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DISSERTAÇÃO DE MESTRADO

INCLUSÃO DE ADITIVOS FITOGÊNICOS NA DIETA DE OVELHAS LEITEIRAS (LACAUNE): EFEITOS SOBRE SAÚDE ANIMAL, EFICIÊNCIA PRODUTIVA, COMPOSIÇÃO E QUALIDADE DO LEITE

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CHAPECÓ, 2020.

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Elaborada por

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como requisito parcial para obtenção do grau de

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RESUMO

Dissertação de Mestrado
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INCLUSÃO DE ADITIVOS FITOGÊNICOS NA DIETA DE OVELHAS LEITEIRA (LACAUNE): EFEITOS SOBRE SAÚDE ANIMAL, EFICIÊNCIA PRODUTIVA, COMPOSIÇÃO E QUALIDADE DO LEITE

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Chapecó 21 de setembro de 2020

Componentes fitoterápicos microencapsulados são uma nova tecnologia usada na alimentação de ruminantes, pois diminuem as perdas dos componentes bioativos durante a passagem pelo rúmen. Tendo em vista o potencial de rentabilidade da comercialização do leite ovino e dos seus derivados, mais estudos surgem no meio técnico e científico. Sabe-se que o leite ovino é rico em sólidos totais, sendo uma ótima opção na fabricação de queijos. Para verificar a eficácia do uso de produtos comerciais à base de plantas como aditivos fitogênicos na alimentação de ovinos, tendo em vista sua influência sobre a qualidade do leite, quantidade produzida e saúde dos animais, foram dirigidos dois estudos. No experimento I foi utilizado um blend microencapsulado à base de timol, carvacrol e cinamaldeído para os animais. Trinta ovelhas Lacaune em lactação (50 ± 3,0 d de lactação) foram divididas em três grupos: Controle (T0), mistura de 150 mg/kg de ração (T150) e mistura de 250 mg/kg de ração (T250). A coleta de sangue e de leite foi realizada antes do início do experimento (dia 0), ao final do período de adaptação (dia 15) e durante o experimento (dia 20). Em amostras de leite, medimos a composição centesimal, a contagem de células somáticas (CCS) e o estado oxidante/antioxidante por meio de espécies reativas de oxigênio (EROs), lipoperoxidação e capacidade antioxidante total. Houve maior produção de leite dos animais que receberam a dieta com a mistura microencapsulada, bem como maior eficiência produtiva e eficiência alimentar, concomitantemente com menor conversão alimentar. Tendência de menor CCS foi obsevado, bem como níveis menores de EROs no leite. No sangue observou-se menor número de neutrófilos e EROs, concomitante aos maiores níveis de globulinas em ovinos de T150 e T250 em relação ao T0. Em resumo, nas ovelhas que consumiram a mistura microencapsulada, encontramos propriedades antiinflamatórias associadas à redução dos radicais livres e ao aumento das globulinas, todos desejáveis para a produção animal. No experimento II, o extrato de pimenta (EP) foi adicionado à ração das ovelhas no período de lactação (meio da lactação) para manter a produção e melhorar a qualidade do leite, bem como preservar sua saúde. O

experimento começou 75 dias após o parto e durou 18 dias. Os animais foram divididos aleatoriamente em três grupos de dez animais cada: T0, usado como controle (sem EP); T200 (200 mg de EP/kg de concentrado) e T400 (400 mg de EP/kg de concentrado). A redução na produção de leite (L) foi menor nas ovelhas T400 nos dias 0 a 18 e 14 a 18 do que no grupo T0. A conversão alimentar foi menor nas ovelhas dos grupos T200 e T400 do que no grupo T0. A interação entre o tratamento e o dia foi observada para proteína, lactose e sólidos totais no leite; ou seja, foi maior nas ovelhas que consumiram PE no dia 18. A CCS no leite foram mais baixas nas ovelhas T400. A contagem total de basófilos, os níveis de proteína e os níveis de albumina foram mais elevados no sangue dos animais do grupo T400. Houve menores níveis de espécies reativas de oxigênio e lipoperoxidação no soro e leite dos animais dos grupos T200 e T400. No 18º dia, o soro de ovelhas que consumiram EP apresentou maiores níveis de tióis não protéicos e atividades da superóxido dismutase. As ovelhas que receberam T200 e T400 passaram mais tempo bebendo e tiveram maior frequência de beber água. Esses resultados sugerem que a inclusão de PE (400 mg/kg) contendo capsaicina no concentrado de ovinos no meio da lactação (após o pico da lactação) minimizou a redução da produção de leite durante o experimento e melhorou a qualidade do leite, bem como estimulando uma resposta antioxidante sistêmica. De modo geral concluímos que a adição dos dois ativos oriundos de plantas form benéficos a saúde dos animais e eficiência produtiva.

Palavras-chave: Timol, carvacrol, capsaicina, fitogênicos, microencapsulados.

ABSTRACT

Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

INCLUSION OF PHYTOGENIC ADDITIVES IN THE DAIRY OF MILK SHEEP (LACAUNE): EFFECTS ON ANIMAL HEALTH, PRODUCTIVE EFFICIENCY, COMPOSITION AND MILK QUALITY

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Chapecó 21 de setembro de 2020

Microencapsulated phytotherapic components are a new technology used in the feeding of ruminants, as they reduce the losses of bioactive components during passage through the rumen. In view of the profitability potential of the commercialization of sheep milk and its derivatives, more studies are being carried out in the technical and scientific environment. Sheep milk is known to be rich in total solids, making it a great choice in cheese making. Two studies were conducted to verify the effectiveness of using commercial plant-based products as phytogenic additives in sheep feed, in view of their influence on milk quality, quantity produced and animal health. In experiment I, a microencapsulated blend based on thymol, carvacrol and cinnamaldehyde was used for the animals. Thirty lactating Lacaune ewes (50 ± 3.0 d of lactation) were divided into three groups: Control (T0), mixture of 150 mg / kg of feed (T150) and mixture of 250 mg / kg of feed (T250). Blood and milk were collected before the beginning of the experiment (day 0), at the end of the adaptation period (day 15) and during the experiment (day 20). In milk samples, we measured centesimal composition, somatic cell count (SCC) and oxidant / antioxidant status using reactive oxygen species (ROS), lipoperoxidation and total antioxidant capacity. There was greater milk production of the animals that received the diet with the microencapsulated mixture, as well as greater productive efficiency and feed efficiency, concomitantly with less feed conversion. Lower SCC trend was observed, as well as lower levels of ROSs in milk. In the blood, a lower number of neutrophils and ROS was observed, concomitant with the higher levels of globulins in T150 and T250 sheep compared to T0. In summary, in the sheep that consumed the microencapsulated mixture, we found an anti-inflammatory effect associated with the reduction of free radicals and an increase in globulins, all desirable for animal production. In experiment II, pepper extract (EP) was added to the ewes' feed during the lactation period (mid-lactation) to maintain production and improve milk quality, as well as preserve their health. The experiment started 75 days after delivery and lasted

18 days. The animals were randomly divided into three groups of ten animals each: T0, used as a control (without PE); T200 (200 mg EP / kg concentrate) and T400 (400 mg EP / kg concentrate). The reduction in milk production (L) was smaller in the T400 ewes on days 0 to 18 and 14 to 18 than in the T0 group. Feed conversion was lower in sheep in groups T200 and T400 than in group T0. The interaction between treatment and day was observed for protein, lactose and total solids in milk; that is, it was higher in ewes that consumed PE on day 18. The SCC counts in milk were lower in the T400 ewes. The total basophil count, protein levels and albumin levels were higher in the blood of animals in the T400 group. There were lower levels of reactive oxygen species and lipoperoxidation in the serum and milk of animals in groups T200 and T400. On the 18th day, the serum of sheep that consumed PE showed higher levels of non-protein thiols and superoxide dismutase activities. The sheep that received T200 and T400 spent more time drinking and were more frequently drinking water. These results suggest that the inclusion of PE (400 mg / kg) containing capsaicin in sheep concentrate in the middle of lactation (after the peak of lactation) minimized the reduction in milk production during the experiment and improved the quality of milk, as well as stimulating a systemic antioxidant response. In general, we conclude that the addition of the two assets from plants is beneficial to animal health and productive efficiency.

Keywords: Thymol, carvacrol, capsaicin, phytogenics, microencapsulated.

SUMÁRIO

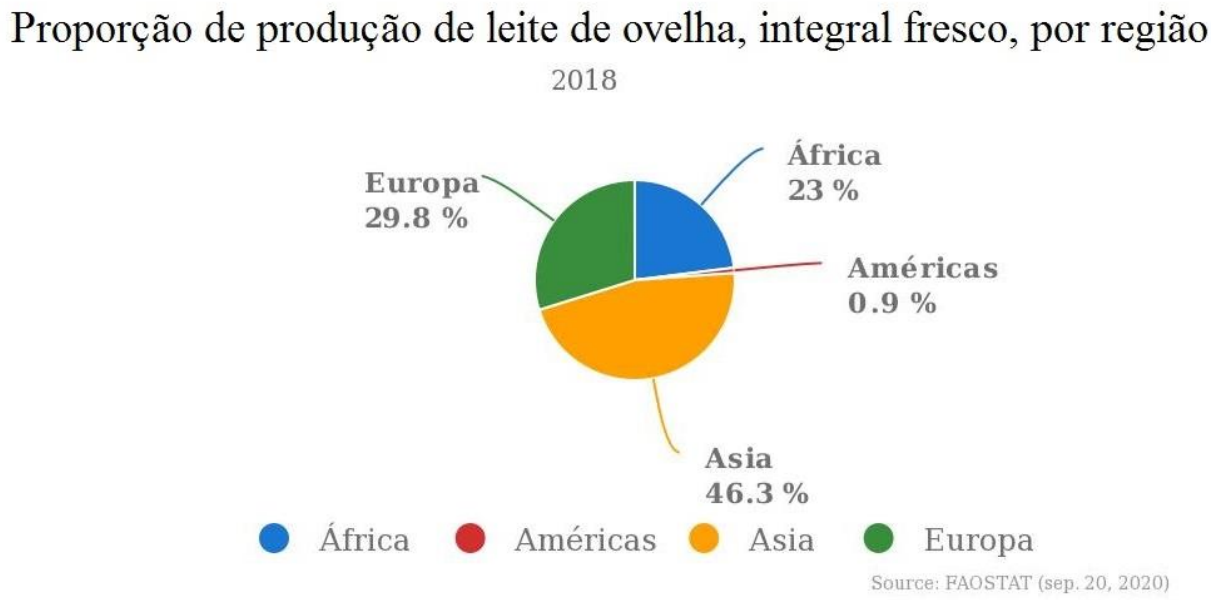
CAPÍTULO I

1. REVISÃO DE LITERATURA

1.1 Panorama da ovinocultura de leite

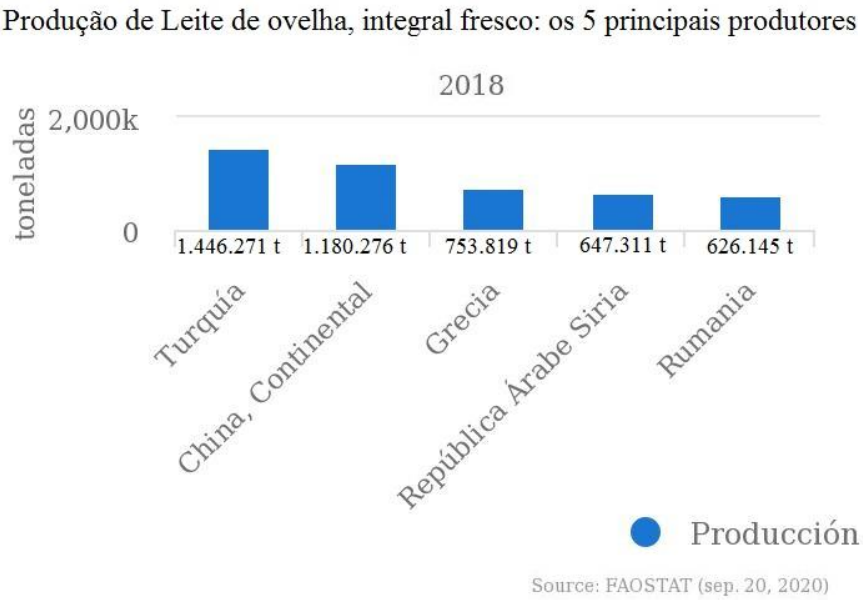
A ovinocultura é considerada desde tempos remotos como forma de subsistência, devido à sua vasta aptidão, já que por meio dela é possível adquirir alimentos como carne e/ou leite, e proteção física com o uso da lã e peles. Esta atividade é realizada em diferentes países, sendo a distribuição mundial apresentada na Figura 1. O maior rebanho de ovinos encontra-se na China Continental com 164.078.900 animais, mais que o dobro do segundo maior na Austrália, com 70.067.316, de uma população mundial total de 803.843.117 animais. Destes, 293.445.524 animais geraram, em 2018, 11.811.328 toneladas de leite. Os cinco países que lideram a produção mundial de leite de ovelha são: Turquia, China Continental, Grécia, Síria/República Árabe e Romênia – Figura 2 (FAO, 2020).

Figura 1- Produção de leite de ovelha por continente.



Fonte: FAO, 2020

Figura 2- Países com maior produção de leite de ovelha



Fonte: FAO, 2020.

Os subprodutos fabricados a partir do leite de ovelha podem ser o queijo, a manteiga e o iogurte. De acordo com os últimos dados da FAO, no período de um ano foram produzidas 788.302 toneladas de queijo, em sua maioria pela Grécia, e 63.258 toneladas de manteiga, principalmente pela Turquia. No Brasil a ovinocultura toma cada vez mais espaço entre as regiões. Em 2017, a região com maior número de estabelecimentos de produção de leite ovino foi a região nordeste, com 82% dos 704 estabelecimentos do país. O volume total de produção nacional neste mesmo ano foi de 1.652.000. Entretanto, a região do país com maior produção foi a Sudeste, com cerca de 36,2%, já a região nordeste produziu 32,0%, a região sul produziu 20,3% e o restante dividido entra regiões Norte e Centro-Oeste (Embrapa, 2019).

Dos 704 estabelecimentos nacionais de produção de leite ovino, apenas 109 fazem a comercialização deste produto, 959.000L. No entanto, dos 53,2% estabelecimentos estarem localizados na região Nordeste, a maior comercialização de leite ocorre na região Sudeste (53%). A renda gerada foi de R$ 2.813.000, sendo 45% da região Sudeste, 41,3% da região Sul e 4,3% da região Nordeste (Embrapa, 2019).

1.2 Leite ovino

1.2.1 Anatomia e fisiologia do úbere

O úbere das ovelhas é composto por duas glândulas localizadas no ventre do animal, na região inguinal. Um tecido elástico chamado de ligamento de suspensão central separa os quartos em direito e esquerdo, além de auxiliar sua sustentação. O corpo do úbere é recoberto internamente por um parênquima glandular formado por ácinos, um conjunto de células epiteliais secretoras. Após a polarização e despolarização destas células ocorre a secreção dos constituintes do leite para o lúmen alveolar, que desce para uma cisterna e, finalmente, para o teto (papila). A formação dos lipídeos do leite ocorre no retículo endoplasmático, a lactose no aparelho de Golgi e as proteínas são formadas nos ribossomos do retículo endoplasmático rugoso (Rouissi et. al., 2020).

A lactação é estimulada pela produção hipofisária de prolactina em conjunto com os hormônios do crescimento (GH), logo após a queda dos níveis de estrogênio e progesterona decorrente do fim da gravidez. A prolactina estimula a produção de leite nas células da glândula mamária (Cunningham, 1999). Conforme há o aumento do estradiol e da progesterona, ao longo da lactação, ocorre a diminuição da produção de leite. Já o GH atua aumentando a circulação sanguínea e a mobilização de reservas corporais (Rouissi et. al., 2020).

1.2.2 Composição do leite de ovelha

O leite de ovelha é um alimento rico nutrientes (Tabela 1), com valores de gordura, lactose, sólidos totais e cinzas superiores ao leite de vaca e de cabra, perdendo apenas para o leite de búfala. Já em relação a proteína, o leite de ovelha possui a maior concentração em comparação as outras três espécies leiteiras domesticas. A gravidade específica do leite de ovelha é maior do que do leite de vaca e de cabra; e a sua alta densidade tem relação com os sólidos não gordurosos. Sua acidez titulável também é superior quando comparado ao leite de vaca e de cabra, indicando maiores concentrações de ácido lático, cítrico e fosfórico (Usman e Mahmood, 2010).

A composição do leite também pode sofrer variação de acordo com o período de produção, ao final da lactação há um aumento na concentração de gordura, proteína e sólidos totais, enquanto o teor de lactose sofre diminuição. Também é influenciada pela dieta, raça, ordem do parto, manejo, condições ambientais, localidade e saúde do animal e do úbere (Park et al., 2007).

Tabela 1 – Composição do leite de ovelha e de vaca.

	Ovelha	**Vaca**
Gordura (%)	7,9	3,6
Sólidos sem gordura	12,0	9,0
Lactose	4,9	4,7
Proteína	6,2	3,2
Caseína	4,2	2,6
Albumina/ globulina	1,0	0,6
Nitrogênio não proteico	0,8	0,2
Cinzas	0,9	0,7
Calorias em 100ml	105	69

Modificado de Anifantakis et al., 1980 e Park et al., 2007.

A alta rentabilidade do leite de ovelha para a fabricação de queijos se dá devido às suas propriedades físico-químicas (Anifantakis et al., 1980). O seu baixo pH, micela de caseína maior e mais cálcio por peso de caseína afetam o tempo de coagulação, taxa de coagulação, firmeza da coalhada e quantidade de coalhada; assim como os teores de caseína influenciam diretamente a formação do gel de coalho, a velocidade de cura e sua firmeza máxima (Park et al., 2007).

A lactose é o carboidrato de maior importância na composição do leite de ovelha, pois é formado a partir da união de uma molécula de glicose e outra de galactose, formando um dissacarídeo (Park, 2006). Tem papel importante na manutenção da homeostase entre a corrente sanguínea e as células alveolares da glândula mamária, onde o leite está sendo sintetizado e secretado no lúmen alveolar e nos ductos do úbere (Larson e Smith, 1974). No leite de ovelha também são encontrados outros carboidratos como oligossacarídeos, glicopeptídeos, glicoproteínas e açúcares nucleotídeos, porém em menores quantidades (Park et al., 2007).

Os lipídeos são os maiores responsáveis pelo aumento nos custos do leite de ovelha e estão presentes principalmente na forma de triacilgliceróis, mas alguns lipídeos simples como os diacilgliceróis, monoacilgliceróis e ésteres de colesterol também podem ser encontrados; além de lipídios complexos como os fosfolipídios e compostos lipossolúveis como os esteróis, ésteres de colesterol, hidrocarbonetos (Park et al., 2007).

A gordura presente no leite e a proporção de ácidos graxos está intimamente ligada à dieta, podendo ser alterada por meio dela. Estudos mostram que alimentações com valores de forragem superiores a 40% do valor de matéria seca e baixos teores de concentrado podem

induzir o aumento na deposição de gordura no leite (Angeles-Hernandez et. al., 2020). A suplementação animal com gordura dietética também é capaz de aumentar o ácido linoléico conjugado (CLA) no leite (Zago et al., 2008). Isto vem atraindo a atenção de pesquisadores na formulação de dietas com a finalidade do enriquecimento da dieta humana por meio da ingestão de um leite ovino rico em ácidos graxos como o vacênico ou o ácido alfa-linolênico (Angeles-Hernandez et. al., 2020). Ovelhas com maior teor de concentrado na dieta apresentam diminuição na concentração de gordura no leite e aumento de proteína (Alba et al., 2019). Pois a um aumento da síntese de propionato no rúmen e diminuição de acetato e butirato, que são ácidos graxos de cadeia curta necessários para a síntese de gordura na glândula mamária. Juntamente a isto, a diminuição de fibras na alimentação diminui ainda mais a proporção acetato: butirato (Angeles-Hernandez et. al., 2020).

A proteína presente no leite está mais relacionada ao suprimento de proteína metabolizável na dieta e produção de proteína microbiana no rúmen do que à ingestão de N de forma isolada (Nudda et al., 2020). Quanto a concentração de CLA, foi observado que houve seu aumento correlacionado à maiores inclusões de forragem na dieta de ruminantes (Angeles-Hernandez et al., 2020). Os microrganismos ruminais através da hidrólise de lipídeos produzem ácidos graxos livres e glicerol e a maioria dos ácidos graxos poli-insaturados (PUFA). Dietas ricas em PUFA podem aumentar a disponibilidade de ácidos graxos disponíveis na glândula mamária e no leite, atuando na inibição da última fase do processo de biohidrogenação no rúmen (Angeles-Hernandez et al., 2020).

1.3 Desafios no setor de produção de leite

Durante as diferentes fases de vida do animal de produção encontramos alguns desafios. No período reprodutivo os animais necessitam uma boa nutrição e escore corporal para sua manutenção, para desprender nutrientes necessários para a fertilidade e para o desenvolvimento de filhotes saudáveis. Estes, por sua vez, se tornarão animais bem nutridos e que em sua vida adulta terão maiores chances de serem animais de grandes índices produtivos.

Entre os desafios encontrados podemos citar a persistência de lactação, a sanidade do rebanho, a mastite, o estresse térmico, o estresse oxidativo e dietas desbalanceadas.

1.3.1 Persistência de lactação

A persistência de lactação pode ser definida como a continuidade de produção após a passagem pelo pico de lactação. Quando calculamos a persistência de lactação devemos levar em consideração a seleção de animais parecidos, a partir do mesmo número de partos e semelhança genética. Também devemos considerar que animais de raças com maior resistência a doenças e maior adaptabilidade apresentam melhores valores produtivos (Cobuci et al., 2003). A persistência de lactação é desejada devido ao seu valor econômico agregado, tendo em vista um animal produtivo. A manutenção de altos valores produtivos aumenta as exigências do animal e, quando não gerenciada, pode causar desordens metabólicas e queda do desempenho reprodutivo. Uma raça que apresenta excelentes resultados quanto ao desempenho zootécnico é a Lacaune. Usada com predileção para a finalidade leiteira no Sul do Brasil, tem sido utilizada em conjunto com East Friesian com intuito de diminuir o coeficiente de endogamia (Ticiani et al., 2013).

O pico de lactação ocorre entre a terceira e quarta semana pós-parto, dependendo se foi parto gemelar ou único (Bianchi, 2018). Em sistemas de produção onde o cordeiro alimenta-se de leite diretamente na mãe a produção do primeiro mês é de extrema importância. Em partos gemelares a produção de leite tende a ser maior, de forma que sustente ambos os filhotes, neste caso o pico de lactação é atingido aos 15 dias pós partos, enquanto que com apenas um cordeiro, o pico ocorre mais tarde, entre a terceira e quarta semana (Blackburn e Cartwright, 1987; Neville, 1999). Neste momento, as mães estão em balanço energético negativo e começam a usar suas reservas corporais (Moore e DeVries, 2020). Uma ovelha que originou dois cordeiros produz cerca de 30 a 40 % mais leite que uma que teve apenas um cordeiro, entretanto, a suplementação muitas vezes ocorre da mesma forma para ambas (Rouissi et al.,2020).

Um ponto a se considerar é que animais de alta produção leiteira tendem a ter uma queda maior na produção após o pico de lactação, enquanto animais com menores produções no início

da lactação tendem a manter o nível de lactação durante todo o período. A curva de lactação é composta por três fases distintas, a primeira é ascendente e compreende o período do parto até o pico de lactação, a segunda fase é linear, sem grande variação na produção, por fim a terceira fase é descendente até o fim da lactação (Cobuci et al, 2003).

O acúmulo de leite na glândula mamária pode diminuir a persistência de lactação, por acelerar o processo de involução e diminuir a taxa de secreção. Desta forma, quanto maior o intervalo entre as ordenhas, menos leite será produzido. O fator responsável pela redução na secreção da glândula é o peptídeo inibidor de feedback da lactação (FIL), formado nas células epiteliais e secretado para os alvéolos. (Cannas et al., 2002).

1.3.2 Sanidade do rebanho

A mastite é um problema de grande importância na produção leiteira, caracteriza-se pela inflamação da glândula mamária proveniente da infecção por microrganismos patogênicos (Dai et al., 2019). O processo inflamatório compromete o tecido glandular a ponto de diminuir a produção de leite, e dependendo da severidade acarreta uma lesão irreversível (Rovai et al., 2015). Além dos altos custos com o tratamento do animal, a queda na produção causa prejuízos significativos, caso não seja tratado corretamente pode levar o animal a morte. Existem duas formas de apresentação da doença, a forma clínica é de fácil identificação, as alterações físicas do leite podem ser observadas no teste de rotina que utiliza a caneca de fundo preto (Langoni et al., 2017). Na mastite subclínica há necessidade de maior número de observação, pois o principal sinal clínico é a diminuição na produção de leite associado a menor qualidade desse leite, sendo um grande problema para as propriedades leiteiras. A identificação da mastite subclínica também pode ser feita através da contagem de células somática (CCS) ou através da *Califórnia Mastitis Test* (CMT) (Alba et al, 2019).

A mastite também pode ocorrer em decorrência de erros na formulação de dietas, que predispõem a inflamações da glândula mamária e o aumento de CCS. A concentração de ureia no leite é inversamente associada à CCS no leite. Deficiência de nutrientes como Se, Zn, Mn, Fe, vitamina A e vitamina C também podem estar associadas a doença (Nudda, 2020). O efeito benéfico de óleos essenciais e fitogênicos utilizados na dieta dos animais vem sendo observado como diminuidor da CCS no leite e melhora na saúde dos animais (Nudda, 2020).

Durante o metabolismo fisiológico, os organismos produzem calor em suas reações. Dependendo das condições ambientais em que se encontram, a temperatura somada a este calor produzido, pode gerar calor excessivo no organismo, resultando no estresse térmico e estresse metabólico (Souza et al., 2012). Quando nos referimos a animais de produção leiteira, o estresse

térmico gera um grande problema de queda de produção em decorrência da diminuição da ingestão de alimentos, aumento na ingestão de água e taquipneia (Dalcin et al., 2016). O aumento no tempo de ruminação também é descrito como indicativo de estresse térmico, mas também pode ocorrer em diversas desordens não associadas a este quadro (Maia et al., 2020).

O aumento da taxa respiratória observada em animais expostos a altas temperaturas ambientais visa reduzir a carga de calor por evaporação respiratória (Silanikove, 2000; Sevi et al., 2001 , Caroprese et al., 2012); em ovinos, a perda de calor por meio do aumento da frequência respiratória é a principal forma de dissipação de calor, pois a sudorese é evitada pela presença de pelagem de lã (Marai et al., 2007). É importante considerar a adaptação do animal às condições climáticas do local onde serão criados, além de adequar o manejo destes animais de forma a minimizar as alterações metabólicas que estes possam apresentar e as perdas econômicas advindas destas alterações. Para manter altos padrões de produtividade é necessário que os animais estejam em conforto térmico, compreendido por uma zona de temperatura agradável. Para que a energia do metabolismo seja aproveitada em sua maior quantidade, em vez de ser utilizada para aquecimento ou resfriamento do organismo (Neiva et al., 2004). Na tentativa de diminuição do calor, os animais diminuem a ingestão com a intenção de diminuir o trânsito intestinal. Desta forma, ocorre a mobilização das reservas corporais de gordura e nitrogênio, visando atender a gliconeogênese e a glândula mamária (Sevi e Caroprese, 2012).

Em ovelhas Sarda, a produção de leite pode ser reduzida em 15% se a temperatura ambiente máxima for superior a 21–24 ° C e em 20% se a temperatura mínima mudar de 9–12 ° C para 18–21 ° C; além disso, o desempenho da produção de ovinos Sarda pode ser reduzido em 20% com o índice médio de temperatura e umidade (THI) passando de 60-65 para 72-75 (Peana et al., 2007). Outros problemas acarretados incluem distúrbios nos equilíbrios hídrico, proteico, energético e mineral, além de afetar as reações enzimáticas, secreções hormonais e os metabólitos do sangue (Marai et al., 2007). As alterações plasmáticas são decorrentes da diminuição de sódio, potássio, cálcio e fósforo, bem como do aumento de cloreto (Sevi e Caroprese, 2012).

Para minimizar os efeitos do estresse térmico na criação de ovinos, algumas alternativas são adotadas, como o manejo alimentar, para alimentos com maior energia, visando menor ingestão e maior disponibilidade energética para a termorregulação, proteínas de baixa degradação ruminal melhorando o catabolismo de nitrogênio, mudar o horário de fornecimento da alimentação para fim do dia em horários mais frescos, uso de aditivos para melhoria das funções imunológicas, respostas fisiológicas e produtivas (Sevi e Caroprese, 2012).

1.3.3 Estresse oxidativo

As reações fisiológicas da célula animal produzem espécies reativas de oxigênio (ROS), que causam danos ao organismo, danificando as células e podendo causar alterações em carboidratos, ácidos nucleicos, lipídeos e protepinas (Silva e Gonçalves, 2010). Em resposta, são produzidas pelo organismo antioxidantes, com intuito de barrar a ação oxidativa. Quando há o desequilíbrio entre agentes oxidantes e antioxidantes ocorre um episódio denominado estresse oxidativo, pois a oxidação celular é determinada por seus efeitos indiretos, como a oxidação de lipídeos, dos grupamentos sulfidril de proteínas, das bases púricas e pirimídicas, que acarretam danos ao DNA celular e no balanço tiol/sulfeto (Birben et al., 2012).

No início do período produtivo, animais com aptidão leiteira podem apresentar um quadro de balanço energético negativo, já que precisam cuidar da alimentação de um filhote, juntamente com seus gastos de energéticos (Moore e DeVries, 2020). Neste período, a alimentação muitas vezes não supre todas as necessidades maternas, favorecendo o estresse oxidativo e problemas de saúde nos animais; além da queda na produção, ocorrem alterações nos ácidos graxos, com aumento da série não esterificada (NEFA) e de beta-hidroxibutirato (BHBA), que são utilizados como biomarcadores do estresse oxidativo (Zhao et al., 2019). A utilização de energia pela glândula mamária e tecidos periféricos aumenta o NEFA plasmático, a peroxidação lipídica e as espécies reativas de oxigênio, já o BHBA é proveniente do metabolismo intermediário da oxidação de ácidos graxos; e para minimizar os efeitos ocasionados pelo estresse oxidativo, como desordens metabólicas e reprodutivas, pesquisadores sugerem uma boa relação entre oxidantes e antioxidantes (Zahrazadeh et al., 2018).

Para que haja o equilíbrio, quando ocorre o aumento de ROS são produzidas substâncias antioxidantes, enzimáticas (superóxido dismutase, catalase e glutationa peroxidase) e não enzimáticas (glutationa) (Caroprese et al., 2019). A superóxido dismutase (SOD) atua na defesa antioxidante das células através da conversão do superóxido (O_2) em peróxido de hidrogênio (H_2O_2) (Halliwell et al., 1993). A catalase transforma o peróxido de hidrogênio em oxigênio e água (Morabito et al., 2017), seu campo de ação é sobre moléculas de baixo peso (Silva e Gonçalves, 2010). A glutationa peroxidase (GSHPx) e a glutationa redutase (GR) são enzimas do ciclo redutor da glutationa. São responsáveis pela redução dos peróxidos transformando a glutationa reduzida (GSH) em glutationa oxidada (GSSG) pela redução de elétrons, que depois é convertida em GSG novamente pela GR (Damasceno et al., 2002; Kinnula, 2005). A inserção de antioxidantes na dieta de animais de produção pode prevenir a ocorrência de mastite, retenção de placenta e aborto (Hoedemaker et al., 2009).

1.3.4 Problemas associados à dieta desbalanceada

A adequada nutrição na criação de ruminantes é de fundamental importância para o funcionamento adequado do sistema reprodutivo, formação do feto, desenvolvimento da gestação e produção de leite. O consumo de nutrientes com balanceamento correto de proteínas, energia, minerais e vitaminas previne diversas patologias e aumenta o rendimento econômico (Sartori e Guardieiro, 2010; Wendorff e Haenlein, 2017).

A nutrição influencia diretamente a função ovariana, além de atuar na produção e disponibilização dos hormônios folículo estimulante, luteinizante e liberador de gonadotrofina (Scaramuzzi e Martin, 2008). A desnutrição durante a gestação de ovelhas pode predispor os cordeiros à problemas metabólicos, com valores hematológicos aumentados de ureia, colesterol, lactato e creatinina, quando há a tentativa de correção da desnutrição dos filhotes através de uma dieta hipercalórica após o nascimento (Khanal et al., 2016). Níveis de desnutrição maternas, mesmo que de grau leve, prejudicam o ciclo da ornitina fetal e reduzem a ureia plasmática materna, acarretando menor síntese de poliamidas e, consequentemente, em menor disponibilidade de substratos para proliferação celular. Nestes casos, o desenvolvimento microvascular renal dos fetos é afetado e os animais apresentarão alterações patológicas (Dunford et al., 2014).

Logo antes da fase de lactação, nas últimas semanas de gestação ocorre aumento da exigência nutricional, pois mais de 80% do desenvolvimento do feto ocorre nesse período. Por este motivo, uma nutrição deficiente neste período terá como consequência menor produção de leite, menor peso e sobrevida dos cordeiros ao nascer (Selmi et al., 2019). Essa é uma preocupação frequente, pois trata-se do período de transição em ovelhas; mas tão quanto importante é o pico de produção, quando os animais têm a capacidade de expressar todo seu potencial genético, devemos fornecer aditivos, suplementos, além de nutrição adequada; a fim de assim ter ovelhas produtivas, muitas vezes com persistência de lactação prolongada.

1.4 Aditivos na nutrição animal e sua relação com a saúde

O Decreto 76.986 de 06 de janeiro de 1976 estabelece que aditivos são substâncias inseridas nos alimentos com a função conservar, modificar ou tornar suas propriedades mais intensas, desde que não alteram seu valor nutricional (Decreto, 1976). Os aditivos alimentares podem ser divididos em seis grupos: texturizantes, corantes, conservantes, nutricionais, aromatizantes, nutricionais e diversos; assim como o grupo dos aditivos conservantes pode ser dividido em antioxidantes, antimicrobianos e antiaglutinantes (Carocho et al., 2014).

A nutrição adequada em ovinos tem grande influência no rendimento econômico, assim como as mudanças alimentares refletem diretamente na secreção de leite, no ganho de peso e na musculatura (Leite, 2013). Estes fatores unidos a proibição do uso de antibióticos como promotores de crescimento pela união europeia (2006), aumentou a busca de aditivos nutricionais na alimentação animal (Araújo, 2010). Em nosso estudo testamos componentes fitogênicos encapsulados e um extrato de pimenta, que serão descritos a seguir.

1.4.1 Biotecnologias na produção de aditivos alimentares

No final da década de 80, a biotecnologia passou a ser utilizada para produção de enzimas para a alimentação animal, com a finalidade de aumentar o valor nutritivo da dieta (Stivari et al., 2014). Entre as biotecnologias utilizadas, destaca-se a microencapsulação que é o empacotamento de partículas (em estado sólido, líquido ou gasoso) a um revestimento em cápsulas extremamente pequenas, que atuam como proteção contra perdas estruturais do componente, de modo que este seja liberado de forma controlada sob condições específicas (Nazzaro et al., 2012; Laurent e Garcia, 2013). Como exemplo, podemos citar o microencapsulamento de probiótico composto por *Saccharomyces cerevisiae*, com alta taxa de sobrevivência mesmo em pH extremamente ácido (pH 1,5), simulando a perda que poderia ocorrer durante a passagem pelo estômago, e liberado apenas em pH próximo a neutralidade, similar ao pH intestinal, pronto para ser absorvido (Laurent e Garcia, 2013).

Compostos naturais, óleos essenciais, extratos vegetais, enzimas, bactérias, polifenóis e aditivos voláteis podem ser microencapsulados para permanecerem mais estáveis e protegidos contra perdas em suas propriedades nutricionais e antimicrobianas, por exemplo (Nazzaro et al., 2012). A microencapsulação de óleos essenciais é importante pois sua volatilidade está relacionada com o baixo peso molecular, o que os tornam suscetíveis a mudanças ambientais, como luz, oxigênio, umidade e temperatura, porém, quando microencapsulados, permanecem estáveis e mantêm sua atividade biológica (Wang et al., 2016). A indústria tem feito o uso dos carboidratos, em forma de goma, como material encapsulante, devido a sua capacidade de

ligação à água. Outro material encapsulante que está ganhando mercado é a mucilagem, em especial a de linhaça e de inhame, neste caso testada para liberação lenta em pH similar ao do ácido clorídrico (Laurent e Garcia, 2013).

O ruminante convive em simbiose com os microrganismos que vivem no rúmen. Os microrganismos trabalham na degradação das fibras e síntese de proteína, fornecendo nutrientes para o animal, enquanto este fornece o ambiente adequado e alimento necessário para a sobrevivência. Esta interação, apesar de muito necessária, também gera algumas perdas energéticas como o metano (Calsamiglia et al., 2015). A administração de óleos essenciais microencapsulados para ovelhas têm resultado em melhorias na fermentação ruminal, aumentando o aproveitamento do alimento fornecido, além de diminuir a emissão do gás metano (CH_4). O maior interesse econômico está voltado para a ideia de que toda a energia poupada é utilizada unicamente para a produção de leite ou carne do animal (Soltan et al., 2018).

1.4.2 Fitogênicos

Os aditivos naturais melhoram a nutrição animal pelo aumento da digestibilidade, da absorção de nutrientes e nitrogênio, além de uma maior eficiência na resposta imune e do sistema endócrino. As ações de aditivos nutricionais à base de plantas podem ser anti-inflamatórias, antibacterianas, antivirais, antioxidantes, coccideostáticas e anti-helmínticas (Kumar et al., 2014). Estudos mostram que os extratos fitogênicos combinados não atuam em apenas um sítio de ação, desta forma, potencializam sua eficácia e evitam modificações nos microrganismos que possam causar resistência (Kissels, 2017). Os aditivos fitogênicos são utilizados principalmente em períodos críticos da produção animal, como na fase de transição (compreendido entre as três semanas que precedem o parto e as três semanas seguintes) (Alba et al., 2020), o início da lactação (Alba et al., 2019) e no período de terminação (Blansh et al., 2016).

Os extratos herbários, comercialmente conhecidos como aditivos fitogênicos, possuem propriedades antioxidantes desejadas, atuando no a combate a radicais livres, na inibição da lipoperoxidação e estimulando a atividade de enzimas antioxidantes. Seu mecanismo de ação está ligado as propriedades redox de seu grupo hidroxila (Materska e Perucka, 2005); assim como também atuam como antimicrobianos, entre as cadeias de ácidos graxos que resulta em fluidificação e expansão, com extravasamento de íons. Este processo pode ser compensado através de bombas iônicas, mas o gasto energético gerado limita o crescimento bacteriano (Calsamiglia et al., 2007).

O timol e o carvacrol são compostos fenólicos com estruturas químicas muito semelhantes, 5-metil-2-(1-metiletil)-fenol e 2-metil-5-(1-metiletil)-fenol, respectivamente; dois dos componentes mais comum nas formulações de fitogênicos. Os compostos fenólicos são produtos do metabolismo secundário das plantas e servem como forma de proteção ou como resultado da adaptação ao estresse (Materska e Perucka, 2005). Originários de plantas aromáticas como o orégano, são extraídos por meio de destilação à vapor, hidrodestilação, dióxido de carbono líquido ou microondas (Bakkali et al., 2008). Devido à sua ação antimicrobiana, são potenciais modificadores da fermentação ruminal, proporcionando acúmulo de aminoácidos e diminuição na emissão de gases (Calsamiglia, 2007). O seu grupo hidroxila permite a formação de pontes de hidrogênio como principal sítio ativo contra microrganismos; assim como acredita-se que o grupo hidroxila trabalhe no transporte transmembrana de cátions e prótons monovalentes (Ultee et al., 2002). Outro mecanismo de ação seria a desnaturação proteica resultando na coagulação de alguns constituintes celulares (Gustafson and Bowen, 1997).

Principal composto bioativo da canela, o cinamaldeido é um fenilpropanóide com propriedades antimicrobianas (Cardozo et al., 2004). Sugere-se que o cinamaldeído seja um modificador de microbiota ruminal, quando em doses elevadas (31,2 mg/L), causando a diminuição da proporção de acetato e aumentado a de propionato (Busquet et al., 2005). Sugere-se que este composto trabalhe através da inibição da metanogênese, mas ainda não há comprovação (Calsamiglia et al., 2007). Diferente do timol e do carvacrol, estudos relatam que o cinamaldeído não interagiu com a membrana citoplasmática, levando a pensar que seu mecanismo de ação está relacionado a algum mecanismo no interior da célula (Helander et al., 1988).

Em nosso primeiro experimento, utilizamos um produto comercial microencapsulado (Enterosan®, Konkreta, Brasil), composto por 21,55 mg de carvacrol, 18,76 mg de timol e 27,62 mg de cinamaldeído por grama de fitogênico (Galli et al., 2020).

1.4.3 Extrato de pimenta

A pimenta (*Capscicum annum L.*) possui componentes que podem ser utilizados como alternativa aos promotores de crescimento na nutrição animal por possuir características estimulantes de processos digestivos, antioxidantes e antimicrobianas, sendo que na sua composição destacam-se os carotenóides, capsaicinóides e o ácido ascórbico (Topuz e Ozdemir, 2007). Entre os capsaicinóides, destaca-se a capsaína (8-metil-N-vanilil-6-nonenamidaCigH27NO3), um carotenóide pertencente ao grupo dos tetraterpenóides, com suas

funções bactericidas, antioxidantes, cicatrizante, anti-hemorrágicas e estimulantes na liberação de endorfinas (Calsamiglia et al., 2007; Dutra et al., 2010). Compostos capsaicinóides são ricos em vitamina C e E, pró-vitamina A e antioxidantes. A propriedade antioxidante pode estar ligada à capacidade de doação de elétrons efeito do grupo –CH=CH-COOH, ao grupo hidroxila e sua posição no anel aromático e sua capacidade de doação de H para estabilizar os radicais livres (Materska e Perucka, 2005).

A capsaicina é sugerida como modificadora da fermentação ruminal, capaz de reduzir a proporção acetato/propionato e N de amônia. Em pH 5,5, foi capaz de aumentar a produção total de AGV, sugerindo que em situações adequadas pode melhorar a utilização dos nutrientes no rúmen (Calsamiglia et al., 2007). Uma propriedade de grande interesse neste composto é o aumento da ingestão de água, que pode estar ligado ao sabor pungente que a pimenta causa. É possível que a capsaicina estimule a peptidólise fornecendo mais peptídeos e AA, além de aumentar a síntese por parte dos microrganismos ruminais e a disponibilidade para o intestino delgado (Calsamiglia et al., 2007).

O aditivo alimentar utilizado no segundo experimento é um produto comercial à base de extrato de pimenta (Capsin®; Nutriquest). A composição química do extrato de pimenta foi de matéria seca (920 g / kg), extrato etéreo (444 g / kg), proteína bruta (64,4 g / kg), fibra detergente neutro (293 g / kg) e fibra em detergente ácido (229 g / kg). A quantificação da capsaicina no extrato de pimenta foi realizada por cromatografia gasosa e revelou concentração de 5,0 g / kg.

1.5 Objetivo geral

Avaliar se a adição de fitogênicos na dieta de ovelhas leiteiras têm benefícios sobre a produção e qualidade do leite, assim como na saúde dos animais.

CAPÍTULO II
MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de dois manuscritos com suas formatações de acordo com as orientações das revistas as quais foram submetidos:

Manuscrito I – Microencapsulated herbal components in diet of Lacaune ewes: impacts on physiology and milk production and quality

Submetido na revista Small Ruminant Research

Manuscrito II – Inclusion of pepper extract containing capsaicin in the diet of ewes in the mid-lactation period: effects on health, milk production and quality

Em correção na revista Society and Development

2.1. MANUSCRITO I

Microencapsulated herbal components in diet of Lacaune ewes: impacts on physiology and milk production and quality

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Abstract

Microencapsulated herbal components are a new technology used in the feeding of ruminants. This study aimed to determine whether the addition of a microencapsulated blend based on thymol, carvacrol, and cinnamaldehyde in dairy sheep feed would improve production efficiency, milk quality, and animal health. Thirty lactating Lacaune ewes (50±3.0 d of lactation) were divided into three groups: Control (T0), 150 mg blend/kg of feed (T150) and 250 mg blend/kg of feed (T250). Milk was measured before the beginning of the experiment (d 0), at the end of the adaptation period (d 15), and during the experiment (d 20). In milk samples, we measured proximate composition, somatic cell count (SCC) and oxidant/antioxidant status through reactive oxygen species (ROS), lipoperoxidation (LPO) and total antioxidant capacity. There was greater milk production of the animals that received the diet with the microencapsulated herbal blend, as well as greater productive efficiency and feed efficiency, concomitantly with less feed conversion. Lower SCC trends, as well as lower ROS and LPO levels in milk. In the blood, we observed a lower number of neutrophils and ROS, concomitant with the greater levels of total protein and globulins in sheep from T150 and T250 compared to T0. In summary, in the sheep that consumed the microencapsulated herbal blend, we found reduction of free radicals and an increase in globulins, as well as lower neutrophil counts. We conclude that the blend consumed by the sheep improved productive performance and milk quality.

Keywords: feed efficiency; antioxidants, animal health, productivity, nutrition.

1. Introduction

Sheep's milk is consumed in various parts of the world; however, the world production of sheep's milk is small compared to that of other ruminants: 11,811,328 tons in 2018 (FAOSTAT, 2018). According to FAO, this production is led by Turkey (1,446,271 tons), followed by China (1,180,276 tons), and Greece (753,819 tons) (FAO STAT, 2018). A sheep's lactation period lasts an average of 150 days (Brito et al., 2006), a relatively short cycle compared to that of cows that produce for 305 days (Jonas et al., 2011). The sheep's lactation peak is close to 30 days postpartum, and it can produce about 4.5 L of milk daily during this period; however, after the peak of production, the average daily production drops to 1.3 L (Brito et al., 2006). Lacaune ewes have production 30% greater than of the East Friesian breed at the beginning of the lactation period; however, the decline in production is faster over time, reaching a decrease of about 8 g in daily production, while the East Frisian loses 2 g (Ticiani et al., 2013). For this reason, it is important to seek alternatives to maintain lactation persistence in sheep, that is, to maintain high levels of production even after the peak of lactation.

Protocols to maintain lactation persistence have been the focus of study with dairy animals (Macciotta et al., 2003; Nagasaku et al., 2007; Ticiani et al., 2013). Essential oils have been studied as modifiers of ruminal fermentation through the modulation of their microbiota, in order to reduce energy losses during digestion and to convert energy to milk or meat production (Calsamiglia et al., 2007; Soltan et al., 2018). The favoring of gram-negative bacteria results in the production of succinate and degradation of lactate in the rumen, which modulates rumen pH. These additives stimulate the formation of propionic acid and reduce methanogenesis, as well as proteolysis and deamination of dietary protein in the rumen (Nicodemo, 2001; Soltan 2018). According to the literature, essential oils can be extracted from aromatic plants using various methodologies, including using steam distillers, hydrodistillation, liquid carbon dioxide, or microwaves (Bakkali et al., 2008). The active components present in

the oils can be found in buds, stems, flowers, fruits, seeds, fruits, branches, roots, or even in the bark (Bakkali et al., 2008).

Most research on this subject has focused on thymol and carvacrol, compounds extracted from thyme, oregano, and cinnamon, with varying chemical structures: 5-methyl-2-(1-methylethyl)-phenol, 2-methyl-5-(1-methylethyl)-phenol and 3-phenyl-2-propenal phenol, respectively (Calsamiglia et al., 2007; Nostro and Papalia, 2012). This interest is related to the mechanism of action thymol and carvacrol that act on the lipid bilayer of bacteria, altering their conformation, and causing destabilization and extravasation of cellular content (Calsamiglia et al., 2007). In an attempt to compensate by means of electric pumps, bacteria expend large amounts of energy and thereby reduce their growth capacity (Calsamiglia et al., 2007; Bakkali et al., 2008; Silva et al., 2012). This property allows stabilization of the rumen environment and improving the use of nutrients by ruminants, in addition to providing protection against the invasion of pathogenic microorganisms, positively impacting animal health and production (Nicodemo, 2001). Thymol and carvacrol modulated the immune system, in addition to having anti-inflammatory, antioxidant, analgesic and spasmolytic effects (Bakkali et al., 2008; Lima et al., 2013). In ruminants, there was stimulation of antioxidant activity and decreased lipid oxidation in the meat of steers that consumed essential oils containing thymol (Monteschio et al., 2017). Cinnamaldehyde is capable of modifying nitrogen metabolism, decreasing the concentrations of ammonia and volatile fatty acids (Cardozo et al., 2004; Busquet, et al., 2006); as well as when cinnamaldehyde associated with thymol and carvacrol increased milk production in multiparous cows (Wall et al., 2014).

Commercial products, based on herbal compounds, have been called phytogenics, or feed additives (Windisch et al., 2008). In ruminant feed, the use of phytogenics has encountered limitations, with emphasis on losses during the passage through their multicavity stomach, as well as degradation by ruminal fermentation (Oliveira et al., 2013); just as we cannot rule out

negative effects on fermentative bacteria. Knowing these problems, researchers developed the microencapsulation process of herbal components, which promotes stability to the phytogenic and releases slowly and under specific conditions (Pereira et al, 2018). According to the literature, the use of a microencapsulated phytogenic also allows the herbal components to be protected from the action of ruminal bacteria, reducing their loss and allowing greater absorption in the intestine (Shen et al., 2017), as the raw material used for microencapsulation it is regulated by pH, that is, the microcapsule opens only at intestinal pH (Laurenti and Garcia, 2013).

Microencapsulated herbal components are a new technology used in the feeding of ruminants. Knowing the biological properties of carvacrol, thymol and cinnamaldehyde, we believe that its inclusion in the diet of sheep can maintain the persistence of lactation. Therefore, the objective of this study was to evaluate whether the addition of a blend of microencapsulated herbal components in the diet of dairy sheep has positive effects on production efficiency, milk quality and animal health.

2. Materials and Methods

2.1. Phytogenic

We used a commercial microencapsulated phytogenic (Enterosan®, Konkreta, Brazil) in our experiment. The commercial product was analyzed to evaluate the guaranteed levels, and these data were previously published by our colleagues Galli et al. (2020): 21.55 mg of carvacrol, 18.76 mg of thymol and 27.62 mg of cinnamaldehyde per gram of phytogenic agent.

2.2. Animals and experimental design

The experiment was carried out on a commercial sheep farm, located in Chapecó, Santa Catarina, Brazil. The experiment lasted 20 d, with the first 15 d involving diet adaptation. Thirty

multiparous Lacaune ewes (3rd delivery) were selected, with an average body weight of 68 ± 3.8 kg and an average of 50 ± 3.0 d of lactation. The animals were separated into three pens with ten animals each, homogeneously with respect to milk production. The stalls (24 m^2) were in a covered shed, with no walls and a hard floor with a bed of wood. The feeders were divided, making it possible to match the supply of feed to the animals individually, that is, the animals were confined during feeding (feed consumption was measured individually).

The groups formed were as follows: without addition of phytogenic, used as a control (T0); 150 mg blend/kg of feed (T150); and 250 mg blend/kg of feed (T250).

In the collective stalls, the sheep were trapped in a bowl in their feeders immediately after milking. Each animal received 1.2 kg/d of concentrate, divided into two daily feedings (0700h and 1700h). Concentrate was offered first and approximately 15 min. later. Every day of the experiment, 100% of the concentrate supplied to the sheep was consumed.

Then, approximately 4.0 kg/d of corn silage (green matter) was divided into three daily feedings (0700h; 1100h and 1700h) (Table 1). The sheep were contained using a headlock to their feeder for 1 h, thereby guaranteeing individual consumption of silage. On days 16, 17, 18, 19, and 20, silage intake also was measured by subtracted orts weight from the amount of feed offered daily. After feed consumption, the animals remain at rest, free in the collective stall, with free access to water until the next feed or milking.

2.3. Analysis of chemical composition of the diet

Samples of silage and concentrate were collected for analysis of chemical composition, stored freezing (–20 ºC) until analysis. The concentrate was ground in a hammer-type mill with a grain size of 1 mm; forage was ground using a knife mill.

The feed samples were analyzed according to AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ethereal extract (EE), method 920.39 and ashes,

method 942.05. The concentration of neutral detergent fiber (NDF) and acid (ADF) were performed according to the methodology of Van Soest et al. (1991; without the addition of sodium sulfite and alpha-amylase). Results are presented in Table 1.

2.4. Milk measurement

The measurement of milk production of the animals was made on d 0, 15 to 20, using a "Milk Meter" (True Test® meter, Auckland, New Zealand). Average of milk production between days 16 to 20 was calculated, and the results were presented as being at d 20 of this study. The total production value was the result of the sum of the two daily milkings.

The productive efficiency (%) was calculated according to a methodology described by Alba et al. (2019), based on the difference between milk production on d 15 and 20 of experiment with that of d 0. Feed conversion was calculated based on the formula: daily feed consumption/daily milk production. The feed efficiency index (IEA) was calculated as the average production of animals in each group divided by the average consumption of feed per animal (Souza, 2003).

2.5 Sample collection

Blood and milk collections were performed on d 0, 15, and 20 of the experiment, at 0700h, with the animals fasting. Restraint was performed manually, using vacuum tubes (4 mL per animal). Blood was collected through puncture of the jugular vein. One of the tubes contained EDTA (ethylenediamine tetra-acetic acid) and was used to collect blood for complete blood counts and blood smears; the other contained clot activator (silica), and was used to obtain the serum for serum biochemical and oxidant/antioxidant analyses. Blood samples with clot activator were centrifuged at 3,800 *g* for 10 min and after separation, the serum was pipetted and placed in microtubes, and subsequently frozen (–20 ºC) until analysis.

The milk samples were collected during the first milking of the d, using a "Milk Meter" type meter (Tru Test®) for homogeneous sampling of milk production. The samples were stored in isothermal boxes with ice in reusable gel (4 ºC), and transported to the laboratory where they were processed.

2.6. Hemogram

Red blood cell counts (RBC), total leukocyte counts (WBC) and hemoglobin concentrations were measured using the semi-automatic analyzer (CC-530 CELM). The leukocyte differential was performed using blood smears stained with a commercial kit (Panotic Rapid, Laborclin), and cell identification was performed using an optical microscope (100x). The hematocrit was obtained after capillary centrifugation at 10,000 rpm for 5 min (Feldman et al., 2000).

2.7. Serum biochemistries

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) activity were evaluated, as well as the levels of total proteins (PT), albumin, urea, triglycerides, cholesterol and glucose. Measurements were performed using a semi-automatic analyzer (BioPlus 2000®), using specific commercial kits (Analisa®, Gold Analisa Diagnóstica, Belo Horizonte, Brazil). Globulin levels were calculated as the difference between albumin and total protein levels.

2.8. Oxidants and antioxidants in serum and milk

Superoxide dismutase (SOD) activity was measured using the auto-oxidation principle of pyrogallol (inhibition in the presence of SOD) with kinetic evaluation of the optical density at 420 nm for two minutes at ten-second intervals, and was expressed as U SOD/mg protein

(Beutler, 1984). Non-protein thiol levels (NPSH) were measured according to Sedlak and Lindsay (1968). Levels of reactive oxygen species (ROS) were obtained after incubation of 10 μL of serum, added in 12 μL dichlorofluorescein in 1 mm at 37 °C for 1 h in the dark (Ali et al., 1992); 488 nm was used for excitation and 520 nm for emission to determine fluorescence and the results were expressed as U DCF/mL. Levels of lipid peroxidation (LPO) were determined according to the method of Monserrat et al. (2003) and the results were expressed as μmol CHP/mL. The analysis of antioxidant capacity against peroxyl radicals (ACAP) was carried out according to Amado et al. (2009) and the results were expressed as U fluorescence/mg protein.

2.9. Proximate composition and milk quality

An automatic infrared analyzer (LactoStar Funke Gerber®) was used to determine the concentrations of proteins, lactose, fat, and total solids. Somatic cell counts (SCC) were measured using semi-automatic equipment (Ekomilk Scan Somatic Cells Analyzer®).

2.10 Statistical analysis

Each animal was considered the experimental unit for all analyses. All dependent variables were tested for normality using Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all were normally distributed. Then, all data were analyzed using the MIXED procedure of SAS, with the Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Productive efficiency, feed intake, feed conversion, and feed efficiency were tested for fixed effect of treatment using animal (treatment) as random effects. All other variables of the study were analyzed as repeated measures and tested for fixed effect of treatment × day. The compound symmetric covariance structure was selected for milk production; the Toeplitz covariance structure was selected for hematocrit, neutrophils, and eosinophils; and the first order autoregressive covariance structure

was selected for all other variables. The covariance structures were selected according to the lowest Akaike information criterion. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$.

3.0 Results

3.1 Milk performance

The results of milk performance are presented in Table 2. Effects of treatment × day ($P = 0.01$) was detected for milk production. T150 ewes had significantly greater milk production only on d 20, compared to T0 ewes. T150 and T250 ewes had significantly greater ($P \leq 0.04$) productive efficiency and feed efficiency, compared to T0 ewes. Although feed intake was not affected by treatments ($P = 0.21$), but T250 ewes had significantly greater ($P = 0.05$) feed conversion when compared to T0 ewes.

3.2 Milk composition and quality

The results of milk composition and quality are presented in Table 3. Effects of treatment × day were not detected for milk concentration of protein, fat, lactose, total solids, and ACAP. Effects of treatment × day was detected ($P = 0.05$) was detected for SCC in milk. T250 ewes have significantly less SCC on d 20 compared T0. Effects of treatment × day and treatment were detected for milk concentration of ROS ($P = 0.01$), i.e. T150 and T250 ewes had significantly lower concentrations on d 15 and 20 when compared to T0 ewes; as well as tendency of reduce to LPO ($P = 0.10$).

3.3 Hemogram

The results of hemogram are presented in Table 4. Effects of treatment × day were not detected ($P \geq 0.11$) for hematocrit, erythrocytes, hemoglobin, lymphocytes, monocytes, or

eosinophils. T250 ewes had significantly fewer leukocytes and neutrophils on d 15, and T150 and T250 ewes had significantly fewer of these cell types on d 20 than did T0 ewes.

3.4 Serum biochemistry

The results of serum biochemistry are presented in Table 5. There were effects of treatment × day for serum concentration of glucose, albumin, triglycerides, urea, AST, and ALT. However, effects of treatment × day (P = 0.05) and effects of treatment x day were detected (P = 0.01) for serum concentrations of total protein; i.e., T150 and T250 ewes had significantly greater concentrations on d 15 and 20, compared to T0 ewes. Effects of treatment × day was detected ($P \leq 0.03$) for serum concentration of globulin; and T150 ewes had significantly greater globulin concentrations on d 15; T150 and T120 ewes had significantly greater concentrations on d 20 when compared to T0 ewes. Effects of treatment x day (P = 0.05) were detected for serum concentration of cholesterol, i.e. T150 ewes had significantly greater concentrations than did T0 ewes. Effects of treatment × day and treatment were detected (P = 0.02) for serum activity of GGT; because T150 ewes had significantly greater concentrations only on d 15, compared to T0 and T250 ewes.

3.5 Oxidant/antioxidant status

The results of serum oxidants/antioxidants variables are presented in Table 6. Effects of treatment × day were detected (P = 0.01) for serum concentration of ROS and T250 ewes had significantly lower concentrations only on d 20, compared to T0 ewes. Effects of treatment × day were not detected ($P \geq 0.18$) for serum levels of LPO, and NPSH, as well as for SOD activity.

4. Discussion

The animals that received the microencapsulated phytogenic had greater feed efficiency, greater production efficiency, lower feed conversion, and greater volume of milk produced (Table 2). This suggests that the use of a phytogenic based on thymol, carvacrol, and cinnamaldehyde improved production. Such results can be explained by the capacity of these compounds to modify rumen fermentation and cause increases in productive performance, owing to their bactericidal, antiparasitic, and antioxidant activities, in order to modify the microorganisms that are part of digestion, in addition to preventing the action of free radicals on the DNA of cells involved in food absorption (Alagawany et al., 2015). Maenner et al. (2011) showed that the addition of essential oils to piglet feed improved performance, with improvement in the feeding efficiency, mainly associated with the ileal digestibility of the amino acids (6.5% improvement) and crude protein (6% to 12% improvement). Through the control of proliferation and inhibition of certain ruminal bacteria, it is possible to modulate ruminal fermentation. Phenolic substances such as thymol and carvacrol interact with bacterial membranes, killing them through the overflow of ions that have a slower replacement and causing energy expenditure that overwhelms the amount of energy required for bacterial growth. Microencapsulated essential oils composed of carvacrol, eugenol, and cinnamaldehyde were supplied to sheep, resulting in an increase in propionate production; the investigators found a decrease in protozoa, suggesting that the growth of propionate-producing microorganisms may be favored by these compounds (Soltan et al., 2018). Propionate is used for the synthesis of glucose and galactose that results in lactose production. Lactose from propionate acts on the mammary gland, increasing milk production (Alves Filho, 2005). Recently, Benchaar (2020) found that the inclusion of 50 mg of carvacrol/kg feed for 30 d did not have significant effects on the performance of milk production in dairy cows, nor did it alter the ruminal fermentation or improve the utilization of nutrients; the authors concluded that this dose was ineffective as a

supplement to increase milk production. In the present study, the use of 150 mg carvacrol/kg feed associated with the other herbal components increased milk production. In particular, there was a possible dose-dependent effect of carvacrol on milk production, a synergistic effect with the other herbal components, or an effect of the microencapsulated form of this carvacrol.

Lower ROS were found simultaneously with the decrease in the number of neutrophils. Neutrophils form an important line of defense, with the ability to engulf pathogens and foreign particles and to eliminate or inactivate them. During the process of elimination of these pathogens, the production of ROS occurs through cytoplasmic organelles of neutrophils. ROS play important bactericidal and bacteriostatic roles; however, their great oxidative potential damages tissues when produced in excess and over long periods, evolving into degenerative diseases (Kielland et al., 2009; Silva, 2015). This leads us to suggest that the significant decrease in LPO and ROS levels in milk and ROS in blood may be a consequence of the drop in the number of neutrophils that occurred on the same day in animals that received the phytogenic (Table 4). Aristatile et al. (2015) found that carvacrol inhibited the formation of free radicals, decreased the concentration of TBARS and LHP and maintained high levels of vitamin C and vitamin E in human neutrophils exposed to UVB radiation, in addition to significantly reducing DNA damage, promoting protection against oxidative stress. Cabello et al. (2015) demonstrated that carvacrol had dose-dependent antioxidant activity, i.e., low concentrations prevented or reduced the increase in the formation of ROS, while high concentrations were pro-oxidant. In this sense, we emphasize that both doses used in this study can be considered antioxidants that are beneficial to the health of sheep with respect to antioxidant/oxidant status.

The ewes fed with the phytogenic had lower SCCs. This can be related both to the decrease in neutrophil counts that occurred in the same period (anti-inflammatory action; Lima et al, 2013), as well as to the decrease in microorganisms (antimicrobial action; Chao et al., 2000) of the compound present in the blend and used here, improving the quality of the milk

produced. Mastitis is one of the causes of substantial economic losses in dairy properties, as this pathology is characterized by inflammation of the udder, with high SCCs in milk as a result of a microbial infection (Alba et al., 2019). The antimicrobial actions of thymol and carvacrol are related to their phenolic compositions, which makes them hydrophobic and capable of increasing the permeability of cell membranes and even their rupture, causing extravasation of the cellular contents and death of microorganisms (Alagawany et al., 2015; Benchaar et al., 2008). It is also reported that gram-positive bacteria are more susceptible to these compounds when compared to gram-negative bacteria that have an extra layer around their cell membrane; however, compounds such as thymol and carvacrol can also act on it by virtue of their low molecular weights (Benchaar et al., 2008).

Cholesterol showed higher values than the reference for sheep (52–76 mg/dL, Meyer and Harvey, 2004) in all treatments, similar results from other studies that reported a physiological increase in cholesterol in ruminants as lactation days progressed (Ruas et al., 2000; Godoy et al., 2004). Nevertheless, the highest value was in the group treated with the lowest doses of phytogenic, the same group with the highest milk production in the final days of our study. High cholesterol levels can also be explained during lactation as a consequence of increased plasma lipoprotein synthesis, or as a result of periods of fasting due to the mobilization of body fat (Gonzáles and Silva, 2008).

The results of our work revealed an increase in total proteins in the animals that received the additive, due to the increase in globulins, since the albumin values did not change. Total serum proteins are formed by the sum of albumin and globulins. By subtracting the value of albumin from the total protein, we obtain the value of globulins (Meyer and Harvey, 2004). Globulins function in plasma transport of metals, lipids, and bilirubin, in addition to actively participating in immune responses (Gonzáles and Silva, 2008). Immunoglobulins provide protection against pathogenic microorganisms, preventing damage to cell surfaces (Nakung et

al., 2004). Hyperglobulinemia may be associated with increased immunoglobulin synthesis, just as it occurs in a vaccine immune response (Jarikre et al., 2019) or after the transfer of colostral immunity providing protection against infections (Hernández-Castellano et al., 2014). Thus, the increase in plasma globulin levels can be associated with an increase in the immune response of supplemented animals.

Greater GGT activity of animals that received the phytogenic may be due to the increase in liver activity in sheep that received the additive. A similar result was reported by Castillo et al. (2012) in cattle supplemented with carvacrol and cinnamaldehyde. GGT is present in cell membranes in various tissues, particularly renal tubular cells and bile duct epithelium (Franciscato et al., 2006); increases in its activity can be a consequence of the increase in the concentration of bile acids and/or cholestasis (Kerr, 2003; Gonzáles and Silva, 2008), which can be correlated to the increase in cholesterol we found in this study.

5. Conclusion

A blend based on thymol, carvacrol, and microencapsulated cinnamaldehyde added to sheep feed after the peak of lactation increased production efficiency and reduced feed conversion. The consumption of the additive by the sheep stimulated humoral immune responses, increasing levels of globulins, as well as reducing neutrophil counts and serum reactive oxygen species substances, and primarily reducing the count of somatic cells in milk. In general, the additive used in the sheep's diet improves milk production and quality.

Conflict of interest

Authors declare they have no conflict of interest.

Ethics committee

This experiment was carried out in accordance with animal welfare practices and approved by the Ethics Committee for the Use of Animals in Research (CEUA / UDESC), protocol number 7308030419.

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Table 1. Ingredients and chemical composition of ingredients and experimental diets.

Ingredients	**As fed (kg/day)**	**Dry matter (DM; kg/day)**		
Corn silage (kg)	4.0	1.32		
Concentrate (kg)	1.2	1.05		
Chemical composition	**Corn silage**	**Concentrate (T0)**	**Concentrate (T150)**	**Concentrate (T250)**
DM, g/kg	329.2	879	881.6	882.4
Ash, g/kg DM	42.1	60.2	64.0	62.0
CP, g/kg DM	86.0	170.6	175.2	174.8
NDF, g/kg DM	333.0	95.6	82.0	84.0
ADF, g/kg DM	178.6	44.0	29.5	34.8
EE, g/kg DM	46.6	44.4	37.1	38.7
TPC (mg GAE/100 g DM)	-	0.006	0.003	0.017
IC_{50} (mg/mL)	-	3.93	2.83	2.58

Ingredients present in 100 kg of concentrate: corn (70%), soybean meal (25%) and buffering lactation nucleus (5%), i.e., ground corn (671 g/kg), soybean meal (277 g/kg), calcitic limestone (10 g/kg), sodium bicarbonate (4 g/kg) and 37 g/kg of premix (calcium min. 180 max. 220 g; phosphorus min. 32 g; sodium min. 40 g; sulfur min. 20 g; magnesium min. 20 g; cobalt min. 16 mg; iodine min. 17 mg; manganese min. 420 mg; selenium min. 730 mg; zinc min. 730 mg; fluorine max. 600 mg; niacin min. 500 mg; vitamin A min. 95000 UI; vitamin D min. 20000 UI; vitamin E min. 350 IU; monensin sodium 1200 mg; *Saccharomyces cerevisiae* 2.1 x 10^{10} CFU).

[2] Note: DM (Dry matter), CP (Crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber) and EE (ethereal extract).

[3] Note: Total phenolic compounds (TPC: mg GAE/100 g DM); Antioxidant activity against DPPH - IC_{50} radical (mg/mL)

Table 2. Milk performance of Lacaune ewes supplemented with dietary phytogenic.

Variables	Treatments[1]			SEM	P-value	
	T0	T150	T250		Treatment	Treatment × day
Production (L)					-	0.01
d 0	2.22	2.21	2.21	0.10		
d 15	2.18	2.14	2.26	0.11		
d 20	2.25^{b}	2.49^{a}	2.35^{ab}	0.09		
Productive efficiency (%)						
d 0 to 15	0.93^{c}	4.56^{b}	8.41^{a}	1.81	0.01	
d 0 to 20	4.18^{b}	13.6^{a}	10.3^{a}	2.11	0.01	
Feed intake						
d 15 to 20	84.1	90.0	85.2	7.74	0.21	
Feed conversion						
d 15 to 20	0.85^{a}	0.82^{ab}	0.81^{b}	0.01	0.05	
Feed efficiency						
d 15 to 20	117.1^{b}	122.8^{a}	122.6^{a}	2.14	0.04	

[1]T0, T150 and T250 represents 0, 150 and 250 g of phytogenic/kg of concentrate, respectively.

[a-c]Differs ($P \leq 0.05$) between treatments each respective day.

Table 3. Milk composition and quality of Lacaune ewes supplemented with dietary phytogenic.

Variables[1]	Treatments[2]			SEM	P-value
	T0	T150	T250		Treatment × day
Milk composition					
Protein (g/kg)					0.87
d 0	5.92	5.85	5.88	0.10	
d 15	5.87	5.81	5.79	0.12	
d 20	5.91	5.86	5.96	0.11	
Fat (g/kg)					0.88
d 0	5.97	5.62	5.50	0.25	
d 15	6.01	5.96	5.74	0.24	
d 20	5.86	6.02	5.85	0.23	
Lactose (g/kg)					0.87
d 0	5.56	5.59	5.62	0.13	
d 15	5.55	5.53	5.60	0.14	
d 20	5.65	5.47	5.57	0.15	
Total solids (g/kg)					0.59
d 0	17.45	17.06	17.00	0.42	
d 15	17.43	17.30	17.13	0.39	
d 20	17.42	17.35	17.38	0.40	
Milk quality					
SCC[1] (x10^3/mL)					0.05
d 0	151.30	143.80	156.10	29.25	
d 15	151.00	131.00	154.40	29.22	
d 20	216.30^{a}	175.90^{ab}	150.20^{b}	29.16	
ROS[1] (U DCF/mg protein)					0.01
d 0	0.66	0.64	0.65	0.55	
d 15	8.37^{a}	4.53^{b}	4.44^{b}	0.59	
d 20	9.15^{a}	4.54^{b}	4.72^{b}	0.49	
LPO[1] (µmol CHP/ mL)					0.10
d 0	395.3	635.7	588.4	144.4	
d 15	412.4	452.7	398.7	106.7	
d 20	432.9^{a}	387.7^{ab}	354.8^{b}	98.71	
ACAP[1] (UF/ mg protein)					0.15
d 0	0.71	0.65	0.67	0.03	
d 15	0.66	0.74	0.71	0.05	
d 20	0.64	0.79	0.82	0.03	

[1] Somatic cell count (SCC), reactive oxygen species (ROS), lipid peroxidation (LPO), and antioxidant capacity against peroxyl radicals (ACAP).

[2]T0, T150 and T250 represents 0, 150 and 250 g of phytogenic./kg of concentrate, respectively.

[a-b]Differs ($P \leq 0.05$) between treatments each respective day.

Table 4. Hemogram of Lacaune ewes supplemented with dietary phytogenic.

Variables	Treatments[1]			SEM	P-value
	T0	T150	T250		Treat × day
Hematocrit (%)					0.33
d 0	31.1	30.9	32.3	0.99	
d 15	31.8	32.7	33.7	0.87	
d 20	31.5	32.8	33.9	0.96	
Erythrocytes (x10^6µL)					0.42
d 0	7.99	8.00	8.15	0.29	
d 15	8.14	8.24	8.16	0.27	
d 20	8.05	8.19	8.68	0.21	
Hemoglobin (g/dL)					0.21
d 0	9.81	9.76	9.87	0.27	
d 15	9.96	9.84	9.65	0.27	
d 20	9.67	9.74	10.2	0.24	
Leukocytes (x10^3 µL)					0.04
d 0	21.2	21.0	20.9	3.13	
d 15	22.4^a	18.6^{ab}	15.0^b	3.01	
d 20	22.6^a	16.1^b	16.0^b	2.97	
Neutrophils (x10^3/µL)					0.02
d 0	10.6	9.78	10.6	1.52	
d 15	14.1^a	10.6^{ab}	7.41^b	1.62	
d 20	13.9^a	8.24^b	8.90^b	1.73	
Lymphocytes (x10^3/µL)					0.31
d 0	8.97	9.73	8.92	0.80	
d 15	7.45	7.06	6.74	0.74	
d 20	7.96	7.01	6.82	0.85	
Monocytes (x10^3/µL)					0.20
d 0	0.15	0.15	0.12	0.03	
d 15	0.15	0.10	0.17	0.07	
d 20	0.10	0.12	0.15	0.05	
Eosinophils (x10^3/µL)					0.11
d 0	1.54	1.40	1.34	0.22	
d 15	0.74	0.84	0.76	0.17	
d 20	0.69	0.74	0.67	0.19	

[1]T0, T150 and T250 represents 0, 150 and 250 g of phytogenic/kg of concentrate, respectively.

[a-b]Differs ($P \leq 0.05$) between treatments each respective day.

Table 5. Serum biochemistry variables of Lacaune ewes supplemented with dietary phytogenic.

Variables[1]	Treatments[2]			SEM	P-value
	T0	T150	T250		Treat × day
Glucose (mg/dL)					0.17
d 0	55.2	53.8	53.3	2.20	
d 15	62.4	66.7	69.4	2.21	
d 20	60.4	67.4	66.7	2.34	
Total Protein (g/dL)					0.05
d 0	6.67	6.51	6.65	0.46	
d 15	7.97^{b}	9.84^{a}	9.02^{a}	0.43	
d 20	7.61^{b}	9.63^{a}	9.23^{a}	0.43	
Albumin (g/dL)					0.40
d 0	3.14	3.25	3.08	0.12	
d 15	3.37	3.96	3.73	0.14	
d 20	3.50	3.61	3.14	0.15	
Globulin (g/dL)					0.03
d 0	3.53	3.26	3.57	0.39	
d 15	4.60^{b}	5.88^{a}	5.29^{ab}	0.37	
d 20	4.11^{b}	6.02^{a}	6.09^{a}	0.37	
Cholesterol (mg/dL)					0.05
d 0	85.6	82.7	84.5	5.01	
d 15	87.9	98.4	99.1	4.25	
d 20	86.0^{b}	101.7^{a}	95.1^{ab}	4.24	

Triglycerides (mg/dL)					0.39
d 0	23.0	22.2	20.83	0.96	
d 15	22.4	22.9	21.7	0.87	
d 20	22.7	23.4	24.3	0.64	
Urea (mg/dL)					0.30
d 0	58.6	62.4	56.1	2.42	
d 15	54.3	56.8	57.4	2.43	
d 20	55.1	53.8	56.7	2.08	
AST (U/L)					0.64
d 0	104.8	110.7	114.3	7.96	
d 15	99.7	106.0	101.3	8.97	
d 20	109.9	106.7	93.0	8.53	
ALT (U/L)					0.93
d 0	13.8	14.9	13.3	0.86	
d 15	14.7	11.9	12.7	0.95	
d 20	13.0	14.1	12.3	0.75	
GGT (U/L)					0.02
d 0	56.9	63.0	59.5	7.68	
d 15	106.3[b]	147.7[a]	105.0[b]	7.32	
d 20	105.5	113.8	100.4	7.32	

[1]Aspartate aminotransferase (AST), Alanine transaminase (ALT) and gamma glutamyltransferase (GGT).

[2]T0, T150 and T250 represents 0, 150 and 250 g of phytogenic/kg of concentrate, respectively.

[a-b]Differs ($P \leq 0.05$) between treatments each respective day.

Table 6. Serum oxidants/antioxidants variables of Lacaune ewes supplemented with dietary phytogenic.

Variables[1]	Treatments[2]			SEM	P-value
	T0	T150	T250		Treat × day
ROS[1] (U DCF/mg protein)					0.01
d 0	0.40	0.52	0.56	0.09	
d 15	0.48	0.42	0.45	0.09	
d 20	0.64[a]	0.51[ab]	0.33[b]	0.09	
LPO[1] (µmol CHP/mL)					0.13
d 0	72.9	80.2	92.0	6.90	
d 15	70.6	74.3	79.1	5.41	
d 20	71.8	67.9	60.7	6.85	
SOD[1] (U SOD/mg protein)					0.36
d 0	5.72	5.51	5.61	0.11	
d 15	5.03	5.17	5.21	0.09	
d 20	5.41	5.32	5.68	0.13	
NPSH[1] (µmol/mL)					0.36
d 0	1.89	1.82	1.86	0.04	
d 15	1.74	1.69	1.81	0.09	
d 20	1.91	1.84	1.90	0.10	

[1] Reactive oxygen species (ROS), lipid peroxidation (LPO), superoxide enzyme dismutase (SOD) and non-protein thiols (NPSH).

[2]T0, T150 and T250 represents 0, 150 and 250 g of phytogenic/kg of concentrate, respectively.

[a-b]Differs ($P \leq 0.05$) between treatments each respective day.

2.2. MANUSCRITO II

Inclusion of pepper extract containing capsaicin in the diet of ewes in the mid-lactation period: effects on health, milk production and quality

Cunha M.G., Alba D.F., Leal K.W., Marcon H., Souza C.F., Baldissera M.D., Paglia E.B., Kempka A.P., Vedovatto M., Zotto A.A. & Da Silva A.S. (2020). Inclusion of pepper extract containing capsaicin in the diet of ewes in the mid-lactation period: effects on health, milk production, and quality. ***Research, Society and Development***, 9(9): 1-16**, eXX**

Inclusion of pepper extract containing capsaicin in the diet of ewes in the mid-lactation period: effects on health, milk production, and quality

Inclusão de extrato de pimenta contendo capsaicina na dieta de ovelhas no período médio de lactação: efeitos na saúde, produção e qualidade do leite

Inclusión de extracto de pimiento que contiene capsaicina en la dieta de las ovejas en el período de lactancia media: efectos sobre la salud, la producción de leche y la calidad

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Abstract

Pepper extract (PE, 5 g capsaicin / kg PE) was added to the feed of the sheep during the lactation period (Day 75-93) to maintain production, improve milk quality, and preserve their health. The groups were: T0, control, (without PE); T200 (200 mg PE / kg concentrate) and T400 (400 mg PE / kg concentrate). The reduction in milk production (L) was smaller in the T400 ewes on days 0 to 18 and 14 to 18 than in the T0 group. Feed conversion was lower in sheep in groups T200 and T400 than in group T0. The interaction between the treatment and the day for protein, lactose and total milk totals was greater in ewes that consumed PE on day 18. The somatic cell counts in milk were lower in the T400 ewes. The levels of total protein and globulin were the highest in the blood of animals in the T400 group. There were lower levels of reactive oxygen species and lipoperoxidation in the serum and milk of animals in groups T200 and T400. On the 18th day, the serum of sheep that consumed PE increased levels of non-protein thiols and superoxide dismutase activities. The inclusion of PE (400 mg / kg) containing capsaicin in sheep concentrate in the middle of lactation (after the peak of lactation) minimized the reduction in milk production during the experiment and improved the quality of the milk, as well as stimulated an antioxidant response systemic.

Keywords: Pepper extract; oxidants; antioxidants; health status; nutrition; lactation.

Resumo

Extrato de pimenta (PE, 5 g capsaicina / kg PE) foi adicionado à ração das ovelhas no período de lactação (Dia 75-93) para manter a produção, melhorar a qualidade do leite, e preservar sua saúde. Os grupos foram: T0, controle, (sem PE); T200 (200 mg de PE / kg de concentrado) e T400 (400 mg de PE / kg de concentrado). A redução na produção de leite (L) foi menor nas ovelhas T400 nos dias 0 a 18 e 14 a 18 do que no grupo T0. A conversão alimentar foi menor nas ovelhas dos grupos T200 e T400 do que no grupo T0. A interação entre o tratamento e o dia para proteína, lactose e sólidos totais no leite foi maior nas ovelhas que consumiram PE no dia 18. As contagens de células somáticas no leite foram mais baixas nas ovelhas T400. Os níveis de proteína total e globulina foram mais elevados no sangue dos animais do grupo T400. Houve menores níveis de espécies reativas de oxigênio e lipoperoxidação no soro e leite dos animais dos grupos T200 e T400. No 18º dia, o soro de ovelhas que consumiram PE apresentou maiores níveis de tióis não protéicos e atividades da superóxido dismutase. A inclusão de PE (400 mg / kg) contendo capsaicina no concentrado de ovinos no meio da lactação (após o pico da lactação)

minimizou a redução da produção de leite durante o experimento e melhorou a qualidade do leite, bem como estimulou uma resposta antioxidante sistêmica.

Palavras-chave: Extrato de pimenta; oxidantes; antioxidantes; estado de saúde; nutrição; lactação.

Resumen

Se añadió extracto de pimienta (PE, 5 g de capsaicina / kg de PE) al alimento de las ovejas durante el período de lactancia (día 75-93) para mantener la producción, mejorar la calidad de la leche y preservar su salud. Los grupos fueron: T0, control, (sin PE); T200 (200 mg PE / kg concentrado) y T400 (400 mg PE / kg concentrado). La reducción en la producción de leche (L) fue menor en las ovejas T400 en los días 0 a 18 y 14 a 18 que en el grupo T0. La conversión alimenticia fue menor en ovejas en los grupos T200 y T400 que en el grupo T0. La interacción entre el tratamiento y el día para proteínas, lactosa y sólidos totales en la leche fue mayor en las ovejas que consumieron PE el día 18. Los recuentos de células somáticas en la leche fueron menores en las ovejas T400. Los niveles de proteína total y globulina fueron más altos en la sangre de los animales del grupo T400. Hubo niveles más bajos de especies reactivas de oxígeno y lipoperoxidación en el suero y la leche de los animales en los grupos T200 y T400. El día 18, el suero de las ovejas que consumieron PE mostró niveles más altos de tioles no proteicos y actividades de superóxido dismutasa. La inclusión de PE (400 mg / kg) que contiene capsaicina en concentrado de oveja en la mitad de la lactancia (después del pico de lactancia) minimizó la reducción en la producción de leche durante el experimento y mejoró la calidad de la leche, además de estimular una respuesta antioxidante. sistémico.

Palabras clave: Extracto de pimienta; oxidantes; antioxidantes; estado de salud; nutrición; lactancia.

1. Introduction

Sheep milk production tends to be impaired in seasons of the year when critical temperatures cause thermal discomfort. This, combined with the drop in the quality of the feed supplied at the end of the lactation peak, can result in large economic losses (Neiva et al., 2004). Persistence of lactation is defined as the ability to maintain production after reaching peak lactation, which occurs between the third and fourth week postpartum (Cannas et al., 2002). Improving the digestion of ruminants has been the focus of research aimed at providing maximum use of the nutrients ingested; the use of active compounds present in plants has garnered substantial attention (Cardozo et al., 2006; Castillo et al., 2012). Modifiers of ruminal fermentation such as capsaicin act by modulating the ruminal microbiota through the inhibition of the growth of some microorganisms and the processing of others; capsaicin decreases the production of acetate and the concentration of ammonia, as well as increasing the production of propionate and the total production of volatile fatty acids (Calsamiglia et al, 2007). According to this researcher, these changes include more acidic pH; therefore, animals that receive feed with higher levels of concentrate are able to make better use of feed (Calsamiglia et al, 2007).

Capsaicin is a bioactive compound found in peppers of the genus *Capsicum* spp., known for its pungent odor. Its chemical name is 8-methyl-N-vanillyl-6-nonenamide, $C_{18}H_{27}NO_3$ (Calsamiglia et al., 2007). It is a crystalline, lipophilic, colorless, and odorless alkaloid (Reyes-Escogido et al., 2011). Among its pharmacological effects, bactericidal and fungicidal activities are particularly important (Zhao et al., 2020). It also has anti-inflammatory properties capable of reducing the expression of interleukins (Choi et al., 2011), as well as analgesic properties that relieve chronic muscle, joint, and neuronal pains that are not responsive to medication (Reyes-Escogido et al., 2011). Capsaicin can be used topically as an ointment for animals and humans as a treatment for peripheral neuropathies, minimizing lameness, and increasing quality of life (Seino et al., 2003, Roberts et al., 2011). Antioxidant properties are also described in studies with capsaicin because they contain phenolic compounds, in addition to the fact that red peppers have a greater antioxidant capacity than do green peppers (Materska & Perucka, 2005). Recently, a study conducted by An et al. (2020) revealed that the addition of a blend based on the oleoresin of *Capsicum* spp. and eugenol improved performance, digestibility of nutrients, immune responses, and antioxidant capacity of sheep, leading us to hypothesize that this feed additive may have positive effects on production, milk quality, and health status of lactating sheep.

In theory, the effect of capsaicin can cause greater water intake; this effect has been the subject of study in ruminants (Rodriguez-Prado et al., 2012). Water is an essential factor for

maintaining the basic functions of the organism, in addition to being closely associated with the animal's zootechnical performance (Nunes, et al., 2011). Studies suggest that the addition of capsaicin to animal feed increases water consumption and can be used to increase production rates in times of low water intake (Zafra et al., 2003; Rodríguez-Prado et al., 2012). Therefore, the objective of the present study was to determine whether adding capsaicin-rich pepper extract to the feed of lactating sheep (mid-lactation period) would maintain milk production and improve milk quality, as well as preserving animal health.

2. Materials and Methods

This scientific research took place through the quantitative experimental method, according to Fonseca (2002), Gil (2008) e Gerhard & Silveira (2009).

2.1 Pepper extract

The feed additive used in this study is a commercial product based on pepper extract (Capsin®; Nutriquest). The chemical composition of the pepper extract was analyzed according to AOAC (2000), being detected the concentration of dry matter (920 g/kg), ether extract (444 g/kg), crude protein (64.4 g/kg), detergent fiber neutral (293 g/kg), and acid detergent fiber (229 g/kg). The quantification of capsaicin in the pepper extract was performed using gas chromatography and revealed a concentration of 5.0 g/kg.

2.2 Animals and experimental design

Thirty multiparous Lacaune ewes (third order of lactation), mid-lactation period (75 to 93 days postpartum) and with an average body weight of 68 ± 3.8 kg were used in this experiment, which was carried out at the end of autumn on a farm located in Chapecó, Santa Catarina, Brazil.

The animals were housed in an open shed on a beaten floor covered with wood shavings, separated by group in three stalls (24 m^2). The groups consisted of ten animals each and were identified as follows: the T0 – control group (without extract), the T200 treatment group (200 mg PE/kg concentrate), and the T400 treatment group (400 mg PE/kg concentrate).

The concentrate (1.2 kg of animal/day) was offered in equal proportions in the morning and afternoon (8:00 AM and 5:00 PM) throughout the experimental period. To guarantee the concentrate intake, animals were restrained in headlocks. The concentrate was produced from soybean meal, ground corn, and a vitamin and mineral complex.

After concentrate was ingested (100%/ewes/day), with the animals remaining restrained in headlocks, corn silage (3.8 kg animal/day) was offered in the morning and

afternoon. It remained for 30 min until the ingestion of most available silage had occurred (between 80 to 90%); then, the sheep were released from the pen and stayed in the collective stall. Afternoon (1:00 PM), the sheep had Tifton chopped hay available in a individual feeder (0.2 kg/animal/day); therefore, the animals were in collective pens, but the feeding was individualized. Water was offered ad libitum.

There was an adaptation period of 13 days (days 1 to 13) to the environment and feed, an adaptation period major that used by our group research (Jaguezeski et al., 2018; Alba et al., 2019, Santos et al., 2019). Between days 14 and 18 of experiment, the intakes of hay, corn silage, and concentrate were measured individually. The leftover food for the day was collected and weighed in order to measure daily intake for five days consecutively.

2.3. Diet analysis

During the experimental period at 1 to 18 (beginning (day 1), middle (day 9), and end (day 18)), samples of silage and concentrate were collected, identified, and stored frozen (–20 ºC). On the day of analysis, the three samples taken during the silage and concentrate experiment for each treatment and were homogenized, forming a single sample used for the processes described below.

2.3.1. Bromatological

First, grinding was carried out; feed was concentrated using a hammer-type mill (grain size 1 mm). For forage, we used a knife-type mill (Silva & Queiroz 2002). The feed samples were analyzed according to the AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ethereal extract (EE), method 920.39, and ash, method 942.05. The concentrations of neutral detergent fiber (NDF) and acid (ADF) were measured according to the methodology of Van Soest et al. (1991; without the addition of sodium sulfite). Results are presented in Table 1.

2.3.2 Determination of total phenolic compounds (TPC) and antioxidant activity by elimination of radicals by DPPH

For quantification of total phenolic compounds (TPC) and antioxidant activity by elimination of radicals by DPPH (IC_{50}), we used the methodology described in detail by Alba et al. (2019). For extraction, 0.5 g of samples (concentrate) were dissolved in 50 ml of distilled water. The mixture was placed in an ultrasonic bath (70 W) for 3 h and remained in the dark for more 3 h. Quantification of TPC was performed using the Folin-Ciocalteu colorimetric method

and free radical scavenging activity in the extracts was determined as the antioxidant reduction capacity of DPPH radical. All tests were performed in triplicate (Table 1).

Table 1. Ingredients and chemical composition of ingredients and experimental diets.

Ingredients	As fed (kg/day)		Dry matter (DM; kg/day)		
Corn silage (kg)	3.80		1.24		
Concentrate (kg)	1.20		1.06		
Hay (kg)	0.20		0.17		
Total (kg)	5.20		2.47		
Chemical composition	Corn silage	Hay	Concentrate (T0)	Concentrate (T200)	Concentrate (T400)
DM, g/kg	327.4	874	885.8	885.2	889.0
Ash, g/kg DM	42.8	34.4	56.3	58.4	57.8
CP, g/kg DM	85.2	99.7	179.5	179.8	179.9
NDF, g/kg DM	343.7	609	89.0	88.2	90.0
ADF, g/kg DM	175.1	217	34.0	35.8	36.0
EE, g/kg DM	47.0	11.7	42.0	42.3	42.0
CFT (mg EGA/100 g DM)	-	-	0.133	0.145	0.158
IC_{50} (mg/mL)	-	-	3.07	2.33	1.54

Ingredients present in 100 kg of concentrate: corn (70%), soybean meal (25%) and buffering lactation nucleus (5%), i.e., ground corn (671 g/kg), soybean meal (277 g/kg), calcitic limestone (10 g/kg), sodium bicarbonate (4 g/kg) and 37 g/kg of premix (calcium min. 180 max. 220 g; phosphorus min. 32 g; sodium min. 40 g; sulfur min. 20 g; magnesium min. 20 g; cobalt min. 16 mg; iodine min. 17 mg; manganese min. 420 mg; selenium min. 730 mg; zinc min. 730 mg; fluorine max. 600 mg; niacin min. 500 mg; vitamin A min. 95000 IU; vitamin D min. 20000 IU; vitamin E min. 350 IU; monensin sodium 1200 mg; *Saccharomyces cerevisiae* 2.1 x 10^{10} CFU).

[2]Note: DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber) and EE (ethereal extract).

[3]Note: Total phenolic compounds (TPC: mg EGA/100 g DM); Antioxidant activity against DPPH - IC_{50} radical (mg/mL).

2.4. Measurement of milk production

The milk produced by the animals was measured at the beginning (day 0) and end (day 14) of the adaptation period, and in the experimental period (days 15, 16, 17 and 18) using a "Milk Meter" True Test® meter, Auckland, New Zealand. The average production of the days on which the experiment occurred was presented. The total production was the result of the sum of the two daily milkings (morning and afternoon). Milk production data from days 0, 14 and 18 were used to calculate the percentage of reduction in milk production during the 18-day experiment in the mid-lactation period. Based on data on milk production and feed consumption, feed conversion was calculated.

2.5. Sample collection

Blood was collected after 12 h of fasting. Blood samples for hematological and biochemical analyses were collected on the first day, after the adaptation period and at the end of the experimental period, on days 0, 14, and 18. Collections were performed with animals fasting for 12 hours of solids, with animals being restrained manually (holding them by the head and flank). Venipuncture from the jugular vein drew blood in vacutainers fitted with specific needles. The tubes used were as follows: with EDTA for blood count and blood smear; and with clot activator (silica) for serum biochemistry and oxidants/antioxidants. To collect serum samples from the clot activator, centrifugation was performed at 5,000 rpm for 10 min.

The serum was pipetted into Eppendorf tubes and frozen (–20 ºC) for further analysis. Milk samples were collected on days 1, 14, and 18, as a final product of complete and homogeneous milking, using "Milk Meter" meters (Tru Test®). All samples were transported to the laboratory in isothermal boxes with ice at –4 ºC.

2.6. Milk analysis

2.6.1 Centesimal composition of milk

The lactose, protein, fat, and total solid concentrations of the milk samples were measured using the LactoStar automatic infrared analyzer, Funke Gerber®, standardized methodology for sheep milk (Alba et al., 2019). Analyses were performed in duplicate.

2.6.2 Somatic cell counts in milk

Somatic cell counts (SCC) present in the milk was performed using a semi-automatic counter (Ekomilk Scan Somatic Cells, Analyzer®). Analyses were performed in duplicate.

2.6.3 Oxidants and antioxidants in milk

Milk samples were used for the analyses. First, protein concentrations in milk samples were measured, and based on this information, the samples were prepared for the analyses described below, according to the specific methodologies for each technique.

The enzymatic activity of superoxide dismutase (SOD) was measured using the method of Beutler (1984), with auto-oxidation in pyrogallol, read by spectrophotometer at 480 nm every 10 seconds for 2 minutes. The results were expressed in U SOD/mg protein.

For the determination of oxygen reactive species (ROS) 10 μL of milk sample, with 12 μL of dichlorofluorescein were incubated at 37 °C for 1 h, without incidence of light (Ali et al.,

1992). Subsequently, 488 nm was used for excitation and 520 nm for emission to determine fluorescence and the results were expressed in U DCF/mL.

The determination of lipid peroxidation (LPO) was according to the method of Monserrat et al. (2003) and the results are expressed in µmol CHP/mL.

Analysis of antioxidant capacity against peroxyl radicals (ACAP) was performed according to Amado et al. (2009) and the results were expressed in FU/mg protein.

2.7. Blood analysis

2.7.1 Hemogram

Red blood cell count (RBC), total leukocyte count (WBC) and hemoglobin concentration were performed using a semi-automatic analyzer (CC-530 CELM). The leukocyte differential was performed by means of blood smears on glass slides stained with its own commercial kit (*Panótico Rápido*, Laborclin), cell identification was performed using an optical microscope (100x). Hematocrit was obtained after capillary centrifugation at 10,000 rpm for 5 min (Feldman et al., 2000).

2.7.2 Serum biochemistries

In serum samples, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) were measured, as were levels of total proteins (TP), albumin, glucose, cholesterol, triglycerides and urea using specific commercial kits (Analisa®, Gold Analisa Diagnóstica, Belo Horizonte, Brazil) and semi-automatic equipment (BioPlus 2000®). Subtracting albumin from total proteins, globulin levels were calculated.

2.7.3. *Oxidants and antioxidants in serum*

The variables LPO, ROS, and SOD were also measured in serum, using the same methodology described above for milk samples (section 2.6.3). Non-protein thiols (NPSH) were measured in serum, according to the methodology described by Sedlak & Lindsay (1968).

2.10 Animal behavior

Animal behavior was evaluated in the morning, right after the individual feeding. As soon as the sheep were released from the headlocks, behavioral analysis continued for a constant period of 90 minutes. The animals were numbered with purple spray (lateral and back-sacral) to facilitate observation. Three trained observers were responsible for data collection for three

consecutive days at the end of the adaptation period (days 11, 12 and 13 of the experiment). The observers rotated among groups between days and treatments, ensuring that all observers have assessed the three groups of sheep. An ethogram predicted the observation of frequency and length of stay, consumption of water, hay and silage, as well as leisure time.

2.11 Statistical analysis

The animal was considered the experimental unit for all analyses. All dependent variables were tested for normality using Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all were normal distributed. Then, all data were analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Lactation efficiency, feed intake, feed conversion, and behavior variables were tested for fixed effect of treatment using animal (treatment) as random effects. All other variables were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment × day, using animal (treatment) as random variables and animal (treatment) as subjects. All results obtained on d 0 for each variable were included as covariates in each respective analysis; however, they were removed from the model when $P > 0.10$. The covariance structures were selected according to the lowest Akaike information criterion. The compound symmetric covariance structure was selected for milk concentration of SOD and ROS. The Toeplitz covariance structure was selected for hematocrit, and eosinophils and the first order autoregressive covariance structure were selected for all other variables. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$.

3.0 Results

3.1 Milk performance, composition and quality

The results of milk performance, composition and quality are presented in Table 2. Effects of treatment × day and treatment were not detected for milk production or concentration of fat. The reduction in milk production (L) was less in the T400 ewes at days 0 to 18 and days 14 to 18 than in the T0 ewes. Effects of treatment were not detected for feed intake, but were detected ($P = 0.01$) for feed conversion, and T200 and T400 ewes had greater feed conversion than did T0 ewes. There was a significant ($P = 0.01$) interaction between treatment and day for protein and ($P \leq 0.05$) for lactose; and T400 ewes had greater concentrations of these variables only on d18 compared to T0 and T200 ewes. Effects of treatment × day ($P = 0.02$) were detected for concentrations of total solids. T200 and T400 ewes had greater milk concentrations of total

solid on d 14 than did T0 ewes, and only T400 ewes had greater concentrations on d 18 than did T0 ewes. Effects of treatment × day and treatment were detected ($P \leq 0.04$) for SCC. T400 ewes had lower counts only on d 18, compared to T0 and T200 ewes.

The results of milk levels of oxidant/antioxidants are presented in Figure 1. Effects of treatment × day and treatment were not detected for milk concentration of SOD. However, effects of treatment × day to be detected ($P = 0.05$) and effects of treatment were not detected ($P = 0.43$) for milk concentration of ROS, and T400 ewes had lower concentrations only on d 14 than did T0 and T200 ewes. Effects of treatment × day ($P = 0.02$) were detected for levels of ACAP, and T200 and T400 had greater levels only on d 18 than did T0 ewes. Effects of treatment × day ($P = 0.05$) were detected for levels of LPO, and T200 ewes had lower levels only on d 14 than did T0 ewes. T400 ewes had lower levels only on d 14, compared to the others.

Table 2. Milk production and composition of Lacaune ewes supplemented with pepper extract.

Variables[1]	Treatments[2]			SEM	*P*-value	
	T0	T200	T400		Treatment	Treatment × day
Production (L)					0.66	0.27
d 0	2.40	2.37	2.38	0.12		
d 14	2.01	2.01	2.04	0.12		
d 18	1.81	1.87	1.97	0.12		
Mean[3]	1.91	1.94	2.00	0.12		
Reduction in milk production (%)						
d 0 to 14	16.2	15.5	14.9	0.08	0.56	
d 0 to 18	24.5[a]	21.5[ab]	17.9[b]	0.09	0.01	
d 14 to 18	9.95[a]	7.14[a]	3.60[b]	0.05	0.01	
Sum of milk production (L)						
d 14-18	9.15	9.25	9.55	0.19	0.54	
Feed intake						
d 14 to 18	79.9	73.1	77.0	13.33	0.43	
Feed conversion						
d 14 to 18	2.19[a]	2.06[b]	2.08[b]	0.10	0.01	
Milk composition						
Protein (g/kg)					0.05	0.01
d 0	3.92	3.99	3.80	0.08		
d 14	3.65	3.84	3.92	0.08		
d 18	3.75[b]	3.87[b]	4.25[a]	0.08		
Mean[3]	3.70B	3.85AB	4.08A	0.07		
Fat (g/kg)					0.62	0.47

d 0	5.24	5.32	5.31	0.19		
d 14	5.48	5.69	5.70			
d 18	5.44	5.76	5.66			
Mean[3]	5.46	5.73	5.68	0.21		
Lactose (g/kg)					0.05	0.01
d 0	5.75	5.90	5.56	0.14		
d 14	5.40	5.64	5.76	0.14		
d 18	5.48[b]	5.69[b]	6.25[a]	0.14		
Mean[3]	5.44B	5.66B	6.00A	0.15		
Total solids (g/kg)					0.11	0.02
d 0	15.29	15.44	14.43	0.38		
d 14	13.80[b]	15.36[a]	15.58[a]	0.41		
d 18	15.48[b]	15.87[ab]	16.75[a]	0.38		
Mean[3]	14.64	15.61	16.16	0.39		
SCC (x 10^3 /mL)					0.01	0.04
d 0	199.7	192.3	209.8	51.3		
d 14	354.9	248.6	244.2	51.3		
d 18	395.4[a]	399.4[a]	144.5[b]	54.0		
Mean[3]	375.1A	324.0A	194.3B	52.3		

[1]SCC, somatic cell count. [2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Differs ($P \leq 0.05$) between treatments by day showed in same line with different letters ([a-b]) (Treat × day). [3]Mean of days 14 and 18 of the experiment, illustrates the effect of the treatment, with different letters ([A-B]) in same line differing statistically ($P \leq 0.05$).

3.2 Hemogram

The results of hemogram are presented in Table 3. Effects of treatment × day and treatment were not detected ($P \geq 0.11$) for erythrocyte counts, hematocrits, hemoglobin levels, or for counts of leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils.

Table 3. Hematological and biochemistry variables of Lacaune ewes supplemented with pepper extract.

Variables	Treatments[1]			SEM	*P*-value	
	T0	T200	T400		Treat	Treat × day
Hematology						
Erythrocytes (x 10^6 µL)	7.94	7.91	7.84	0.33	0.98	0.83
Hematocrit (%)	31.85	30.69	31.17	0.89	0.62	0.82
Hemoglobin (g/dL)	9.66	9.30	9.36	0.23	0.52	0.45
Leukocytes (x 10^3 µL)	11.13	11.78	12.72	1.69	0.76	0.65
Neutrophils (x 10^3 µL)	4.24	4.93	5.23	0.59	0.37	0.51
Lymphocytes (x 10^3 µL)	5.43	5.63	5.73	0.93	0.96	0.75
Monocytes (x 10^3 µL)	0.04	0.05	0.03	0.01	0.61	0.12
Basophils (x 10^3 µL)	0.01	0.02	0.06	0.02	0.10	0.11

Eosinophils (x 10^3 µL)	1.34	1.20	1.66	0.31	0.51	0.13
Biochemistry						
Glucose (mg/dL)	60.28	63.39	60.56	2.43	0.62	0.93
Albumin (g/dL)	3.71	3.81	3.68	0.12	0.73	0.98
Cholesterol (mg/dL)	75.18	82.03	77.36	3.70	0.42	0.66
Triglycerides (mg/dL)	26.42	26.31	24.77	1.07	0.47	0.29
AST (U/L)	123.97	114.30	117.60	7.80	0.68	0.63
ALT (U/L)	20.63	18.53	20.03	0.74	0.14	0.16
GGT (U/L)	109.35	106.31	111.97	6.27	0.81	0.21

[1]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Note: There was no statistical difference (P>0.05) between treatments, as well as there was no interaction between treatment versus day for the variables presented in this table. We present the treatment average considering the 14th and 18th days of the experiment, with the "day 0" being used only as a covariate. OBS: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT).

3.3 Serum biochemistry

The results of serum biochemistry are presented in Table 3 and 4. Effects of treatment × day and treatment were not detected for serum concentration of glucose, albumin, cholesterol, triglycerides, AST, ALT, or GGT. However, effects of treatment (P = 0.05) and interaction (days 14 and 18) were detected for serum concentration of total protein and globulin, and T400 ewes had greater concentrations than did T0 ewes. Effects of treatment × day (P = 0.04) were detected for serum concentration of urea, and T400 ewes had greater concentrations only on d 18, compared to T0 ewes.

Table 4. Protein response (total protein, globulin, and urea) of Lacaune ewes supplemented with pepper extract.

Variables[1]	Treatments[2]			SEM	P-value	
	T0	T200	T400		Treatment	Treatment × day
Total Protein (g/dL)					0.03	0.05
d 0	8.54	8.35	9.54	0.22		
d 14	8.22[b]	8.94[ab]	8.97[a]	0.21		
d 18	8.60[b]	8.90[ab]	10.3[a]	0.22		
Mean[1]	8.41B	8.92AB	9.63A	0.20		
Globulin (g/dL)					0.05	0.04
d 0	4.83	4.60	5.96	0.17		
d 14	4.62[b]	5.08[ab]	5.39[a]	0.18		
d 18	4.74[b]	5.00[b]	6.53[a]	0.19		
Mean[1]	4.68B	5.04B	5.96A	0.19		
Urea (mg/dL)					0.11	0.04
d 0	42.9	37.3	37.5	3.11		
d 14	36.6	43.4	39.1	3.11		

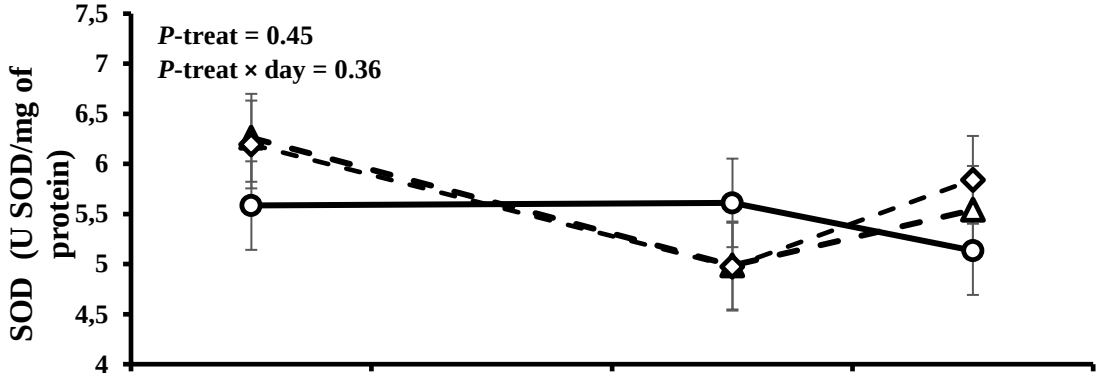
d 18	39.4[b]	45.3[ab]	49.2[a]	3.12
Mean[1]	38.0	44.3	44.1	3.11

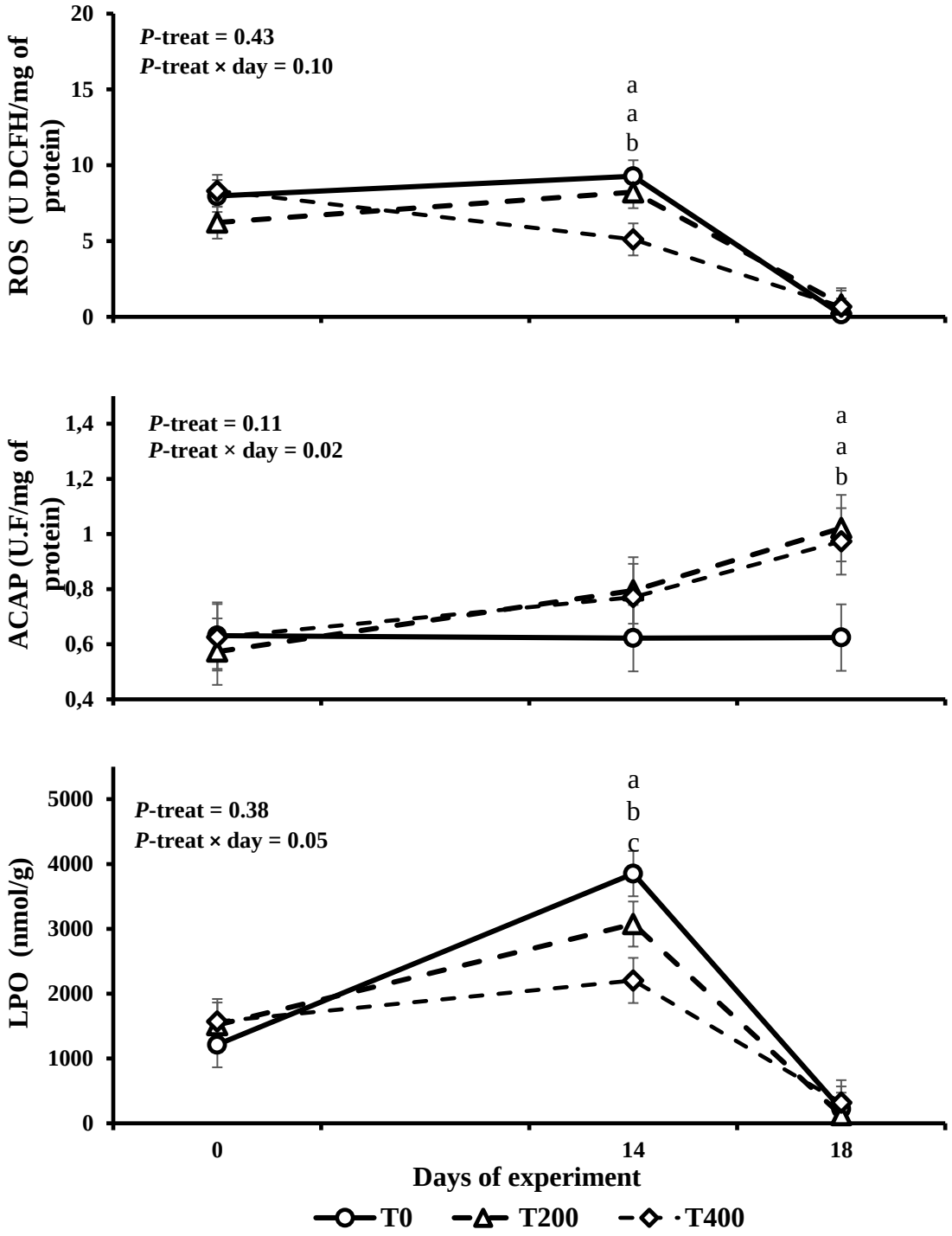
[2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Differs ($P \leq 0.05$) between treatments by day showed in same line with different letters ([a-b]) (Treat × day). [3]Mean of days 14 and 18 of the experiment, illustrates the effect of the treatment, with different letters ([A-B]) in same line differing statistically ($P \leq 0.05$).

3.4 Serum oxidant/antioxidant status

The results of serum oxidants/antioxidants variables are presented in Figure 2. Effects of treatment × day to be detected (P = 0.05) and were not detected (P = 0.47) for serum concentrations of NPSH. T200 and T400 ewes had greater concentrations only on d 18 compared with T0 ewes. Effects of treatment × day and treatment were detected (P = 0.01) for serum activity of SOD. T200 and T400 ewes had greater activities on d 14 and 18 than did T0 ewes. However, effects of treatment × day (P = 0.02) were detected for serum concentration of ROS. T400 ewes had lower concentrations only on d 14, compared to T0 and T200 ewes. Effects of treatment × day and treatment were detected (P = 0.01) for serum levels of LPO. T400 ewes had lower LPO levels on d 14 and 18 than did T0 and T200 ewes.

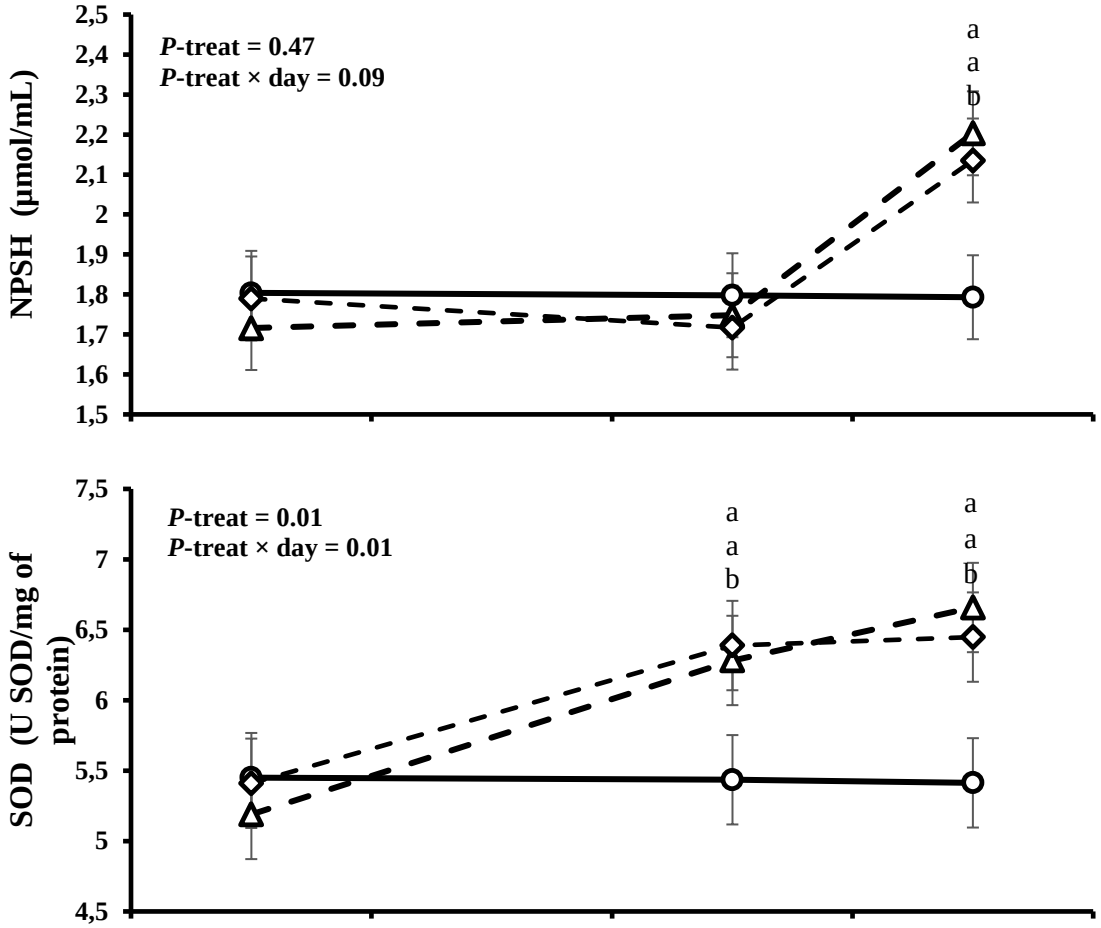
Figure 1. Superoxide dismutase (SOD) activity, and reactive oxygen species (ROS), total antioxidant capacity (ACAP) and lipoperoxidation (LPO) levels in milk of Lacaune ewes supplemented with pepper extract. T0, T200, and T400 represent 0, 200, and 400 mg of pepper extract/kg of concentrate, respectively.

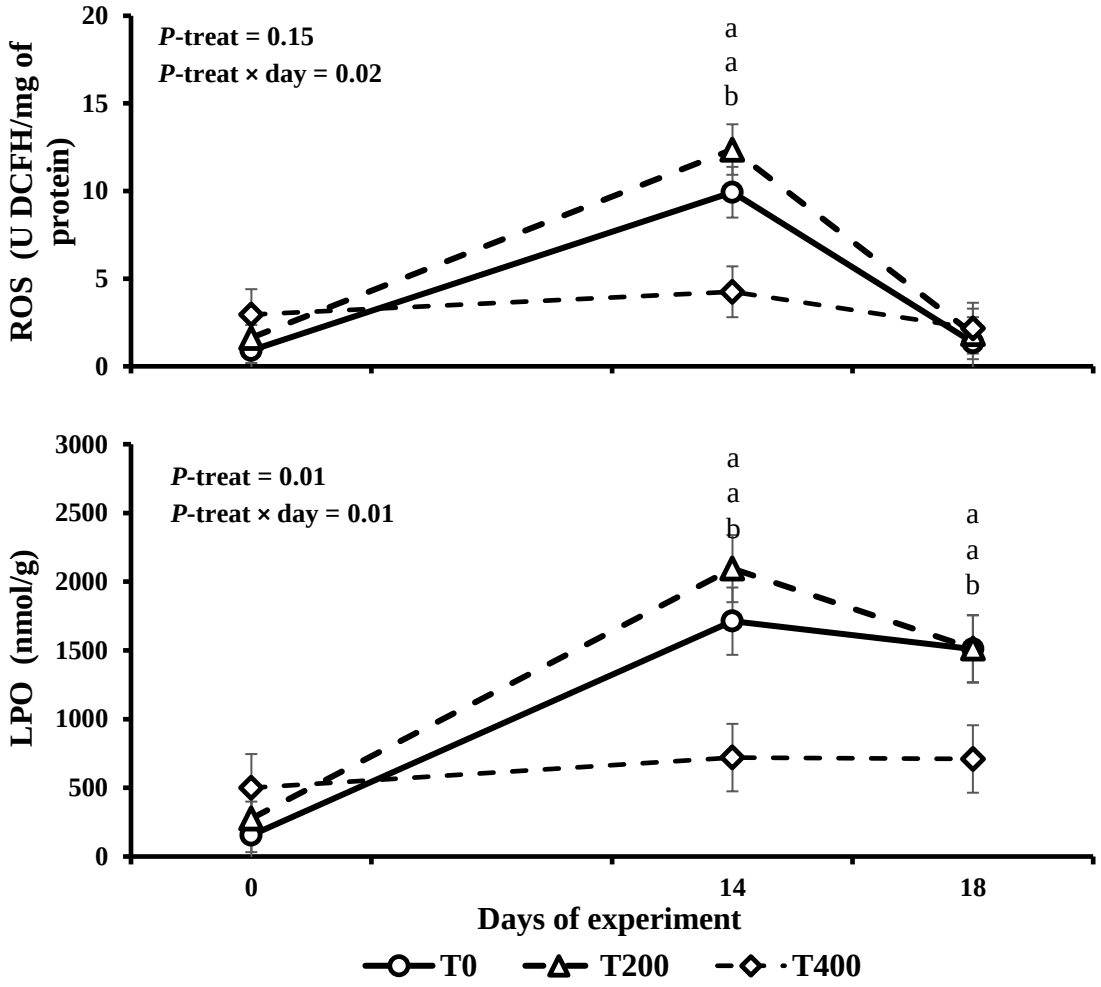




[a-c]Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM.

Figure 2. Levels of non-protein thiol (NPSH), superoxide dismutase (SOD) activity, reactive oxygen species (ROS) and lipoperoxidation (LPO) levels in serum of Lacaune ewes supplemented with pepper extract. T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate.





[a-b]Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM.

3.5 Behavior

The results of behavior variables are presented in Table 5. Effects of treatment were not detected ($P = 0.12$) for time spend eating silage and hay or remaining idle as well as the frequency of consuming hay. However, T200 and T400 ewes spent more time drinking water ($P = 0.01$) and had greater frequency ($P = 0.04$) of water drinking. T200 and T400 ewes ($P = 0.05$) to consume silage less frequently.

Table 5. Behavior of Lacaune ewes supplemented with pepper extract.

Variables[1]	Treatments[2]			SEM	P-value
	T0	T200	T400		Treat
Time (min)					
Silage	11.25	7.60	8.25	1.30	0.12
Hay	7.90	9.80	11.30	2.07	0.52
Water	0.80[b]	2.05[a]	1.69[a]	0.36	0.01
Idle	70.05	71.30	69.30	2.69	0.87
Frequency (n[o])					

Silage	2.35[a]	1.70[b]	1.60[b]	0.24	0.05
Hay	1.75	1.50	1.60	0.37	0.89
Water	0.70[b]	1.45[a]	1.28[a]	0.21	0.04

[1]Time (minutes when the animals remained in activity, observed for 90 min) and Frequency (times when the animals went to activity, observed for 90 min).
[2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate.
[a-b]Differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.

4. Discussion

The sheep that consumed pepper extract were in lactation phase when milk production started to decrease; however, for the sheep that consumed 400 mg PE/kg of concentrate, this reduction was smaller than that of the control group (T0). In addition, the inclusion of PE in the feed reduced production costs, because the feed conversion of these animals was lower. Furthermore, the milk quality was better when the sheep consumed pepper extract because the milk of these animals had higher concentrations of solids, resulting in lower lipoperoxidation levels and somatic cell counts. Raising solids levels is desirable for sheep milk producers because a large part of contemporary production is destined for production of co-products; and a higher concentration of solids increases production yield. We believe that the lower SCCs were a consequence of lower circulating leukocyte counts; however, this was not confirmed, because there were no differences among groups with respect to these variables. It was not clear to us how the intake of pepper extract reduced SCCs; further studies are needed to investigate the mechanisms involved.

The increase in protein concentration in milk produced by animals that received pepper extract containing capsaicin at the highest dose may be related to the greater availability of amino acids synthesized by the ruminal microbiota, which corroborates the results of a study conducted by An et al. (2020), who found that feed containing 50 mg of a blend containing oleoresin from *Capsicum* spp./kg diet for 15 days improved the digestibility of nutrients, as well as sheep performance. This was also reported in a study with beef cattle that were cannulated and supplemented with *Capsicum* oil, resulting in increased ruminal concentrations of amino acids, probably due to improvements in the microbial synthesis of proteins deposited in milk and meat (Cardoso et al., 2006).

The animals that received PE with capsaicin cause an increase lactose levels at the end of the experiment. Capsaicin decreased secretion and/or the response to insulin, causing higher blood glucose levels (Van de Wall et al., 2005, 2006). Oh et al. (2017) suggested that capsaicin may increase the availability of glucose to the mammary gland (through its action on insulin secretion), which may explain the greater levels of lactose synthesis. Another effect is the

mobilization of fat with reduction in adipose tissue and increased serum levels of free fatty acid. Capsaicin increased the number of defense cells such as neutrophils and lymphocytes (Franco-Penteado et al., 2006; Takano et al., 2007; Oh et al., 2015). For these reasons, we imagine that the increase in globulins was related to this same event, as a result of greater production of immunoglobulins. The administration of *Capsicum* oleoresin to cattle increased the number of eosinophil counts (Oh et al., 2015); instead, we observed an increase in basophil counts. Corroborating this effect on the immune response, An et al. (2020) found that 50 and 80 mg of a blend containing oleoresin from *Capsicum* spp. caused an increase in the production of IgG and IgM; the authors concluded that this caused a stimulating effect on the humoral immune response of sheep supplemented with a blend containing this additive.

The animals that received less capsaicin showed higher water consumption, both in terms of frequency and in terms of time drinking. In similar studies, higher water intake was also observed, followed by even higher feed consumption on the part of animals that received capsaicin (Cardozo et al., 2006; Rodríguez-Prado et al., 2012). Increased water consumption is believed to be related to the pungent properties of the pepper extract. For the production of milk, it is necessary to ingest large amounts of water that pass into the bloodstream and become available to the mammary gland. The addition of capsaicin to cattle feed increased milk production (Oh et al., 2015); we believe that the smallest reduction in milk production in the T400 sheep observed in our experiment may be related to this greater water intake by the animals that received pepper extract, because water consumption positively correlates with milk production.

The concentrate containing pepper extract consumed by the sheep had a greater amount of total phenolic compounds and greater antioxidant activity (Table 1). According to the literature, phenolic compounds such as capsaicin are known for their high antioxidant activity and their ability to protect against the formation of free radicals through the power to reduce their hydroxy groups (Materska & Perucka, 2005; Zhuang et al., 2012). Therefore, as expected, the antioxidant defense responded to the feed, as serum levels of non-protein uncles and SOD activity were higher in sheep in the groups that received PE; this was probably reflected in higher levels of total antioxidants in milk.

Endogenous antioxidant enzymes such as SOD and CAT are the primary mediators of intracellular defenses against oxidative stress by neutralization of free radicals (Mates et al., 1999). For this reason, the increase in SOD activity we observed is also an indication of better ability to remove superoxide anion, an important free radical (Vinã et al., 2018), possibly explaining the decrease in levels of serum ROS, produced mainly in cellular respiration. In our

study, ROS levels were lower in the serum and milk of sheep that consumed PE; this suggests that levels of lipid peroxidation in milk were also lower. An et al. (2020) reported that 50 and 80 mg of a blend containing oleoresin from *Capsicum* spp./kg feed increased non-enzymatic antioxidant activity, as well as stimulating the activity of enzymes such as SOD, CAT, and glutathione peroxidase that have direct effects on oxidants and consequently on the reduction of lipoperoxidation, similar to what was observed in the present study. These results of the antioxidant status of milk when analyzed together with the lower CCS and the higher percentage of solids allow us to conclude that the inclusion of pepper extract improved milk quality.

5. Conclusion

Pepper extract (main 400 mg PE/kg of concentrate) containing capsaicin in the feed of sheep during the mid-lactation period (after the peak of lactation) minimized the reduction in milk production during the experiment and improved feed conversion. The milk produced by these animals that consumed PE had higher levels of protein and lactose, that is, a greater number of total solids, which is desirable for the industrialization and production of derivatives such as yogurt, ice cream, and cheese. There were lower somatic cell counts and levels of lipid peroxidation in milk secondary to the increase in total antioxidants. This suggests that pepper extract may be a potential feed additive that can improve milk quality. Higher levels of globulins indicate that consumption of concentrate with pepper extract stimulated a humoral immune response, combined with increased antioxidant defenses and decreased levels of oxidation in the blood.

We suggest that further studies be carried out with greater challenges so that the already positive results are exacerbated. Studies at different times, in longer periods, where unfavorable environmental conditions and / or low production animals exist. In addition to differentiated assessments, to elucidate the exact mechanisms of action that caused the results obtained, for example: the ruminal microbiota.

Ethics committee

This experiment was carried out in accordance with animal welfare practices and approved by the Ethics Committee for the Use of Animals in Research (CEUA/UDESC), protocol number 1027070519.

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3. CONSIDERAÇÕES FINAIS

A adição de uma junção de fitogênicos microencapsulados à base de timol, carvacrol e cinamaldeído na alimentação de ovelhas lactantes, verificada no experimento I foi benéfica, pois houve maior produção de leite dos animais que receberam a dieta com a mistura microencapsulada, bem como maior eficiência produtiva e eficiência alimentar, concomitantemente com menor conversão alimentar. Estes resultados apontam para o seu envolvimento com as bactérias ruminais, mudando o perfil de aproveitamento do alimento fornecido. Tendências mais baixas de SCC, bem como níveis mais baixos de ROS no leite foram verificadas. No sangue observou-se menor número de neutrófilos e ROS, concomitante aos maiores níveis de globulinas em ovinos de T150 e T250 em relação ao T0. Este efeito era esperado graças ao potencial antioxidante que a mistura propunha. Em resumo, nas ovelhas que consumiram a mistura microencapsulada, encontramos um efeito antiinflamatório associado à redução dos radicais livres e ao aumento das globulinas, todos desejáveis para a produção animal.

No experimento II, houve menor redução na produção de leite nas ovelhas que receberam o extrato de pimenta, o que era esperado, graças ao melhor aproveitamento dos nutrientes disponíveis na dieta. A conversão alimentar foi menor nas ovelhas dos grupos que receberam o produto. A interação entre o tratamento e o dia foi observada para proteína, lactose e sólidos totais no leite; ou seja, foi maior nas ovelhas que consumiram o extrato de pimenta no final do experimento. As contagens de células somáticas no leite foram mais baixas nas ovelhas que receberam a maior dose, além do aumento na contagem total de basófilos, os níveis de proteína e os níveis de albumina, mostrando seu potencial anti-inflamatório. Menores níveis de espécies reativas de oxigênio e lipoperoxidação no soro e leite dos animais dos grupos que consumiram o extrato de pimenta, bem como maiores níveis de tióis não protéicos e atividades da superóxido dismutase nos animais que receberam o produto, mostrando o benefício de seu potencial antioxidante. As ovelhas que receberam a capsaicina passaram mais tempo bebendo e tiveram maior frequência de beber água. Esses resultados sugerem que a inclusão de PE (400 mg / kg) contendo capsaicina no concentrado de ovinos minimizou a queda na produção de leite, após a passagem pelo pico de lactação, durante o experimento e melhorou a qualidade do leite, bem como estimulando uma resposta antioxidante sistêmica.

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ANEXOS

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Fitogênico encapsulado na alimentação de ovelhas lactantes: impactos sobre a produção, composição e qualidade de leite, perfil imunológico e digestibilidade ", protocolada sob o CEUA nº 7308030419 (ID 000878), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 12/04/2019.

We certify that the proposal "Phylogeny encapsulated in lactating sheep feeding: impacts on milk production, composition and quality, immunological profile and digestibility", utilizing 30 Ovines (30 females), protocol number CEUA 7308030419 (ID 000878), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 04/12/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2019 a 12/2019 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Fêmeas	idade:	2 a 4 anos	N:	30
Linhagem:	Iacaune			Peso:	60 a 70 kg		

Local do experimento: O experimento ocorrerá em uma propriedade particular parceira, de produção exclusiva de ovinos. Esta fica situada na cidade de Chapecó, Santa Catarina, Brasil.

Lages, 11 de abril de 2020

Ubirajara Maciel da Costa
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

em aberto
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Av. Luis de Camões, 2090 - Centro Agroveterinário - Bairro Conta Dinheiro Lages/SC CEP: 88520-000 - tel: 55 (49) 32899129
Horário de atendimento: 2ª a 6ª das 8h às 18h : e-mail: ceua@cav.udesc.br
CEUA N 7308030419

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Extrato de pimenta na alimentação de ovelhas lactantes: impactos sobre a produção, composição e qualidade de leite, perfil antioxidante, anti-inflamatório e comportamento animal ", protocolada sob o CEUA nº 1027070519 (ID 000954), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 18/07/2019.

We certify that the proposal "Pepper extract in lactating sheep feeding: impacts on milk production, composition and quality, antioxidant profile, anti-inflammatory and animal behavior", utilizing 30 Ovines (30 females), protocol number CEUA 1027070519 (ID 000954), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 07/18/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 06/2019 a 12/2019 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Ovinos	sexo:	Fêmeas	idade:	2 a 4 anos	N:	30
Linhagem:	lacaune			Peso:	60 a 70 kg		

Local do experimento: Cabanha chapecó - conveniada com udesc oeste

Lages, 11 de abril de 2020

Ubirajara Maciel da Costa
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

em aberto
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina