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EFEITO ANTI-HELMÍNTICO DA SUPLEMENTAÇÃO COM *Mimosa caesalpiniifolia* EM CAPRINOS SUBMETIDOS A UMA FLORESTA TROPICAL DECÍDUA.

NATHALIE SILVA PINTO

CHAPADINHA

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Dissertação apresentada ao Programa de Pós Graduação em Ciência Animal da Universidade Federal do Maranhão, como requisito parcial para obtenção do título de Mestre em Ciência Animal.

Orientador: Lívio Martins Costa Junior.

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“Não é o que você faz, mas quanto amor você dedica no que faz que realmente importa”.

(Sta. Madre de Tereza Calcutá)

À Deus, à Nossa Senhora e à minha família que são as razões do meu viver.

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SUMÁRIO

LISTA DE SIGLAS E ABREVIAÇÕES

ADF	Acid Detergent Fiber
AH	Acid Detergent Fiber
BC	Bite categories
BW	Body Weight
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CP	Crude Protein
CT	Condensed Tannin
DM	Dry Matter
DMI	Dry Matter Intake
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
FAPEMA	Fundação de Amparo à Pesquisa do Maranhão
FTD	Floresta Tropical Decídua
FEC	Faecal Egg Count
FECR	Faecal Egg Count Reduction
GIN	Gatrointestinal Nematodes
IBGE	Instituto Brasileiro de Geografia e Estatística
IMS	Ingestão de Matéria Seca
INMET	Instituto Nacional de Meteorologia
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MM	Mineral Matter
MS	Matéria Seca
NDF	Neutral Detergent Fiber
OH	Hidroxila
OM	Organic Matter
OPG	Ovos por grama de fezes
PEG	Polietilenoglicol
PSM	Plant Secondary Metabolities
TC	Tanino Condensado
TDF	Tropical Decidous Forest

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RESUMO

A inclusão de produtos e subprodutos de plantas ricas em taninos, na dieta de pequenos ruminantes é uma alternativa no controle de *Haemonchus contortus*, haja vista a resistência dos parasitos ao tratamento tradicional baseado na utilização de bases químicas. O uso de florestas tropicais decíduas (FTD) é um recurso importante na produção de caprinos, devido à grande diversidade de plantas forrageiras ricas em proteínas e taninos condensados, que podem ser importantes no controle de nematódeos gastrointestinais. Mas como esta vegetação heterogênea sofre mudanças drásticas devido ao efeito climático, ocorrendo alterações na disponibilidade de alimento ao longo do ano, vale a pena avaliar o efeito de uma suplementação em animais submetidos a mesma. O objetivo do estudo foi avaliar o efeito da suplementação com *Mimosa caesalpiniifolia* sobre *H. contortus* em caprinos sob pastejo em FTD. Para confirmar a atividade *in vitro* da amostra de *M. caesalpiniifolia,* testes de eclodibilidade de ovos e desembainhamento larval foram realizados. Vinte e quatro caprinos, machos, castrados, mestiços anglo nubiano, de seis a oito meses de idade, foram infectados com uma única dose de 4000 larvas infectantes de *H. contortus*, 28 dias antes do início do experimento. Os animais passaram uma semana em adaptação à dieta experimental, após isso foram distribuídos em dois grupos de acordo com a contagem de ovos por grama de fezes (OPG) e peso corporal. Grupo I: animais infectados que receberam suplemento diário contendo *M. caesalpiniifolia* (128,7 mg de tanino condensado (CT) / kg) e Grupo II: animais infectados que receberam suplemento diário sem inclusão de *M. caesalpiniifolia*, ambos os grupos terão acesso à vegetação heterogênea das 8:00 às 16:00. Todos os animais receberam suplementação isoproteica e isoenergética. Foram realizados OPG três vezes por semana. Além disso, um ensaio de eclodibilidade de ovos foi realizado uma vez por semana para ambos os grupos. O método de observação direta foi aplicado em 4 caprinos para registrar as espécies de plantas consumidas e categorias de bocado (CB), por meio de gravações de áudio. Os circuitos de pastagem duraram 8 h por dia, foram realizados quatro dias de observação. Após 28 dias de alimentação experimental, todos os animais foram abatidos humanamente e a contagem de vermes adultos foi realizada. No ensaio *in vitro*, observou-se um efeito inibitório no desembainhamento de 90,27% e 86,12% nas concentrações de 1,2 mg / ml e 0,6 mg / ml, respectivamente. Após o tratamento com 10 mg/ ml (maior concentração) foi observado no teste de eclodibilidade um efeito inibitório de 42%. Não houve efeito da dieta experimental sobre ganho de peso e a eficiência na redução da contagem de ovos fecais (FECR) foi de 25,1% para os animais que foram suplementados com *Mimosa caesalpiniifolia* em comparação com os animais que não receberam essa planta na suplementação, na última semana de experimento. Não houve efeito significativo da suplementação com *M. caesalpiniifolia* na redução do número de adultos. (P>0,05). Um total de 20 espécies de plantas da FDT foi consumido. Os valores médios de IMS foram de 133,7 g / dia para animais suplementados com tanino e de 68,9 g / dia para animais não suplementados com tanino, diferença significativa (P <0,05). A ingestão de PB (g/MS/ dia) para os animais suplementados com tanino foi maior que para os taninos não suplementados, com média de 14,2 e 6,2, respectivamente (P <0,05). Os animais suplementados com tanino ingeriram 7,1 g/dia e os animais não suplementados ingeriram 4,1 g/dia de TC na FTD (P>0,05). Existe um potencial significativo no uso de *M. caesalpiniifolia* na dieta de caprinos para o controle de *H. contortus*, mas há necessidade de outros estudos para esclarecer com mais detalhes como o efeito anti-helmíntico dos taninos ou outros polifenóis presentes na planta em estudo, se desenvolve. O uso de uma FDT é uma alternativa possível, na nutrição de caprinos, mas depende do conhecimento sobre a qualidade da dieta e o equilíbrio de nutrientes.

Palavras-chave: *Haemonchus contortus*; Taninos Condensados; Vegetação Heterogênea.

ABSTRACT

The inclusion of products and by - products of plants rich in tannins in the diet of small ruminants is an alternative in the control of *Haemonchus contortus*, due to the resistance of the parasites to the traditional treatment based on the use of chemical bases. The use of deciduous tropical forests (FTD) is an important resource in goat production, due to the great diversity of fodder plants rich in protein and condensed tannins, which may be important in the control of gastrointestinal nematodes. However, as this heterogeneous vegetation undergoes drastic changes due to the climatic effect, occurring changes in the availability of food throughout the year, it is worth to evaluate the effect of a supplementation in animals submitted to it. The objective of this study was to evaluate the effect of *Mimosa caesalpiniifolia* supplementation on *H. contortus* in goats under grazing on FTD. To confirm the in vitro activity of the *M. caesalpiniifolia* sample, egg hatchability tests and larval sampling were performed. Twenty-four male, castrated, Anglo-Nubian mestizos, six to eight months old, were infected with a single dose of 4000 infective larvae of *H. contortus* 28 days before the start of the experiment. The animals spent one week in adaptation to the experimental diet, after which they were distributed in two groups according to the egg count per gram of feces (OPG) and body weight. Group I: infected animals receiving a daily supplement containing *M. caesalpiniifolia* (128.7 mg of condensed tannin (CT) / kg) and Group II: infected animals receiving a daily supplement without *M. caesalpiniifolia*, both groups will have access to heterogeneous vegetation from 8:00 am to 4:00 p.m. All animals received isoprotein and isoenergetic supplementation. The FEC were carried out three times a week. In addition, an egg hatchability assay was performed once a week for both groups. The direct observation method was applied in 4 goats to record the species of plants consumed and categories of bite (CB), through audio recordings. The pasture circuits lasted 8 hours per day, four days of observation were carried out. After 28 days of experimental feeding, all animals were slaughtered humanely and adult worms were counted. In the in vitro assay, a 90.27% and 86.12% draw inhibitory effect was observed at the concentrations of 1.2 mg / ml and 0.6 mg / ml, respectively. After treatment with 10 mg / ml (higher concentration) an inhibitory effect of 42% was observed in the hatchability test. There was no effect of the experimental diet on weight gain and the efficiency of the FECR reduction was 25.1% in the supplemented group compared to the supplemented group in the last week of the experiment. There was no significant effect of *M. caesalpiniifolia* supplementation on the reduction of the number of adults. ($P > 0.05$). A total of 20 species of FDT plants were consumed. The medium values of DMI was 133.7 g/day for tannin supplemented animals and 68.9 g/day for tannin non-supplemented animals, it was significantly difference ($P<0.05$). The intake of CP (g/DM/day) for tannin supplemented animals was greater than for tannin non-supplemented animals, medium values were of 14.2 and 6.2, respectively ($P<0.05$). Tannin supplemented animals ingested 7.1 g/day and non-supplemented animals ingested 4.1 g/day of TC in TDF ($P>0.05$). There is significant potential in the use of *M. caesalpiniifolia* in the goat diet for the control of *H. contortus*, but there is a need for further studies to clarify in more detail how the anthelmintic effect of tannins or other polyphenols present in the plant under study, develops. The use of an FDT is a possible alternative in the nutrition of goats, but it depends on the knowledge about the quality of the diet and the nutrient balance.

Keywords: Haemonchus contortus; Condensed Tannins; Heterogeneous vegetation.

CAPÍTULO 1

CONSIDERAÇÕES GERAIS

1. Introdução

O rebanho nacional de caprinos alcançou 8.851.879 cabeças, sendo 8.109.672 cabeças na Região Nordeste (91,6%), o Brasil concentra hoje o 22º rebanho mundial de caprinos (IBGE, 2014; MAPA, 2014). A caprinocultura de corte no Brasil é uma alternativa de desenvolvimento econômico e social, capaz de reduzir a marginalização, constituindo-se em instrumento gerador de emprego e renda no campo, porém a cadeia produtiva de carne caprina no país é ainda bastante frágil, havendo deficiência de entrosamento e de conhecimento dos problemas dos diferentes atores em relação às dificuldades das diversas áreas que compõem a cadeia por isso é necessário que haja fortalecimento da cadeia produtiva, com base no estabelecimento de uma política nacional para o setor, que possibilite o desenvolvimento de pólos de produção de caprinos e processamento da carne e produtos derivados que tenham maior atração nos mercados interno e externo (COUTO 2001; MEDEIROS 2003).

A criação de pequenos ruminantes é uma das mais importantes atividades econômicas no semiárido nordestino, sendo desenvolvida por todas as classes sociais (Gomes et al., 2007; SILVA, 2004). Estas atividades são um atrativo, pois apresentam retorno financeiro rápido devido ao ciclo curto de produção, com aproximadamente 270 dias (VAZ, 2007).Com vantagens sobre carne bovina, principalmente pelos baixos teores de gorduras, colesterol e calorias, a carne caprina tão logo terá sua oferta consolidada, dos pontos de vista quantitativo e qualitativo (Santos et al., 2009).

Os rebanhos caprino e ovino apresentam baixo nível de desempenho (GUIMARÃES, 2006). Situação essa observada, quando se avalia o desempenho das explorações desses pequenos ruminantes, caracterizada por baixa produtividade, pequena agregação de valores aos produtos e reduzida adoção de tecnologias e procedimentos gerenciais, resultando em animais com baixa velocidade de crescimento, abate tardio, baixo rendimento de carcaça, baixa produção leiteira e altas taxas de mortalidade (Lima et al., 2006). A ovinocaprinocultura, ainda utiliza práticas de manejo e tecnologias nem sempre adequadas, o que favorece o aumento dos problemas de saúde (Martins Filho e Menezes, 2001), sendo os nematódeos gastrintestinais um dos principais entraves ao crescimento deste segmento, que representando o maior e mais grave problema sanitário dos pequenos ruminantes, chegando a inviabilizar economicamente a criação (VIEIRA, 2008).

Os animais são parasitados por diferentes espécies ao mesmo tempo, sendo que as mais importantes e comuns nas regiões tropicais são: *Haemonchus contortus, Trichostrongylus colubriformis*, *Strongyloides* spp., *Cooperia* spp. e *Oesophagostomum columbianum* (Amarante et al., 1997; Buzzulini et al., 2007).

O controle destas parasitoses é imprescindível para o sucesso dos sistemas de produção de ruminantes. O uso indiscriminado de drogas antiparasitárias pelos criadores, sem que se faça estudo sobre a prevalência do agente parasitário, tem provocado o aparecimento de resistência dos parasitos à ação dos medicamentos utilizados, podendo constituir-se em um dos principais problemas sanitários da produção animal (Paiva et al., 2001). As implementações de alternativas de controle deverão reduzir o número de vermifugações, em que os anti-helmínticos devem ser utilizados apenas de maneira complementar em esquemas de manejo que visem minimizar a utilização das drogas e, ao mesmo tempo, maximizar a produtividade do rebanho.

2. *Haemonchus contortus*

2.1. Ocorrência e Distribuição Geográfica

O *H. contortus* é um nematódeo gastrintestinal hematófago altamente patogênico, principalmente para pequenos ruminantes, com distribuição nas regiões tropicais e subtropicais (Besier et al., 2016; KOTZE; PRICHARD, 2016). Esse parasito é capaz de estabelecer-se e viver em diferentes ecossistemas, essas adaptações são caracterizadas devido a padrões específicos de transcrição e expressão gênica (KAPUR; SOOD, 1987). Condições ambientais quentes e úmidas são necessárias para a sobrevivência dos estádios de vida livre de *H. contortus*, as mesmas determinam as distribuições geográficas e sazonais do parasito e a prevalência do mesmo, particularmente nas zonas climáticas tropicais de ambos os hemisférios (O' Connor et al., 2006).

Na região Nordeste do Brasil, o período chuvoso promove elevações significativas na pluviosidade, criando condições ideias de temperatura e umidade para sobrevivência das larvas na pastagem e o seu maior desenvolvimento em menor tempo possível, por isso há elevada prevalência de *H. contortus* nessa região, que parece estar associada às condições favoráveis a manutenção de seu ciclo de vida (Silva et al., 2003).

2.2. Biologia

As fêmeas de *H. contortus* eliminam seus ovos junto com as fezes do hospedeiro, e em condições ideias os ovos e as larvas se desenvolvem no ambiente até larvas de terceiro estágio

(L3), em aproximadamente cinco dias. Do ovo eclode a larva de primeiro estágio (L1), que desenvolver-se à para larva de segundo estágio (L2), ambas são rabtidiformes e alimentam-se de bactérias encontradas no bolo fecal do hospedeiro, o próximo estágio larvar é o terceiro (L3) que é a forma infectante com cerca de 650 a 825 µm de comprimento, a bainha da cauda é média e termina de forma aguda, possuem 16 células intestinais, esôfago tipo filariforme e possui uma bainha protetora, portanto não se alimenta. A larva infectante é bastante móvel e migra para a pastagem (SANCHO, 2009; ONYAH; ARSLAN, 2004). Os ruminantes se infectam ao ingerir larvas infectantes durante o pastejo, ao passar pelo rúmen a bainha protetora é removida, depois disso a larva alcança o abomaso, penetra na mucosa e atinge o quarto estágio larvar (L4) após 48 horas, a partir daí desenvolvem uma lanceta perfurante na extremidade anterior, que lhes permitem obter o sangue dos vasos sanguíneos da mucosa do abomaso do hospedeiro, existe ainda um quinto e último estágio larvar, antes de se tornarem-se adultos (SOULSBY, 1987; ZAJAC, 2006). Segundo Amarante (2014) os vermes adultos medem de 1 a 3 cm de comprimento e são facilmente observados a olho nu, as fêmeas são maiores que os machos e em condições favoráveis a capacidade de oviposição das mesmas varia entre cinco a dez mil ovos por dia (Ueno e Gonçalves, 1998).

2.3. Sintomatologia Clínica e Diagnóstico

A sintomatologia clínica de *H. contortus* que é anemia, perda de peso e edemas subcutâneos está relacionada à característica hematófaga dos vermes adultos e dos últimos estágios larvais (BOWMAN, 2014; DUNN, 1978; LEVINE, 1980). Nos casos de infecção grave, animais são encontrados mortos e a maioria daqueles que sobrevivem tem sinais de anemia grave, encontram-se fracos, debilitados, apáticos e com pequeno ganho de peso (FREITAS, 1982; Cavalcante et al., 2009).

Se os animais não forem tratados, as mortes continuarão e as perdas irão aumentar ao longo dos dias. Entretanto, é comum entre os indivíduos, uma variação na susceptibilidade à hemoncose, caracterizando tanto a resistência ao estabelecimento do verme como a resiliência aos efeitos da infecção (capacidade de recuperação da perda sanguínea ou boas condições hematológicas, prévias à infecção). Não é frequente, mas pode acontecer que todos os indivíduos de um grupo, infectados com *H. contortus*, apresentem sintomatologia clínica grave ou morram caso não sejam tratados. (ROBERTS; SWAN, 1982).

A hemoncose foi descrita pela primeira vez na África, em sistemas de criação em pastejo extensivo, onde os animais encontram-se frequentemente desnutridos, devido ao grande efeito sazonal sobre esses ambientes (ALLONBY; URQUHART, 1975). Os sinais visíveis de anemia foram explorados como um indicador rápido e simples no diagnóstico de *H. contortus,* através do método FAMACHA, desenvolvido na África do Sul, que envolve a avaliação da coloração da mucosa conjuntival (VAN WYK; BATH, 2002). Após a contenção dos animais, os olhos são examinados, e a cor da conjuntiva é comparada contra um conjunto de cinco cores padronizado, que variam de um rosa avermelhado (normal) a branco (anemia severa) (Besier, et al., 2016).

O FAMACHA foi criado para a identificação dos animais que precisamde tratamento, reduzindo assim a seleção de parasitos resistentes, haja visto a frequente vermifugação de todos os animais do rebanho. Assim, há redução na proporção de vermifugações e os reincidentes são identificados e destinados ao abate, haja vista que o grau do FAMACHA é uma característica com alta herdabilidade, tanto para ovinos (RILEY; VAN WYK, 2009) como para caprinos (Mahieu et al., 2007).

A contagem de ovos nas fezes não é muito preciso ou sensível para determinar o nível da infecção, devido a relação variável entre o número de vermes no trato gastrintestinal do hospedeiro e o número de ovos nas fezes, além disso é comum uma variação ampla da carga parasitária entre animais do mesmo rebanho (BARGER, 1985; Whitlock et al., 1972). Felizmente, o diagnóstico feito por meio do OPG tem maior valor para *H. contortus* do que para outros nematoides que pertencem à família Trichostrongyloidea, porque existe uma relação relativamente forte entre a carga parasitária e a produção de ovos (COADWELL; WARD, 1982), e entre contagem total de vermes adultos e o OPG em caprinos (Rinaldi et al., 2009).

As técnicas mais utilizadas para realização de contagem de ovos nas fezes em laboratório são variações do método Mac Master (WITHLOCK, 1948), onde os ovos de helmintos são contados por flotação, em uma solução salina adicionada às fezes. Se houver necessidade de mais sensibilidade, faz-se a contagem de um maior número de câmaras ou examina-se uma maior quantidade de fezes, porém técnicas mais sensíveis, como o FLOTAC já foram desenvolvidas (Rinaldi et al., 2011).

2.4 Controle

2.4.1 Tratamento com Anti-helmínticos

Existem seis grupos de anti-helmínticos que são produzidos para o uso contra o *H. contortus*, dentre as principais bases químicas utilizadas, estão as lactonas macrocíclicas, imidatiazóis e bezimidazóis (Melo et al., 2003). A escolha do anti-helmíntico e do momento certo de sua utilização é fruto de um balanço entre a necessidade de tratamento e a prevenção, dos custos econômicos e mão de obra requerida, e se há possibilidade no desenvolvimento de resistência anti-helmíntica (BESIER, 2016). Porém, o uso indiscriminado desses compostos químicos por parte dos produtores, teve como consequência a seleção de nematoides resistentes a essas bases utilizadas no tratamento dos animais (VIEIRA; CAVALCANTE, 1999; MOLENTO, 2004; VIEIRA, 2008).

O aumento significativo de indivíduos que não são afetados por doses que seriam letais para maioria da população de indivíduos sensíveis da mesma espécie (VIEIRA, 2008) e quando não se verifica redução de adultos ou ovos excretados nas fezes após as vermifugações (Silvestre et al., 2002), ambos são conceitos adotados para resistência.

2.4.2 Controle Alternativo

Têm sido desenvolvida uma busca constante por métodos alternativos de controle, devido a resistência amplamente difundida aos anti-helmínticos sintéticos (Hay et al., 1997), por isso, é necessário preconizar a associação desses métodos adicionais com a utilização correta dos anti-helmínticos para controlar as infecções, com uma menor frequência de tratamentos possível e sem evitar por completo a exposição dos ruminantes aos parasitas, uma vez que este contato é necessário para o estímulo à resposta imune (Cezar et al., 2008).

O tempo de pastejo baseado em informações epidemiológicas visa minimizar a ingestão de larvas infectantes pelos animais e a contaminação excessiva das pastagens por ovos de *H. contortus* e reduz o risco de hemoncose (Besier et al., 2016). O pastejo rotacionado consiste na divisão da área de pastagem em piquetes que recebem elevada densidade animal por curtos períodos. Depois da retirada dos animais, há um intervalo para a recuperação do ponto ideal de pastejo. Este manejo visa prover o melhor aproveitamento da pastagem do ponto de vista nutricional, porém, pode ser compatível a um propósito anti-parasitário caso o período de permanência em cada piquete seja inferior ao período de desenvolvimento das larvas infectantes oriundas de ovos depositados nas fezes dos animais e o período de intervalo seja suficiente à destruição/inviabilidade destas larvas (Cezar et al., 2008). Outra alternativa é a alternância de caprinos ou ovinos com bovinos, reduzindo a contaminação das pastagens e das helmintoses dos ruminantes (SOUTHCOTT; BARGER, 1975; AMARANTE, 2004).

O controle biológico consiste no uso de antagonistas naturais para restringir a um limiar subclínico e economicamente aceitável a ação de parasitas por meio da diminuição da fonte de infecção para os hospedeiros finais. Há vários antagonistas naturais de nematódeos descritos, entre eles: vírus, bactérias, amebas, fungos, nematódeos de vida livre, anelídeos e artrópodes (Grønvold et al., 1996). Os atuais métodos de controle biológico de nematódeos enfrentam barreiras de custo/benefício, aplicabilidade e segurança da obtenção de resultados (WALLER, 2002).

O potencial da seleção genética de animais com alta resistência (WOOLASTRON; BAKER, 1996) e ou resiliência (BISSET; MORRIS, 1996) às infecções por nematoides, e, consequentemente a redução no uso de anti-helmínticos, tem sido reconhecida por muitos anos. A habilidade de animais em uma boa condição nutricional em resistir à infecções por nematoides e a suportar os efeitos das mesmas, é reconhecido há muito tempo (COOP; HOLMES, 1996). Há uma relação de proporcionalidade entre a qualidade da dieta e a intensidade da infecção do hospedeiro, sendo que sua imunidade aos parasitos é diminuída em condições de restrição nutricional (Cezar et al., 2008).

3. Compostos Anti-helmínticos de Produtos Naturais

A utilização de produtos naturais é outro método alternativo, que consiste na utilização de plantas e seus subprodutos no combate aos nematódeos gastrintestinais de ruminantes (ATHANASIADOU; KYRIAZAKIS, 2004), em geral dentro da medicina natural essa prática, é a mais antiga, o homem pré-histórico aprendeu com os animais, a distinguir as plantas comestíveis daquelas que podiam ajuda-lo a curar suas doenças (RATES, 2001).

A utilização de plantas tem surgido como uma alternativa para diminuir os problemas da resistência anti-helmíntica no controle de nematoides gastrintestinais em animais, sendo indicada, principalmente, por reduzir o custo dos tratamentos e prolongar a vida útil dos produtos sintéticos disponíveis no mercado (VIEIRA; CAVALCANTE, 1991).

A ação terapêutica dos extratos vegetais ou óleos essenciais está frequentemente associada a metabólitos secundários (Chagas, et al., 2004), entre eles encontram-se os taninos (Hoste et al., 2006). Os taninos são um grupo complexo de compostos polifenoicos que possuem elevado peso molecular, funcionam como defesa contra predadores (herbívoros, patógenos ou plantas competidoras por nutrientes e luz), conservadores de energia e nitrogênio, e protetores contra os efeitos deletérios dos raios ultravioletas (ACAMOVIC; BROOKER, 2005; WAGHORN; MCNABB, 2003). Tais compostos podem ser divididos de acordo com sua estrutura química e propriedades em dois grupos: taninos condensados (TC) e hidrolisados.

3.1 Taninos Condensados

Taninos condensados ou não hidrolisáveis são oligômeros e polímeros formados pela ligação de dois ou mais monômeros de flavan-3-ol (catequina) ou flavan-3-4diol. Essa classe de taninos também é denominada proantocianidina devido à produção de pigmentos avermelhados da classe das antocianidinas, tais como cianidina e delfinidina (Zuanazzi, 2000). A presença ou não da hidroxila (OH), assim como sua localização na estrutura dos monômeros de flavan-3-ol resulta em diferentes classificações dos taninos condensados. Desta forma os TC podem ser divididos em tipo 1, aquele que apresenta uma hidroxila na posição C- 5 do anel A e tipo 2, que não apresenta hidroxila na mesma posição (Zuanazzi, 2000).

Os taninos hidrolisáveis amplamente distribuídos em espécies arbóreas, em concentrações que podem alcançar 200 g/kg de matéria seca (MS) (REED, 1995), apresentam um açúcar central (D-glicose) na sua estrutura, ligado a grupos de ácido gálico (galotanino) ou ácido elágico (elagitanino) (HAGERMAN, 2002). O consumo excessivo pode resultar em alta mortalidade em caprinos e ovinos, devido a liberação do ácido gálico através da hidrólise dos ésteres de galoil, sendo metabolizado a compostos fenólicos tóxicos e estes absorvidos no rúmen (Reed et al., 2000).

Os taninos presentes nas espécies vegetais podem agir de forma benéfica ou adversa quando utilizados na alimentação animal, dependendo do tipo (condensado ou hidrolisável) (MAKKAR, 2003), concentração na planta, espécie vegetal, composição nutricional da dieta, categoria e estado fisiológico do animal (KUMAR; SINGH, 1984). Acredita-se que os TC se ligam com a cutícula dos nematoides, que é rica em prolina e hidroxiprolina alterando suas propriedades físico-químicas (THOMPSON; GEARY, 1995; Athanasiadou et al., 2000; Hoste et al., 2006), agindo assim de forma direta. Acredita-se também que a ação indireta do TC é resultado da estimulação da resposta imune do hospedeiro (KAHN; DIAZ-HERNANDEZ, 2000), devido ao fato desses compostos protegerem as proteínas da degradação ruminal, estando mais disponíveis para a absorção no intestino delgado (SCHWAB, 1995; BARRY; MCNABB, 1999; MIN; HART, 2003).

3.2 Plantas Taniníferas

Os taninos condensados estão presentes em concentrações variadas na maioria das leguminosas forrageiras tropicais (MAKKAR; BECKER, 1994) podendo alcançar até 300 g/kg de matéria seca (MS) (MUELLER-HARVEY, 2001).A utilização de TC oriundos de espécies como *Acacia mearnsii* (MAX, 2010), *Ceratonia siliqua* (Lanza et al., 2001), *Onobrychis viciifolia* (Hoste et al., 2005) e *Lotus corniculatus* (Min et al., 2001), mostraram efeitos positivos sobre o ganho de peso, produção de leite, produção de lã e atividade anti-helmíntica. As pesquisas realizadas utilizando-se forrageiras taniníferas na alimentação de ruminantes são de interesse econômico, social e ambiental (Silva et al., 2016). Makkar (2007) confirma a bioatividade de plantas no controle de nematódeos gastrintestinal em pequenos ruminantes, fazendo uso de extratos de folhas, frutos, ou sementes oriundas de diferentes regiões do mundo e obtidas com diferentes técnicas.

Hoste et al. (2005) registraram redução do número de OPG dos gêneros *Teladorsagia* e *Trichostrongylus* em caprinos leiteiros alimentados com feno de *O. viciifolia* (25,2 g de TC/kg de MS). Em grande parte dos sistemas de criação de pequenos ruminantes são utilizadas espécies taniníferas presentes no bioma brasileiro caatinga, característico da região Nordeste. Dentre estas podem ser citadas a *Caesalpinea bracteosa*, *Mimosa hostilis*, *Mimosa caesalpiniifolia* e *Bauhinia cheilantha* (Gonzaga Neto et al., 2001; Guimarães-Beelen et al., 2006).

3.3 *Mimosa caesalpiniifolia*

Formada por quatro mil espécies distribuídas em sessenta gêneros a família *Mimosaceae* é característica de regiões tropicais e subtropicais (Nunes et al., 2008). O gênero *Mimosa* é composto por espécies de plantas herbáceas e arbustivas, distribuídas predominantemente na América Central e do Sul. No Brasil há cerca de 340 espécies do gênero *Mimosa*, 60% dessas são muito comuns em diferentes regiões (GRETHER, 2000; SILVA; SECCO, 2000).

A composição química do gênero *Mimosa* inclui derivados triptofanos alcaloides (Moraes, et al., 1990), isoprenóides (diterpenos, triterpenos, caratenóides e esteroides (Kudritskaya et al., 1988), ácidos fenólicos e flavanóides (Souza et al., 2008).

Popularmente conhecida como “sabiá”, “angiquinho-sabiá” e “sansão-do-campo”, a *M. caesalpiniifolia* é uma planta nativa do Cerrado e da Caatinga Brasileira, é encontrada principalmente na região Nordeste (CARVALHO, 2007). Além disso, suas folhas secas ou verdes são usadas com frequência na nutrição de ovinos e caprinos, devido a seu alto teor proteico e mineral (Vieira et al, 2005).O extrato cetônico de *M. caesalpiniifolia*foram eficazes na inibição do desembainhamento larval e da eclodibilidade de ovos de *H. contortus e Trichostrongylus colubriformes* (Brito et al., 2017). In vivo o efeito da *M. caesalpiniifolia* na redução de vermes adultos nos grupos experimentais que receberam sabiá e sabiá+ polietilenoglicol (PEG) foi de 57,7 e 66,9%, respectivamente (Brito et al., 2018).

3.4 Floresta Tropical Decídua

A definição floresta tropical decídua (FTD) é bastante ampla, referem-se, em geral, às formações que ocorrem em regiões com duas estações bem definidas, uma seca e uma chuvosa (MURPHY; LUGO, 1986; Nascimento et al., 2004). No Brasil, ela está distribuída na maior parte no bioma do Cerrado, mas existem fragmentos no domínio da Caatinga e nas áreas de transição (PEDRALLI, 1997; SILVA, 2011). A FTD do Brasil é caracterizada por uma temperatura média anual de 25 ° C e a precipitação média anual varianado entre 700 e 2000 mm, com pelo menos três meses de chuva por ano (Sánchez-Azofeifa et al., 2005) e apresentando uma grande diversidade de espécies vegetais (OLIVEIRA-FILHO, 2006).

A vegetação heterogênea pode servir como base em dietas para ruminantes (GAUTIER; MOULIN, 2004). É necessário vincular o comportamento ingestivo dos ruminantes com a diversidade das plantas, considerando ambos os aspectos como processos interdependentes (Agreil et al., 2010), a avaliação do comportamento em uma FTD é realizada por meio de um monitoramento contínuo dos bocados realizados pelos animais dentro da pastagem, baseado no método de observação direta (MOD) (Agreil and Meuret, 2004). As categorias de bocados são identificadas usando uma tabela de codificação adaptada às condições do FDT por González-Pech et al. (2014).

Durante a estação seca em uma FTD, ovinos e caprinos consumiram de forma semelhante as espécies de plantas, os ovinos realizam menos bocados por dia do que os caprinos, bocados esses menores e mais leves (González-Pech et al., 2015).

Várias espécies de plantas da FTD contêm compostos secundários com alguma atividade anti-helmíntica e essas leguminosas contendo taninos foram submetidas a uma ampla gama de estudos como nutracêuticos potenciais (Torres-Acosta et al., 2012; Sandoval-Castro et al., 2012).

4. OBJETIVOS

Objetivo Geral

Avaliar o efeito de um suplemento contendo folha de sabiá sobre *Haemonchus contortus* e o comportamento ingestivo de caprinos em uma Floresta Tropical Decídua.

Objetivos Específicos

- Identificar a influência da ingestão de um suplemento com *Mimosa caesalpiniifolia* sobre o comportamento ingestivo em vegetação heterogênea;
- Determinar o efeito anti-helmíntico *in vitro* do extrato acetônico de *M. caesalpiniifolia*;
- Determinar o efeito anti-helmíntico *in vivo* da inclusão da folha de *M. caesalpiniifolia*;
- Avaliar o desempenho produtivo, determinando o ganho de peso dos animais.

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CAPÍTULO 2 [1]

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[1]Neste capítulo consta o artigo “Feeding behavior of goats in a tropical deciduous forest during the dry season supplemented with *Mimosa caesalpiniifolia*” que foi escrito de acordo com as normas para a publicação no periódico Small Ruminant Research.

Feeding behavior of goats in a tropical deciduous forest during the dry season supplemented with *Mimosa caesalpiniifolia*.

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Abstract

The use of tropical deciduous forest (TDF) is an important resource in the goat's production, due to the great diversity of fodder plants rich in protein and condensed tannins, which may be important in the control of gastrointestinal nematodes. However, as this heterogeneous vegetation on drastic changes due to the climatic effect and the availability of feed changes throughout the year it is worth evaluating the effect of a supplementation in animals submitted to grazing. This study aimed at describing the feeding behavior of goats supplemented with *Mimosa caesalpiniifolia* in a TDF during the grazing in the dry season. Four male goats, castrated, Anglo Nubian crossbreed, aged six to eight months were distributed into two groups according to fecal eggs count and body weight. Group I: infected animals receiving concentrate with tanniniferous food from *M. caesalpinifolia* (128.7 mg condensed tannin (CT)/kg) and Group II: infected animals receiving concentrate without *M. caesalpiniifolia*.All animals received isoproteic and isoenergetic supplementation and have daily access to TDF from 8:00 am to 4:00 pm. The continuos bite monitoring was based on the direct observation method. A total of 20 species of FDT plants were consumed. The medium values of DMI was 133.7 g/day for tannin supplemented animals and 68.9 g/day for tannin non-supplemented animals, it was significantly difference ($P<0.05$). The intake of CP (g/DM/day) for tannin supplemented animals was greater than for tannin non-supplemented animals, medium values were of 14.2 and 6.2, respectively ($P<0.05$). Tannin supplemented animals ingested 7.1 g/day and non-supplemented animals ingested 4.1 g/day of TC in TDF ($P>0.05$). There is significant potential in the use of *M. caesalpiniifolia* in the goat diet for the control of *H. contortus*, but there is a need for further studies to clarify in more detail how the anthelmintic effect of tannins or other polyphenols present in the plant under study, develops. The use of an FDT is a possible alternative in the nutrition of goats, but it depends on the knowledge about the quality of the diet and the nutrient balance.

Key Words: Heterogeneous vegetation; Artificial Infection; Small ruminants; Direct observation

Introduction

Livestock production systems worldwide rely largely on conventional feedstuffs. The current world feed crisis highlights the need to improve the use of local resources for animal nutrition, such as fodder trees and shrubs (Alonso-Díaz et al., 2010). The tropical deciduous forest (TDF) is an ecosystem characterized by a complex and heterogeneous array of different plant species and represents the most important source of feeding materials for sheep and goats produced in semi-intensive farms in tropical areas. Tropical trees and shrubs are important sources, for small ruminants of proteins and plant secondary compounds such as condensed tannins (CT) (Torres-Acosta et al., 2017; Ben Salem et al., 2005; Sandoval-Castro et al., 2005). The definition of these forests is quite broad, they refer, in general, to the formations trees occurring in regions with two well-defined dry and rainy seasons (Murphy and Lugo, 1986; Nascimento et al., 2004). In Brazil they are distributed, for the most part, within the Cerrado biome, also, fragments on the Caatinga domain and in transition areas (Pedralli, 1997; Silva, 2011).

Several plant species of the TDF contain secondary compounds with some anthelmintic (AH) activity and these tannins containing legumes have been subjected to a wide range of studies as potential nutraceuticals (Torres-Acosta et al., 2012; Sandoval-Castro et al., 2012). These include herbaceous plants, trees, and shrubs, which are perennials or annuals, and which are used by small ruminants in a wide range of feeding systems (Hoste et al., 2015). Condensed Tannin could also have a positive role for small ruminants in the TDF, they can counteract the high protein content of diet harvested in the TDF by complexing with protein, reducing the amount of protein digested in the rumen (Van Soest, 1994). Besides that, CT might also have beneficial effects on ruminant physiology, production and health (Ramírez-Restrepo et al., 2005; Hoste et al., 2006). Modern parasite management involves alternative approaches seeking to achieve an integrated and more sustainable control of helminth infections (Hoste and Torres-Acosta, 2011).

The *Mimosa caesalpiniifolia* is a native tannin-rich plant characterized by rapid growth, high capacity for regeneration and drought resistance (Podadera et al., 2015), commonly found in Brazilian Caatinga and Cerrado biome, mainly in the TFD from Brazilian Northeast region. This shrub is readily consumed by ruminants and is used as a food supplement in the dry season in Brazil because of its high crude protein content, which varies between 18.8 and 28.6% (Vieira et al., 2005). Triterpenes and phenolic compounds were identified from various parts (leaves, fruits, flowers, twigs and stem barks) of *M. caesalpiniifolia* (Lopes Citó et al., 2010). The *M. caesalpiniifolia* leaves were effective *in vitro* and *in vivo* against small ruminant gastrointestinal nematode (Brito et al., 2017, 2018).

Heterogeneous vegetation may serve as the basis of a complex diet for ruminants (Gautier and Moulin, 2004). To better utilize the plant secondary metabolites, are necessary studies to identify plant selection, feeding behavior, by small ruminants and possibly self-medication under grazing or browsing conditions (Hoste et al. 2015). It is important to associate the ruminant feeding behavior with plant diversity (Agreil et al., 2010). This study aimed at describing the feeding behavior of infected goats supplemented with concentrate containing *M. caesalpiniifolia* in a TDF during the grazing in the dry season.

Materials and Methods

Study site and collect of plant material

The study was performed in São Luís, Maranhão, Brazil (02º37’01’’S and 44º16’19’’W) from October to November 2017 (dry season). The accumulated rainfall during the study was less than 100 mm and the mean temperature was 34.5 ºC, with maximum of 40.6 ºC and minimum of 28.7 ºC (INMET, www.inmet.gov.br).

The leaves of *M. caesalpiniifolia* were collected in a city of Chapadinha, Maranhão, Brazil (03º44'30"S and 43º21'37"W) in march 2017. The leaves collected were dried under shade and crushed in disintegrator and ground with a mesh 0.25mm. The bromatological analysis were measured dry matter (DM) 105ºC (expressed in g/ Kg of green matter), mineral matter (MM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and crude protein (CP) (values expressed in g/ Kg of dry matter). Bromatological analyses were performed according to AOAC (2011) and Van Soest et al. (1991). The CT content was estimated using the HCl-butanol method (Porter et al., 1986) and were expressed as a leucocyanidin equivalent.

Experimental animals and Feeding conditions

All procedures were approved through the Ethics Committee for the Animal Experimentation of the Federal University of Maranhão, Brazil, under number 2311501861.

Twenty four male goats, castrated, Anglo Nubian crossbreed, six to eight months old were distributed into two groups according to body weight (BW) and fecal eggs counts performed as described by Gordon and Whitlock (1939). Two goats of each group were select randomly for the direct observations, the animals had 19.4 ± 3 kg of body weight (BW). The supplement was provided as soon as the animals returned from pasture and amount provided was determined according to body weight of the animal (3.07% of BW), the animals were weighed weekly and the amount of feed was adjusted. Group I: infected animals supplemented with tanniniferous food from *M. caesalpiniifolia* (128.7 mg CT/ Kg BW/day) equivalent to 1.32 g of *M. caesalpiniifolia* powder/ Kg BW /Day, and Group II: infected animals non supplemented with *M. caesalpiniifolia*. Two supplements feed were used: (1), with inclusion of the leaf powder of *M. caesalpiniifolia* (60.4% corn; 34.5% epicarp manioc bran; 4.3% *M. caesalpiniifolia*; 0,7% soybean) (2), without inclusion of plant (57.4% corn; 40% epicarp manioc bran; 2.5% soybean and 2% calcareous).

The animals of both groups had daily access to TDF from 8:00 am to 4:00 pm, the grazing circuit was covered in 8h. After finishing the grazing circuit, animals were introduced to individual pens where they received a supplement isoproteic and isoenergetic and fresh water ad-libitum.

Feeding behavior observation

The continuous bite monitoring was based on the direct observation method (DOM) (Agreil and Meuret, 2004) used to determine the feeding behavior of small ruminants. Experimental animals have gone through a period of adaptation to the presence of observers. Animals adapted to the presence of the observer could be observed at a distance greater than 1 m without affecting or modifying their activity. Familiarization period lasted 30 days and helped to avoid stress due to the presence of observers during the study. The animals adapted to the observation process allowed the observers to accurately identify the plant species and the parts of plants harvested along the grazing circuit. Four observers were trained in the identification of plant species in the TDF. All plants were herborized and identified, and a sample was deposited at the Herbarium of Maranhão (MAR), located in Federal University of Maranhão, Brazil (Almeida Jr, 2015).

The bite categories (BC) were identified using the coding grid of bites adapted to the conditions of the TDF by González-Pech et al. (2014). It included 33 different BC, each of which had a different monosyllabic code. The technique of manual harvesting described by Agreil and Meuret (2004), and recently validated by Bonnet et al. (2015), was used to determine the weight (g/DM) of each BC for the different plant species consumed. Samples included leaves, stems, leaves with stems, flowers, pods, fruits, mixtures of dry leaves and mixtures of pods from the relevant plant species and size of the correspondent BC. These samples were collected at the beginning and end of dry season, in the same location where the flock grazed.

Direct observations were performed in two weeks, an experimental animal was observed for 8h for four days. Experimental animals were closely followed while observers registered on a voice recorder the plants consumed as well as the corresponding BC. An observation period was performed during two weeks with two observation days each.

Audio recordings of plant species and BC codes were captured in respective spreadsheets for each experimental animal. After the direct observation, all the species ingested by the animals in TDF and their respective BC, were collected manually, including weighing of bits and the bromatological analysis of plants. With the weight and the nutritional composition, it was possible to determine the intake of the macronutrients (g/day).

Voluntary dry matter intake (DMI) of each animal was estimated as the product of BC weight (DM) and the total BC number from all the plants consumed. Based on DMI (g/ day), the crude protein (CP) and CT intake were determined.

Statistical Analyses

The data concerning on the estimation of DMI and DMI of *M. caesalpinifolia*, captured from audio recordings of direct observations, were compared by means of Student Test (GraphPad Inc., San Diego, CA, USA).

The BC were re-grouped into small bites (from 0 to 5 cm) and large bites (from 6 to 20 cm), to analyze the size of bites and were re-grouped according to the weight (g DM) into: (a) light (0.001–0.212 g), (b) medium (0.213–0.378 g), (c) heavy (0.379–0.477 g) and (d) extra-heavy (0.478–6.733 g). Such subdivisions corresponded to the quartiles of the BC weight data.

According Kolmogorov-Smirnov test and Levene's test the variables number of bites, size and weight of BC were not normally distributed and unequal variances, so it was used Chi-square tests to compare size and weight of bites and number bites displayed by goats. The results referring to the intake of DM, CP and CT were submitted to an analysis of variance by means of a two-way ANOVA

Results

From a total of 20 plant species of TDF consumed, fourteen were shrubs, two dicots herbaceous, two creep/climbers and two grasses, from which 15 were consumed by both experimental groups (Table 2). All plants consumed by tannin supplemented animals, were also consumed by tannin non-supplemented animals. The goats received concentrate without tanniniferous food from *M. caesalpinifolia* fed exclusively of four shrubs (*Davilla* sp., *Randia armata, Senna alata* and *Waltheria indica*) and one dicot herbaceous (*Cecropia pachystachya).*

The foliage of shrubs (*Mimosa caesalpiniifolia, Ouratea cuspidata, Ouratea* sp.*, Randia armata, Sida acuta, Vismia guianensis, Bluataparon vermiculare, Mimosa pudica* var *tetranda, Waltheria indica*, *Senna alata, Staelia virgata, Casearia arborea, Myrciaria tenella* and *Davilla* sp.) represented 65.8% and 58.0% of DMI for tannin supplemented and non-supplemented animals, respectively. The foliage of dicots herbaceous *(Mangifera indica)* represented 0.2% of DMI tannin supplemented animals and (*Cecropia pachystachya and Mangifera indica*) represented 1.0% of DMI for tannin non-supplemented animals. Grasses *(Cyperus laxus* and *Panicum maximum* var *massai*) represented 32.3% of DMI for tannin supplemented animals and 36.6% for tannin non-supplemented animals. Creep/Climber represented 1.7% of DMI tannin supplemented animals and 4.4% of DMI tannin non-supplemented animals

The total value of the number of bites were 2,885 for goats who received supplementation with *M. caesalpiniifolia* (Min: 1,303, Max: 1,582), and 1,263 for goats that received supplementation without *M. caesalpiniifolia* (Min: 540, Max: 723) (Table 2). There was no significant difference between the groups (P>0.05).

Were identified a total of 110 plant-bite combinations, from which 82 were harvested by goat tannin supplemented and 101 were harvested by goat tannin non-supplemented, plant species providing 97.6% and 89.6% of the respective DMI. Every plant species varied from one to twelve type of BC included leaves, stems, leaves with stems, flowers, pods, fruits and mixtures of dry leaves.

From the shared 82 plant-bite combinations, tannin supplemented animals performed (1,731/60%) small size bites and (1,154/40%) large size bites, harvested more small bites than tannin non-supplemented animals (P<0.005) (Table 3).

From the shared 93 plant-bite combinations, tannin supplemented animals performed different proportions of light (16.6% or 479/2,885) than tannin non-supplemented animals (9.8% or 124/1,263) (P<0.001). There was significant difference between the groups for consume of medium bites (P>0.05). Tannin non-supplemented animals harvested fewer heavy bites (42.6% or 538/1,263) and bigger extra-heavy bites (30.1% or 380/1,263) than tannin supplemented animals (57.7% heavy bites (1,665/2,885) and 11.4% extra-heavy bites (330/2,885)) (P<0.05) (Table 3).

The medium values of DMI was 133.7 g/day for tannin supplemented animals and 68.9 g/day for tannin non-supplemented animals, it was significantly difference (P<0.05). The intake of CP (g/DM/day) for tannin supplemented animals was greater than for tannin non-supplemented animals, median values were of 14.2 and 6.2, respectively (P<0.05). Tannin supplemented animals ingested 7.1 g/day and non-supplemented animals ingested 4.1 g/day of CT in TDF (P>0.05), considering the intake of TC coming of supplement, the animals supplemented ingested 8.5 g/day, intake higher than non-supplemented animals (Table 4). The supplement with *M. caesalpiniifolia* did affect the feed intake, the tannin supplemented and tannin non-supplemented animals had an average for this variable of 346 and 496g, respectively (P<0.05) (Figure 1).

Discussion

Goats as mixed or intermediate feeders that consume grasses and browses. These animals have developed different preferences for feed where goats generally consume a wider array of plant species and show a preference for a varied diet that consequently keeps the land area better grazed (Bojkovski et al., 2014). Considering the vastness of the potential plant pharmacy, animals are surrounded by powerful pharmacological substances and have ample opportunity to self-medicate (Rahmann and Seip, 2007; Kearney et al., 2016). Many tannin-rich plants have been studied as candidates with nutraceutical potential (Butter et al., 2000; Hoste et al., 2005). The condensed tannins present in several plants from TDF are considered responsible for the reduction in worm burden and/or for female fecundity, decreasing nematode egg excretion in the feces of the host and subsequently reducing pasture contamination, which leads to lower host re-infections (Hoste and Torres-Acosta, 2011; Martínez-Ortíz-de-Montellano et al., 2010; Lopes et al., 2016). *M. caesalpiniifolia* is Brazilian native tannin-rich plant readily consumed by ruminants in TDF with anthelmintic effect (Brito et al., 2017, 2018).

The ruminants perform species-specific grazing behavior depending by climatic variations and the type of vegetation, that strongly influence other behaviors (Bojkovski et al., 2014). The TDF of Brazil is characterized by an average annual temperature of 25 °C and annual average rainfall varies between 700 and 2,000 mm, with at least three months of rainfall per year (Sánchez-Azofeifa et al., 2005) and has a high species wealth (Oliveira-Filho, 2006). The goats in the present study fed at the total 20 plants, predominantly shrubs. More widely diet were observed in feed behavior of sheep and goats in a Yucatan TDF with a total of 33 plant species (González-Pech et al. 2015). Native TDF from Yucatan Peninsula has more than 260 legume species (Flores et al., 2006) and involves different types of plants, including trees, shrubs, vines, herbs and grasses (González-Pech et al. 2014).

The foliage shrubs contributed most of their DMI in both groups; goats prefer to feed on shrubs (Nefzaoui et al., 1993; Bartolome et al., 1998). The shrub that most contributes to DMI in tannin supplemented animals was *M. caesalpiniifolia* (26.3%) and tannin non-supplemented animals was *Ouratea cuspidate* (12.2%). Meanwhile, grasses contributed most of their DMI in Group II (35.84%) than Group I (33.95%). During the dry season goats consumed large and heavy bites (González-Pech et al., 2015). In this study were observed more heavy and extra heavy bites (69.15% or 1,995/2,885). In four hours of grazing and twelve days of direct observation reach 3,634 bites/day in the TDF of Yucatan (González-Pech et al., 2015). In the present study the direct observation was performed in eight hours of grazing and four days of direct observation, the number of bites was of 703 bites/day.

The TDF represent a source of plant secondary compounds such as CT (Torres-Acosta et al. 2017) and the goat species is better adapted to the consumption of taniniferous forages, which is related to mechanisms of secretion of proline-rich salivary proteins able to connect of tannin, thus avoiding the complexation of with dietary protein (Landau et al., 2000) due to the better capacity of the microbial population to metabolize them using the available energy for its development (Acamovic and Brooker, 2005), therefore the expressive value of DMI of *M. caesalpiniifolia*, besides that the previous experience of animals is important when using plants rich in tannins in the diet (Silva et al., 2016), the supplementation with *M. caesalpiniifolia* determined a better adaptation to the tannins plants.

Feeding tanniferous forages has both positive and negative effects. High concentrations of condensed tannins are known to reduce feed digestibility, feed intake and consequently lower production (Engel, 2002). By their capacity to bind with proteins, condensed tannins affect dietary protein availability, inactivate digestive enzymes, irritate the GI tract and can cause systemic toxicity (Landau et al., 2000). The inverse relation between high tannin levels in forage and palatability, voluntary intake, digestibility and nitrogen retention has long been established in several herbivores [5-7]. However, several ruminant species seem to tolerate (or even prefer) considerable amounts of tannins in their diets [10,11].

Voluntary consumption of ruminants may be reduced in the presence of high concentrations of tannin (Silva et al., 2016), so there was lower intake of the compound supplement of *M. caesalpiniiIfolia* when compared to the consumption of the supplement without the plant. The animals supplemented with condensed tannin compensated for this deficit by using TDF, ingesting more MS, CP and CT. Supplementation with *M. caesalpiniifolia* did influence in the total DMI (g/day), however the DMI of *M. caesalpiniifolia* was different between the experimental groups. The use of a TDF is a possible alternative for nutrition of goats, because it is an available resource and worldwide distribution, however greater knowledge about balancing and nutritional value and quality of the diet are necessary.

Sheep supplemented with condensed tannins had enhanced rumen fermentative activity to degrade tannin-rich browse, due to the appearance and proliferation of tannin-tolerant bacterial species and/or to the stimulation of changes in the existing bacteria to enhance their tolerance to these phenolic compounds (Ammar et al., 2009). Being naturally predisposed to consuming browse, goats are better suited to tolerate and detoxify natural toxins of such forages, particularly plant secondary metabolite (Hoste et al., 2010). Goats include browse as the major component of their diet whenever possible; therefore, browsing goats rely on the presence of tannin-adapted ruminal flora, superior nitrogen recycling and salivary adaptations (Hoste et al., 2010; Landau et al., 2000). A good animal performance in a heterogeneous vegetation depends on the amount of tannin in the diet and the fibrous and protein content dietary. To understand the consumption of condensed tannins and of other compounds present in most plants found in a heterogeneous vegetation is an important part of a better evaluation of the use of the same as an alternative method in the control of gastrointestinal infections of small ruminants.

Competing interests

The authors declare that they have no competing interests.

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Captions:

Table 1. Composition of experimental supplements.

Table 2. Species, habits and bromatological analyses (% DM except where stated) of plants consumed by goats in the tropical deciduous forest of São Luís during the dry season.

Table 3. Number of bites of different size (cm) and weight (g of dry matter) consumed by goats in the tropical deciduous forest of São Luís, and their observed frequency goats at dry season when considering all the BC. Different letters in the same column indicate significant difference (Chi-square test).

Table 4. Macronutrient intake of goats (g/day) browsing 8 hours per day in the tropical deciduous forest of São Luís, Brasil. P value reported of 2way ANOVA.

Figure 1. Feed intake of the group control and treated. The goats were provided concentrate without tannin (non-supplemented group) and with tanniniferous food from *Mimosa caesalpiniifolia*. * Significant difference ($p < 0.05$).

Table 1.

Ingredientes (%)	With *Mimosa caesalpiniifolia*	Without *Mimosa caesalpiniifolia*
Corn	60.4	57.4
Epicarp manioc bran	34.5	40
M. caesalpiniifolia	4.3	
Soybean	0.7	2.5
Calcareous		2

Table 2.

Plant Species	Habit of Plants	Chemical Composition			
		DM% Fresh Basis	OM*	CP*	CT**
Blutaparon vermiculare (dry stems)	Shrub	92,6	97,3	4,7	0,5
Casearia arborea	Shrub	93,0	97,1	8,4	38,6
Davilla sp.	Shrub	91,6	90,6	8,5	7,9
Mimosa caesalpiniifolia	Shrub	92,4	95,7	20,7	10,4
Mimosa caesalpiniifolia (dry leaves)	Shrub	92,2	80,5	10,4	1,7
Mimosa pudica var. *tetrandra*	Shrub	92,9	96,4		
Myrciaria tenella	Shrub	91,4	97,5	10,5	11,2
Ouratea sp.	Shrub	91,5	96,0	9,5	21,1
Ouratea cuspidata	Shrub	92,2	96,1	8,9	15,4
Randia armata	Shrub	92,3	94,4	11,8	1,1
Senna alata	Shrub	92,6	92,6	14,6	4,2
Sida acuta	Shrub	96,9	97,1		
Staelia virgata	Shrub	93,0	78,1		
Staelia virgata (dry plant)	Shrub	90,2	93,4	8,7	1,8
Vismia guianensis	Shrub	92,4	97,7	8,0	28,6
Waltheria indica	Shrub	91,4	93,5		
Cecropia pachystachya	Dicot herbaceous	90,6	90,5	21,6	0,5
Mangifera indica	Dicot herbaceous	92,4	93,5	6,6	4,9
Securidaca lanceolata	Creep/Climber	93,3	97,3	15,4	0,1
No identification (leaves)	Creep/Climber	92,2	91,9	18,5	1,7
No identification (stems)	Creep/Climber	92,6	96,7	17,8	0,2
Cyperus laxus	Grass	92,4	93,5	8,0	5,6
Panicum maximum var. *Massai*	Grass	93,9	92,2	13,0	0,3

Dry Matter expressed in g/kg Fresh Matter
* Values expressed in g/kg of Dry Matter
** Values expressed in gram equivalents of leucocianidine/kg of Dry Matter

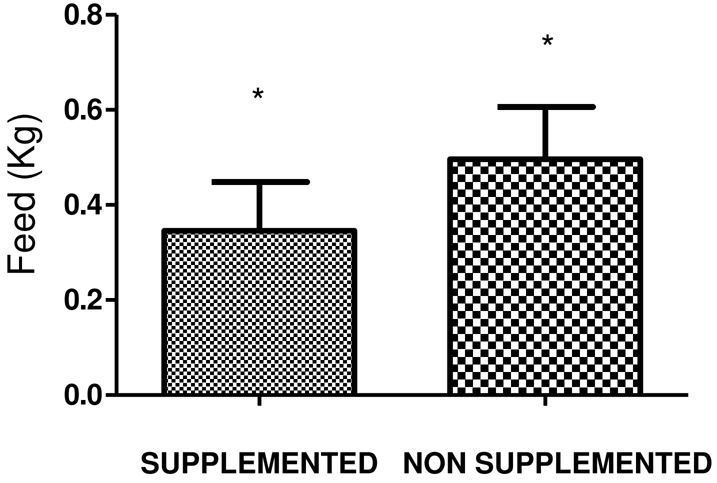
Table 3.

Animals	Total of Bites	Bites size (cm)		Bites weight (g dry matter)			
		Small (0 - 5)	Large (6 - 20)	Light (0.001 – 0.212)	Medium (0.213 – 0.378)	Heavy (0.379 – 0.477)	Extra-Heavy (0.478 – 6.733)
Supplemented	2,885	1,731^{a} (60.0%)	1,154 (40.0%)	479^{a} (16.6%)	411 (14.3%)	1,665^{a} (57.7%)	330^{a} (11.4%)
Non Supplemented	1,263	774^{b} (61.3%)	489 (38.7%)	124^{b} (9.8%)	221 (17.5%)	538^{b} (42.6%)	380^{b} (30.1%)

Table 4.

Nutritional Component	Medium Value (g/day)	
	Supplemented	Non Supplemented
DM (TDF)	133.7 ± 3.5[a]	68.9 ± 15.4[b]
PB (TDF)	14.2 ± 0.3[a]	6.2 ± 2.2[b]
TC (TDF)	7.1 ± 1.1[a]	4.1 ± 0.6[a]
TC (Concentrate)	1.4	-
TOTAL TC	8.5[a]	4.1 ± 0.6[a]

Figure 1.



CAPÍTULO 3 [1]

[1]Neste capítulo consta o artigo "Anthelmintic effect of suplementation with *Mimosa caesalpiniifolia* on goats grazing in tropical deciduous forest"que foi escrito de acordo com as normas para a publicação no periódico Veterinary Parasitology.

Anthelmintic effect of suplementation with *Mimosa caesalpiniifolia* on goats grazing in tropical deciduous forest

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Abstract

The inclusion of products and byproducts of plants rich in tannins, in the diet of small ruminants, have demonstrated efficacy in the control of *Haemonchus contortus*. The objective of the study was to evaluate the effect of supplementation of an important tanninipherous plant, *Mimosa caesalpiniifolia*, against *H. contortus* in goats grazing in tropical deciduos forest (TDF). To confirm the activity of the sample of *M. caesalpiniifolia* in vitro tests of egg hatching and larval exsheathment were performed. Bromatological analysis and chromatographic profile of the sample plant, were performed. Twenty-four male goats, castrated, Anglo nubian crossbreed, six to eight months old were infected with a single dose of infective larvae of *H. contortus*, 28 days prior to the start the experiment. The animals spent a week in adaptation to experimental diet after which they were distributed into two groups according to fecal eggs count and body weight. Group I: infected animals receiving concentrate with tanniniferous food from *M. caesalpiniifolia*. (128.7 mg condensed tannin (CT)/kg) and Group II: infected animals receiving concentrate without *M. caesalpiniifolia*. All animals received isoproteic and isoenergetic supplementation and have daily access to TDF from 8:00 am to 4:00 pm. Animals were weighed weekly and faecal egg counts and faecal cultures performed twice weekly. In addition, egg hatching assay were performed once a week for both groups. After 28 days of experimental feeding, all animals were humanly slaughtered and adult worm populations were estimated. In the *in vitro* an inhibitory effect of 90.3% and 86.1% at concentrations of 1.2 mg/ml and 0.6 mg/ml, respectively with the lowest IC50 value of 0.46 mg/mL (97% CI 0.407-0.524 mg /mL).After treatment with 10 mg/ml (higher concentration) was observed on hatchability an innibittory effect of 42%. There was no effect of experimental diet on weight gain and efficiency on reduction of faecal egg counts (FECR) was 34.5% in the Group I compared to the Group II in the last week of experiment. There was no significant effect of the supplementation with *M.caesalpiniifolia* on the reduction of adult worm burdens. (P>0.05). There is significant potential in the use of *M. caesalpiniifolia* in the goat diet for the control of *H. contortus*, but there is a need for other studies to clarify in more detail how the anthelmintic effect of the tannins or other polyphenols present in the plant studied.

Key words: *Haemonchus* contortus; Tannins; Control

Introduction

Haemonchosis is recognized in its main endemic zones as the most economically important parasitic nematode (McLeod, 2004; Perry et al., 2002), chiefly due to the common occurrence and potential for heavy mortality rates in small ruminants. The effective prevention of haemonchosis is essential for the sustainable management of sheep and goats in regions where *Haemonchus contortus* is endemic, due especially to the threat of animal mortalities. Although the seasonal epidemiology of *H. contortus* infection in relation to weather patterns is well-established in the majority of climatic zones (O'Connor et al., 2006).

Synthetic anthelmintics (AH) have been the main tool to control these parasitic diseases in sheep and goats, for more than 50 years for two reasons, first of the increasing societal demand to reduce the use of chemical compounds in agriculture and livestock breeding, and second of the constant development and diffusion of resistance to AHs in gastrointestinal nematodes (GIN) populations especially in small ruminants (Gaudin et al., 2016).

The use of plants has emerged as an alternative to reduce the problems of anthelmintic resistance in the control of gastrointestinal nematodes in animals and is indicated mainly by reducing the cost of treatments and prolonging the useful life of the synthetic products available in the market (Vieira and Cavalcante, 1999). Forages containing condensed tannin (CT) with AH properties represent one of the novel promising option, including resistant parasites (Heckendorn et al., 2006; Gaudin et al., 2016). The tannins are polyphenolic compounds, contained in various parts of the plant, belonging to the flavonoid groups (Mueller-Harvey and McAllan, 1992). Condensed tannins are present at varying concentrations in most tropical forage legumes (Makkar and Becker, 1994) and can reach up to 300 g / kg dry matter (DM) (Mueller-Harvey, 2001).

There is a hypothesis that CT bind to the cuticle of the nematodes, which is rich in proline and hydroxyproline, altering its physico-chemical properties (Thompson and Geary, 1995; Hoste et al., 2006). The indirect action of CT is believed to be a result of the stimulation of the host immune response (Kahn and Diaz-Hernandez, 2000), due to the fact that these compounds protect proteins from ruminal degradation, being more available for absorption in the small intestine (Schwab, 1995; Barry and Mcnabb, 1999; Min and Hart, 2003).

The *Mimosa caesalpiniifolia* is a native plant (legume) in Brazilian Caatinga and Cerrado vegetation, and is widely found in the Brazilian Northeast region. This species is popularly known as “unha de gato”, “sabiá” and “sansão do campo”, and presents a high capacity for adaptation and regeneration of the soil, as well as is tolerant to acid soils (Carvalho, 2007; Agra et al., 2008). Considering its chemical composition, many triterpenes and phenolic compounds were identified from various parts (leaves, fruits, flowers, twigs and stem barks) of *M. caesalpiniifolia* (Lopes Citó et al., 2010).

The *M. caesalpiniifolia* cetonic extracts were effective in the in vitro inhibition of larval exsheathment and egg hatchability (Brito et al., 2017). Fed goats with dry leaves of *M. caesalpiniifolia* reduced worm burden in 57.7% (Brito et al., 2018). (animais em confinamento).

The tropical deciduous forest (TDF) is an ecosystem characterized by a complex and heterogeneous array of different plant species and represents the most important source of feeding materials for sheep and goats produced in semi-intensive farms in tropical America. (Torres-Acosta et al., 2017). Heterogeneous vegetation may serve as the basis of a complex diet for ruminants (Gautier and Moulin, 2004). It is important to associate the ruminant feeding behavior with plant diversity (Agreil et al., 2010). Several plant species of the tropical deciduous forest contain secondary compounds with some AH activity (Torres-Acosta et al., 2012).

The necessity for effective anthelmintics for the treatment and prevention of haemonchosis is hard to overestimate, given the potential for animal mortalities if left unchecked. Although anthelmintics should always be used in conjunction with nonchemical strategies as part of an IPM approach, the potential for rapid increases in *H. contortus* populations requires effective treatment at appropriate times (Besier et al., 2016).

The objectives of the current study were, to determine the effect of suplementation with *M. caesalpiniifolia* in goats fed in native pasture on established populations of *H. contortus* in goats and to assess the consequences of these two treatments on animal productivity.

Materials and methods

All procedures were approved through the Ethics Committee for the Animal Experimentation of the Federal University of Maranhão, Brazil, under number 2311501861.

Plant material and extract preparation

The leaves of *M. caesalpiniifolia* were collected in a city of Chapadinha, Maranhão, Brazil (03º 44' 30" S and 43º 21' 37" W) in March 2017. The leaves collected were dried under shade and crushed in disintegrator and ground with a mesh 0.25mm. Freeze-dried, powdered plant leaves (6.0 g), was extracted by maceration until exhaustion using acetone: water mixture (7:3) as extracting solvent. The crude extract was filtered and result concentrate was rotaevaporated at 40 ° C.

Chemical Analysis

The bromatological analysis were measured dry matter (DM) 105ºC (expressed in g / kg of green matter), mineral matter (MM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and crude protein (CP) (values expressed in g / kg of dry matter). Bromatological analyses were performed according to (AOAC, 2011) and (Van Soest, et al., 1991).

The CT content was estimated using the HCl-butanol method (Porter et al., 1986) and were expressed as a leucocyanidin equivalent (Table 1).

In order to trace the chromatographic profile, the extract was solubilized in 2.0 mL of methanol / water (9:1) and applied to C18 cartridge (Strata C18 -E , Phenomenex), the sample was filtered through membrane filters (Simplepure PTFE 0.22µm, Allcrom) and diluted solution in methanol/water (8:2) to obtain an approximate solution concentration of 5.0 mg/ml. A Shimadzu model HPLC system (Shimadzu Corp., Kyoto, Japan) was used, consisting of a solvent delivery module with a double-plunger reciprocating pump, UV-VIS detector (SPA-10A. The column used was Luna 5µm C18 100 A (250 µm x 4.6 µm. The elution solvents used were A (2% acetic acid in water) and B (% acetic acid in methanol). The samples were eluted according to the following gradient: 95% A/5% B as initial in 30 min. Flow rate was 1 mL/min, and the run time 50 min. Column temperature was 20 ºC. The sample injection volume was 10 mL. Data were collected and processed using LC Solution software (Shimadzu).

In Vitro assays

Infected eggs and L3

Eggs and third stage larvae (L3) were obtained from a donor goats experimentally infected with a monospecific stump of *H. contortus*. Fresh faeces were collected and mixed with warm water (37°C); the eggs were then recovered from solution in 25-µm sieves. Recovered eggs were added to a saturated NaCl solution and centrifuged (3000 rpm) for 3 min; floating eggs were recovered using a 25-µm sieve (Coles et al., 1992). Eggs were washed three times to eliminate the remaining salt and were re-suspended in distilled water. According to the technique described by Roberts & O'Sullivan, following the method Ueno and Gonçalves (1998), feces were cultured for recovery of third stage larvae (L3).

Egg Hatching Assay (EHA)

A suspension of 100 eggs/well was placed in a plate, and 100 µL of each treatment (methanol at 3% and the extract) was added. The extract was diluted in 3% methanol at concentrations that ranged from 10 mg/mL to 0.3 mg/ml, decreasing by halves. Tests were performed with four replicates. The plate was incubated at 27°C and RH > 80% for 48 h. Larvae and unhatched eggs were counted under an inverted microscope. The test was based on the method described by Coles et al., (1992).

Larval Exsheathment Inhibition Assay (LEIA)

This test was performed according to Bahuaud et al. (2006). The extracts were diluted in 3% methanol and evaluated at concentrations that ranged from 1.2 mg/mL to 0.075 mg/mL, decreasing by halves. The negative control was performed with 3% methanol. The L3 larvae were incubated in the different treatments for 3 h at 22 °C. After incubation, the larvae were washed and centrifuged (3000 rpm) three times with PBS. Approximately 1000 larvae/tube were subjected to the artificial exsheathment process by contact with sodium hypochlorite (2.0%, w/v) and sodium chloride (16.5% w/v). Tests were performed with four replicates. The percentages of larval exsheathment process were monitored at 0-, 20-, 40- and 60-min intervals by observation under an inverted microscope.

***In Vivo* Assay**

Animals and Feed Management

Twenty-four male goats, castrated, crossbreed anglo nubian, aged six to eight months were used in the study. The animals were had 18.5± 5.5 kg of body weight (BW) at the start of the trial, they were distributed into two groups according to fecal eggs counts and body weight (BW). Group I: infected animals receiving concentrate with tanniniferous food from *M. caesalpiniifolia*. (128.7 mg CT/ Kg (BW)/day) equivalent to 1.32 g of *M. caesalpiniifolia* powder/ Kg (BW) /Day and Group II: infected animals receiving concentrate without *M. caesalpiniifolia*.The animals were submitted to TDF with grazing starting at eight o'clock in the morning and ending at four o'clock in the afternoon, adding a grazing time of eight hours per day and received isoproteic and isoenergetic supplementation.

Two concentrated feed were used: (1), with inclusion of the leaf powder of *M. caesalpiniifolia* (60.4% corn; 34.5% epicarp manioc bran; 4.3% *M. caesalpiniifolia*; 0,7% soybean) (2), without inclusion of plant (57.4% corn; 40% epicarp manioc bran; 2.5% soybean and 2% calcareous).

The supplement was provided as soon as the animals returned from pasture and amount provided was determined according to body weight of the animal (3.07% of BW), the animals were weighed weekly and the amount of feed was adjusted. Water and mineral supplements were offered *ad libitum*. The feed intake was assessed by subtracting from the value provided the leftover value.

Experimental design

The experiment lasted 28 days, starting at the (D0), 30 days after the artificial infection (D-30). Before the artificial infection it was made anthelmintic treatment with Monepantel 5mg/kg. A control by egg counting was performed with three negatives results of faecal egg counts (FEC) on three consecutive days. After that the animals were infected with a single dose of 4000 third stage larvae of *H. contortus*. The period of adaptation permitted animals to adapt progressively to the new diet before the feeding experiment itself (Figure 1).

After four weeks of experimentation, all animals were slaughtered and adult worm populations were determined. All animals were treated with Toltrazurila (Baycox® 5%, 20mg/kg) to control coccidian.

Parasitological measurements

During the experimental period faecal samples were collected rectally third weekly for determination of FEC, using a modified McMaster procedure (Gordon and Whitlock, 1939, two of these samples were also intended for eggs hatchabilility test. Faecal culture as described by Roberts and Sullivan (1950) were held fortnightly.

The percentage reduction of FEC was calculated based on the formula described by Dash et al. (1998), $100 \times (1 - [T_2/T_1][C_1/C_2])$ with arithmetic means. In the formulae T is treated and C is control, 1 is pre-treatment and 2 is post-treatment.

The slaughtered animals were eviscerated and the abomasum will be collected, this organ was separated by double ligatures with string in the anterior and posterior region and, later, they will be opened individually with scissors from where all the content will be collected. The mucosa was carefully scraped to remove the worms, it was done by a manual collection process with curved needles.

The washes of the organs were passed through sieves under running water. The worms retained in the sieves were transferred to another container with the aid of spatulas. The adult worms were placed in a vial containing formalin solution (5%) at room temperature, for further quantification and identification according to Ueno & Gonçalves (1998). The slides were assembled using Hoyer's solution (Anderson, 1954) for nematode counting and identification.

The anthelmintic efficacy in the group consuming *M. caesalpiniifolia* leaf powder was estimated by comparing the worm burdens of the treated group with that of the control group, by means of equation described by Wood et al. (1995):

Efficacy % = ((geometric mean control group – geometric mean treated)/ geometric mean control group) x 100.

Statistical analysis

Data on FEC, worm burdens, feed intake and weight gain were submitted to analysis of variance and means were compared by Student t test (GraphPad Inc., San Diego, CA, USA). In vitro test results were used to determine the concentration required to inhibit 50% of hatching and larval exsheathment (IC50) with respective 95% confidence intervals (95% CI) using GraphPad Prism 7.0 software (GraphPad Inc., San Diego, CA, USA) according to the technique described by Roditakis et al. (2005).

Results

Chemical analysis

The leaves of *M. caesalpiniifolia* samples showed 17.4% of crude protein and 9.7% of condensed (Table 1). With the aid of the ultraviolet detector (UV/Vis) coupled to the HPLC system, it was detected that the extract of leaves *M. caesalpiniifolia*, present great complexity and diversity in the matrix, evaluated by the large number of peaks eluted along the chromatographic run. With the aid of HPLC-UV/Vis, the chromatographic data was processed and the chromatograms obtnained in selective wavelengths for a determined a secondary metabolite class. We checking for the presence of flavonoids and derivatives of phenolic acids and tannins derivativies of catechin by the presence of spectra with bands characteristic of these classes in sample analyzed (Figure 2).

***In Vitro* Assay**

After treatment with 10 mg/ml (higher concentration) was observed on hatchbility an innibittory effect of 42%. An inhibitory effect on larval exsheathment was observed in *M. caesalpinifolia* acetone extract tested of 90.3% and 86.1% at concentrations of 1.2 mg/ml and 0.6 mg/ml, respectively with the lowest IC50 value of 0.46 mg/mL (97% CI 0.407-0.524 mg /mL).

***In Vivo* Assay**

The nematode most prevalent was *Haemonchus* spp. in both groups (98 to 99%). The inclusion of leaf dust from the study plant did not influence the body weight of goats in the different groups studied (P>0.05).

The efficiency on FECR were 7.4%; 12.7%; 4.2% and 25.1% lower for tannin supplemented animals compared to the tannin non-supplemented animals in the four weeks of experiment, respectively (Figure 3A). The mean arithmetic faecal egg count of tannin supplemented animals in pre- treatment was 1,600 and 681.8 in the fourth experimental week. There was no intervention of supplementation with *M. caesalpiniifolia* on egg hatchability.

There was no significant effect of the supplementation with *M. caesalpiniifolia* on the reduction of adult worms burdens, the group that consumed the plant had a mean of 692.7 nematodes and the control showed mean of 757.9 (P>0.05) (Figure 3B). However, some animals in the treated group presented a smaller number of adult worms when compared to the individual results of the control animals.

Discussion

The extracts of plants rich in tannins have been demonstrated to have higher ovicidal and larvicidal activity than other extracts, depending on the structure of the CT monomers present in the plant (Molan et al., 2003). *In vitro* activity of *M. caesalpiniifolia* acetone extract were showed with larval exsheathment inhibition of 99.8% and 97.3% at 1.2 and 0.6 mg/ml, respectively. *M. caesalpiniifolia* cetone extract the showed egg hacthing inhibition of 80.7% and 82.3% at 1.0 and 2.0 mg/ ml, respectively (Brito et al., 2017). These differences in the anthelmintic activity of the same plant, which has been related to factors depending on the parasite (stage, species), the host (sheep, goats, cattles) and/or the plant (species, variety, stage of growth) factors (Hoste et al., 2006).

The previous experience of animals is important when using plants rich in tannins in the diet (Silva et al., 2016). Inexperienced animals adapted only for six days to a taniniferous diet had weight loss, when the adaptation time was elevated to 48 days there was a higher intake of DM and consequently an improvement in weight gain. (Bem Salem et al., 2005), There were no significant live weight diferences between groups of this study and the period of adaptation to the diet lasted one week. The mean feed intake of the treated group was lower than that of the control group, demonstrating a reduction in consumption by the animals that received *M. caesalpiniifolia*.

The *M. caesalpiniifolia* had no effect on performance of hair sheep at pasture, the group received the plant showed lower total gain and mean daily gain 6.0 kg and 56.8 g/animal/day compared with the group Control (Campos et al., 2009)

The fodder comsumption of *Lysiloma latisiliquum*showed that in the second period of the trial, from D29 to D36, the FEC data in the treated group was lower than those in the control group. On D36, the faecal egg excretion was 31% lower in the group received *L. latisiliquum* compared to the control group (Martínez-Ortíz-de-Montellanoa et al., 2010). In the group treated with *M. caesalpinifolia* the reduction in faecal egg counts was 34.5%, between D21 and D27, in the present study.

The use of *M. caesalpiniifolia* in the diet of goats submitted to a TDF did not influence the hatchability of the eggs. Tanniniferous food from *Bauhinia pulchella* decreased pasture contamination with gastrointestinal nematodes from goats, was observed that the egg hatch percentage in the treatment group was significantly lower than that in the control group in 7 out of 9 weeks assessed. The egg hatch percentage of the *B. pulchella* group gradually increased during the experiment (Lopes et al., 2016). The post mortem results of the study that Brito et al. (2018) performed, showed a significant reduction of *H. contortus* adult worm burden in goats fed the powder of *M. caesalpiniifolia*

Conclusion

M. caesalpiniifolia leaf acetone extract showed larval exsheathment inhibitory activity on *H. contortus*. The supplementation with dry leafs of *M. caesalpiniifolia* is a promising alternative for the control of *H. contortus* infections in goats and the current study is the first one assessing the *in vivo* AH activity of this plant on goats fed in TDF. But there is a need for other studies to clarify in more detail how the anthelmintic effect of the tannins or other polyphenols present in the plant studied.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Chemical composition (% of dry matter) of *Mimosa caesalpinifolia*

Mimosa caesalpinifolia	
Condensed Tannins[a]	9.7
Dry Matter	88.3
Neutral Detergent Fiber	73.0
Acid Detergent Fiber	64.1
Lignin	42.9
Crude Protein	17.4

[a] Condensed tannins are expressed as the leucocyanidin equivalent.

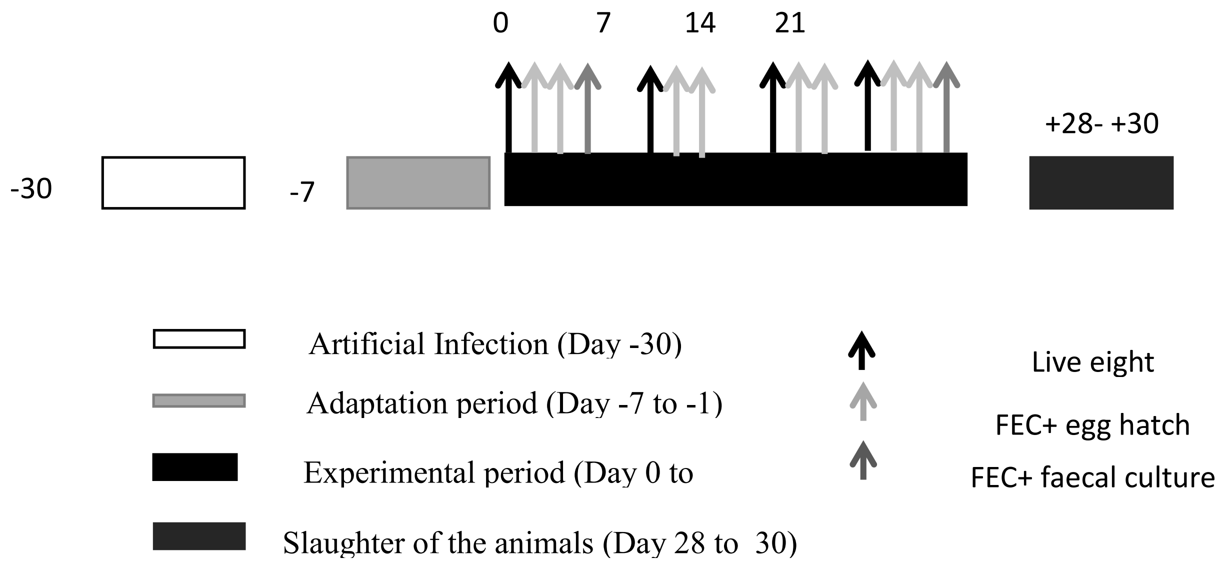


Figure 1. Schematic timeline of experimental procedures.

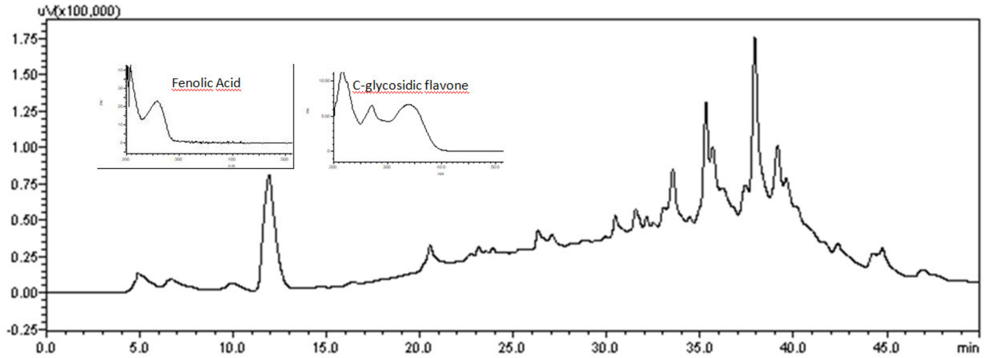
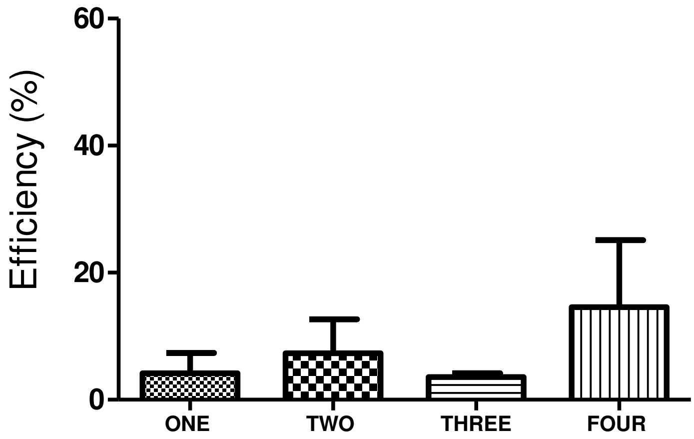


Figure 2. Typical chromatogram obtained by analysis HPLC-UV/Vis (270 nm). Sample collected from UFMA.

A



B

Figure 3. A) Percentage reduction of faecal egg count (FECR) of the supplemented group compared with non supplemented group. The values are reported as the mean values of one week pre-treatment and four weeks of treatment. B) Adult worm burdens of experimental goats. The goats were provided concentrate without tannin (non supplemented group) and with tanniniferous food from *Mimosa caesalpinifolia*. No Significant difference ($p > 0.05$).