0	48.1	7.37	0.61
N	60.2^{x}	60.7^{x}	54.5^{y}	6.25	0.09
True digestibility, %					
OM	81.8	81.6	80.6	2.74	0.75
N	89.4^{x}	87.2^{y}	88.4^{xy}	1.03	0.07

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

Table 6. Effect of tannin and managing the grazing schedule on N partition

	Treatment[1]				
Item	AM	AMt	PM	SEM	*P*-value
N intake, g/d	468	467	459	23.2	0.17
N fractions intake,[2] g/d					
$A+B_1$	180	180	176	7.5	0.11
B_2+B_3	279	278	274	17.8	0.27
C	9.0	9.0	9.0	1.10	0.99
BUN, mg/dL	13.2	13.3	14.0	1.56	0.47
MUN, mg/dL	20.9	18.9	22.6	2.25	0.23
Milk N excretion, g/d	119	116	118	9.70	0.55
Milk N/N intake	0.25	0.25	0.26	0.18	0.44
Fecal excretion					
Fecal output, kg DM/d	7.1^{xy}	7.0^{y}	8.0^{x}	0.87	0.06
N, g/d	181	182	207	22.0	0.12
Fecal N/N intake	0.40^{y}	0.40^{y}	0.45^{x}	0.062	0.09
ADIN, g/d	8.3^{b}	17.6^{a}	10.3^{b}	1.29	<0.01
Urine excretion					
Urine volume, L/d	21.6	20.8	19.7	2.19	0.16
N, g/d	176^{a}	162^{b}	163^{b}	13.6	0.03
Urine N/N intake	0.38^{x}	0.35^{y}	0.35^{y}	0.019	0.09
Urea-N, g/d	105^{a}	90^{b}	91^{b}	16.1	0.01
Urea-N/urine N	0.59^{x}	0.55^{y}	0.55^{y}	0.060	0.09
Manure N excretion,[3] g/d	363	350	376	15.6	0.29
Urine N/fecal N	1.07^{a}	0.93^{b}	0.80^{c}	0.171	<0.01

Urine N/creatinine[4]	14.5[a]	13.1[b]	13.5[b]	1.05	0.02

[1]AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = morning TMR without TA and afternoon grazing.

[2]N fractions, $A+B_1$ = soluble N, B_2+B_3 = potentially degradable N, and C = undegradable N.

[3]Manure N excretion (g/d) = urinary N excretion (g/d) + fecal N excretion (g/d).

[4]Urine N and creatinine concentration in g/L.

Pozo et al. Figure 1.

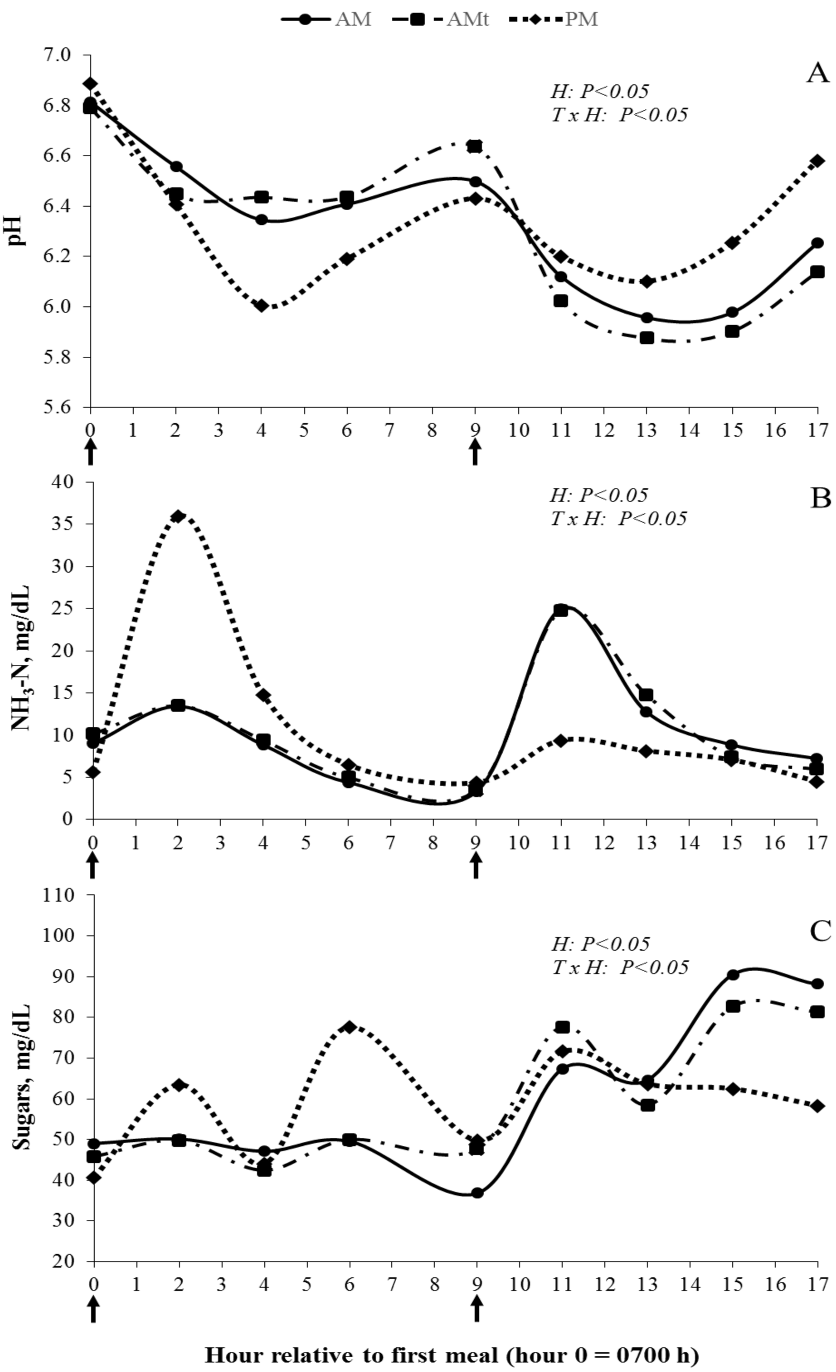

Figure 1. Effect of tannin and managing the grazing schedule on kinetics of ruminal pH (A) and concentration of NH_3-N (B), and reducing sugars (C).

AM = morning grazing and afternoon TMR without TA; AMt = morning grazing and afternoon TMR added with 15 g TA/kg of DM; PM = and morning TMR without TA and afternoon grazing. The arrows indicate the meals.

CONCLUSÃO

Conclui-se que a utilização de duas estratégias de fácil aplicação na pratica como a inclusão de baixas quantidades de TA na RTM e a mudança do horário de pastejo de manha para a tarde são eficazes para reduzir a excreção de N urinário sem impactar negativamente a digestão de nutrientes nem o desempenho produtivo de vacas leiteiras.

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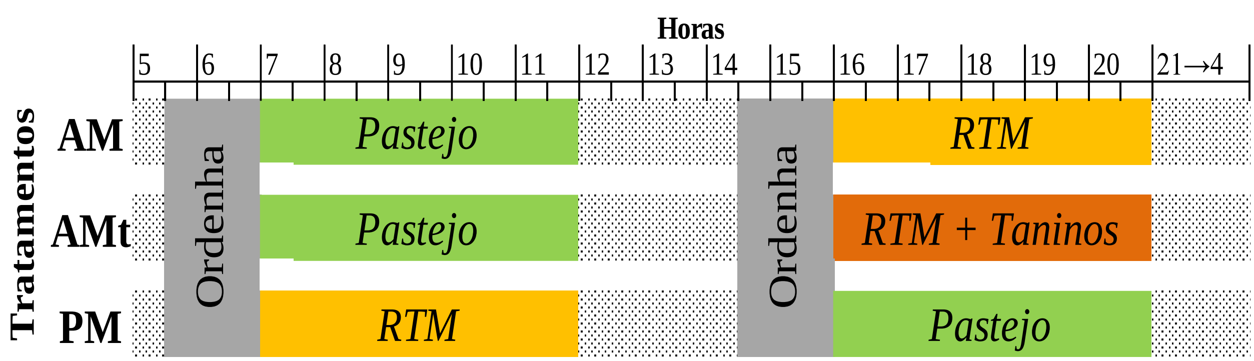
ANEXOS

ANEXO 1. Publicações incluídas na avaliação do efeito de extratos taníferos sobre a digestibilidade da matéria orgânica e as excreções de N.

Referência	Especie	Alimento	Fonte de ET	Niveis de ET (% MS)
Komolong et al., 2001	Ovinos	Feno de alfafa	*Schinopsis spp.*	0 a 5,45
Carulla et al., 2005	Ovinos	Azevém + trébol vermelho + alfafa	*Acacia mearnsii*	0 y 4
Bengaly et al., 2007	Caprinos	Alfafa + concentrado	*Acacia mearnsii*	0 a 3
Al Dobaib, 2009	Ovinos	Feno de alfafa	*Schinopsis spp.*	0 a 2,25
Kozloski et al., 2012	Ovinos	Azevém	*Acacia mearnsii*	0 a 6
Orlandi et al., 2015	Bovinos	Aveia + concentrado	*Acacia mearnsii*	0 a 2,7

ANEXO 2 - Tratamentos e manejo alimentar durante o dia.



AM = pastejo de manha e RTM sem tanino de tarde; AMt = pastejo de manha e RTM com 15 g/kg de MS de tarde; PM = RTM sem tanino de manha e pastejo de tarde.

ANEXO 3 – Determinacoes experimentais.