UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

COMPOSTOS BIOATIVOS NO TRATAMENTO DE LESÕES INFLAMATÓRIAS NA PRODUÇÃO ANIMAL

Autora: Ana Carolina Viscardi Plefh
Orientador: Profª. Drª. Paula Toshimi Matumoto-Pintro

MARINGÁ
Estado do Paraná
2021

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Dissertação apresentada, como parte das exigências para obtenção do título de MESTRE EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá – Área de Tecnologia de Produtos Agropecuários.

MARINGÁ
Estado do Paraná
2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

Plefh, Ana Carolina Viscardi

PP724c

Compostos bioativos no tratamento de lesões inflamatórias na produção animal / Ana Carolina Viscardi Plefh. -- Maringá, PR, 2021.
0 f.: il. color., figs., tabs.

Orientadora: Profa. Dra. Paula Toshimi Matumoto-Pintro.
Dissertação (Mestrado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, 2021.

1. Antioxidante - Cravo-da-índia. 2. Lactoferrina bovina. 3. Mastite. 4. Produção animal. I. Matumoto-Pintro, Paula Toshimi, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.2

Jane Lessa Monção - CRB 9/1173

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TITULAÇÃO: Mestre em Zootecnia - Área de Tecnologia de Produtos Agropecuários.

APROVADA em 22 de fevereiro de 2021.

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(Orientadora)

“Bendirei o Senhor, que me aconselha;
na escura noite o meu coração me ensina!
Sempre tenho o Senhor diante de mim.
Com Ele à minha direita, não serei abalado.
Por isso o meu coração se alegra
e no íntimo exulto;
mesmo o meu corpo repousará tranquilo,
porque Tu não me abandonarás,
nem permitirás que eu desça.
Tu me farás conhecer a vereda da vida,
a alegria plena da tua presença,
eterno prazer à Tua direita.”

Trecho de Salmos 16

A Deus,

Aos meus pais José e Eliana,

À minha irmã Natalia,

Aos meus avós maternos Helena e Pedro e minha avó paterna, Santília,

Aos meus animais de companhia Jack, Berenice e Francisco.

Dedico.

AGRADECIMENTOS

A Deus.

À Universidade Estadual de Maringá, por ter possibilitado desenvolver este trabalho.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela bolsa de estudos.

À Profª Drª Paula Toshimi Matumoto-Pintro, pela dedicada orientação, ensinamentos, amizade e confiança na minha pessoa e no meu trabalho.

Ao Departamento de Zootecnia, UEM, em especial ao Prof. Dr. Leandro Dalcin Castilha, Profª Drª Claudete Regina Alcalde e Profª Drª Tatiana Carlesso dos Santos, que muito contribuíram para a realização deste trabalho.

Ao Departamento de Física, UEM, em especial à Profª Drª Franciele Sato e Drª Lidiane Vizioli, que muito contribuíram para a realização deste trabalho.

À Fazenda Experimental de Iguatemi, pelo fornecimento de seus animais e de suas instalações para a execução deste trabalho.

Aos professores do Programa de Pós-Graduação em Zootecnia, da UEM, pelos valiosos ensinamentos.

Aos Grupo de Estudos em Alimentos Funcionais e aos colegas do laboratório, pelo auxílio no desenvolvimento do trabalho.

A todos que, direta ou indiretamente, contribuíram para a realização deste trabalho.

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RESUMO

O aumento da produção animal voltado à produção de bens de consumo acarreta problemas relacionados com a sustentabilidade, bem-estar animal e queda de produção provenientes de sistemas mal manejados. O uso de compostos bioativos no tratamento de doenças inflamatórias e reincidentes: mastite em cabras leiteiras e úlceras de decúbito em coelhos, foram objetos de estudo deste trabalho. O cravo-da-índia no tratamento tópico das úlceras calcaneres em coelhos, demonstrou resultados satisfatórios quanto aos processos de regeneração celular e ação antioxidante sobre as lesões inflamatórias. O tratamento preventivo da mastite em cabras leiteiras, com base no uso de lactoferrina bovina intramamária, foi significativo quanto à diminuição de infecções mamárias e melhora na qualidade do leite. A administração de cravo-da-índia e lactoferrina bovina como compostos ativos dos tratamentos tópicos foram eficientes e demonstraram boa relação custo/benefício em substituição aos tratamentos antimicrobianos e anti-inflamatórios convencionais.

Palavras-chave: antioxidantes, cravo-da-índia, inflamação, lactoferrina bovina, mastite.

ABSTRACT

The increase in animal production for consumer goods production brings problems of sustainability, animal welfare and low production from poorly maintained systems. The use of bioactive compounds in the treatment of inflammatory and re-incident diseases: mastitis in dairy goats and decubitus ulcers in rabbits, were objects of study of this research. The use of clove in the topical treatment of leg ulcers in rabbits demonstrated satisfactory results regarding the processes of cell regeneration and antioxidant action on inflammatory lesions. Preventive treatment of mastitis in dairy goats based on the use of intramammary bovine lactoferrin was significant in terms of reducing breast infections and improving milk quality. The administration of clove and bovine lactoferrin as active compounds of topical treatments were efficient and demonstrated good cost-benefit ratio to replace conventional treatments.

Keywords: antioxidants, bovine lactoferrin, clove, inflammation, mastitis.

I. INTRODUÇÃO

O sistema de produção animal brasileiro se destaca no cenário mundial, com o aumento da capacidade produtiva, eficiência e sustentabilidade, devido ao foco em: alimentação, genética, sanidade e processos reprodutivos (EMBRAPA, 2020). Voltada à produção de alimentos e bens de consumo de origem animal, a produção animal engloba grande parte do sistema agropecuário, desde industrias a atualizações técnicas que buscam expressar o potencial de produção, mantendo o dinamismo entre as bases fisiológicas e o equilíbrio com o ambiente (EMBRAPA, 2018). O aumento do consumo e procura por qualidade, propiciou a diversificação produtiva, aumentando a criação de animais e produtos que antes não eram tão consumidos e procurados (KAC, 2015), como é o caso da produção de coelhos para corte e produção de leite de cabra.

A produção e o consumo de carne de coelho ainda são pequenos quando comparados com outras culturas, mas já fazem parte da realidade comercial dos brasileiros (KAC, 2015). As criações comerciais de coelhos fazem pouco uso de tecnologias e, apresentam problemas: ciclo reprodutivo exaustivo para as matrizes, podendo causar danos nos membros, lesões de decúbito, esterilidade de machos quando em desconforto térmico, entre outros agravantes produtivos (HECKER, 2011). As técnicas que possibilitam a criação racional e sustentável de animais para que possam alcançar altas taxas de produtividade (MACHADO & FERREIRA, 2011) vem sendo aprimoradas para manter a qualidade de vida, o bem-estar animal aliado ao aumento da produção.

A caprinocultura leiteira é uma alternativa eficaz dentro da agricultura familiar, principalmente nas regiões Nordeste e Sudeste do país (FELISBERTO, OLIVEIRA & CORDEIRO, 2016). A facilidade de manejo aliados a pequena área produtiva, pequeno volume de alimentos e procura por produtos derivados diferenciados e com alto teor de

proteínas, propiciou à produção maior valor agregado (EMBRAPA, 2016). Há fatores que limitam o aumento produtivo de leite de cabra, como: potencial genético do rebanho, sazonalidade da produção, qualidade de alimento, controle reprodutivo, presença de doenças e falta de sanidade e bem-estar animal (GONÇALVES et al., 2008). A alta e frequente prevalência de mastite em caprinos assume importância cada vez maior e é influenciada por uma variedade de fatores relacionados ao animal, ao patógeno e ao ambiente (PEIXOTO, et al., 2010). Uma vez que a mastite engloba parte da sanidade animal que reflete diretamente na produtividade, há preocupação com os sistemas de manejo e controle de doenças empregados.

O controle e tratamento de injúrias na produção animal é objeto de estudo na indústria farmacêutica e fitoterápica. O uso de produtos naturais ricos em compostos bioativos tem promovido interesse médico (NASSAR et al., 2007) e, nas últimas décadas, várias proteínas de origem animal se tornaram interessantes, como é o caso da lactoferrina bovina, apresentando eficiência no tratamento de lesões inflamatórias e cancerígenas, obtendo resultados satisfatórios e, aproximadamente sessenta por cento dos fármacos anticâncer provêm de fontes animais (ORANGI et al., 2016). A medicina animal emprega o uso de medicamentos de várias formas e concentrações (ANSEL, 2006), e, na procura de melhor relação custo/benefício, a fitoterapia e o uso de compostos derivados de plantas têm apresentado bons resultados na saúde (OZAKI et al. 2006). O cravo-da-índia apresenta alto teor de eugenol e outros compostos bioativos que possuem propriedades antissépticas, bactericidas, fungicidas e anti-inflamatórias (DUARTE, 2014) podendo ser aliado a outros tratamentos, profilaxia e cura de lesões.

As pesquisas em profilaxia e tratamentos alternativos de doenças, a fim de substituir o uso de fármacos químicos são relevantes para proporcionar bem-estar animal no meio produtivo e possuem boa relação custo/benefício. O presente trabalho visa o

estudo de compostos bioativos, lactoferrina bovina e *Syzygium aromaticum* (cravo-da-índia), no tratamento preventivo e curativo de injúrias inflamatórias na produção de coelhos e cabras de leite.

1. REVISÃO BIBLIOGRÁFICA

1.1. Compostos bioativos

Compostos bioativos são substâncias presentes em alimentos, especiarias e plantas, que influenciam as atividades fisiológicas ou celulares, causando efeito benéfico à saúde (KRIS-ETHERTON et al., 2004). Presentes em pequenas quantidades nos alimentos (KRIS-ETHERTON et al., 2002) têm efeitos mais sutis que os nutrientes: modificam o risco de doenças, mas não suprem as deficiências nutricionais. Um número crescente de compostos bioativos tem sido identificado como potencialmente importantes: antioxidantes, inibidores e indutores de enzimas, inibidores de receptores e expressão gênica (KRIS-ETHERTON et al., 2004). Os antioxidantes são compostos bioativos de natureza química que retardam ou impedem a formação de radicais livres (HALLIWELL & TRIDGE, 1999) que é um evento associado ao metabolismo aeróbio (KRIS-ETHERTON et al., 2004). Os radicais livres possuem meia-vida curta, são altamente reativos e reagem com moléculas que se localizam em torno do seu sítio de formação (SOARES et al., 2015). Os antioxidantes interceptam esses radicais oxidantes e fazem reparação de lesões inflamatórias induzidas pela oxidação (TRIBBLE, 1999), possuem habilidade em doar elétrons e são moduladores de vias de sinalização antioxidante e anti-inflamatória (KRIS-ETHERTON et al., 2004; SOARES et al., 2015). Os compostos bioativos têm a capacidade de modular a via Nrf2/Keap1 – fator de transcrição nuclear, agindo indiretamente no estresse oxidativo e na expressão do fator de

transcrição NFκB, atuante na resposta inflamatória (SOARES et al., 2015).

Nos últimos anos, houve aumento significativo nos estudos de compostos naturais na medicina e na indústria alimentícia. A indústria passou a buscar alternativas aos conservantes químicos tradicionalmente empregados como antimicrobianos, substituindo-os por produtos naturais de plantas e óleos vegetais (RADÜNZ et al., 2019). A medicina faz o uso de medicamentos de várias formas e concentrações (ANSEL, 2006), usando a fitoterapia, que faz o uso de compostos derivados de plantas e moléculas orgânicas, apresentando bons resultados na saúde (OZAKI et al. 2006; ORANGI et al., 2016). O cravo-da-índia é uma especiaria rica em compostos bioativos com capacidades antioxidantes, regenerativas e antimicrobianas (CHAIEB, et al., 2007) e a lactoferrina bovina, que também é objeto de estudo neste trabalho, apresenta eficiência no tratamento de lesões inflamatórias e cancerígenas (ORANGI et al., 2016).

1.1.1. Cravo-da-índia (*Syzygium aromaticum)*

O cravo é uma planta pertencente à família Myrtaceae e ao gênero *Syzygium*, conhecido pelo nome científico *Syzygium aromaticum* (MERRILL & PERRY, 1939). Os principais produtos do cravo são as flores, amplamente utilizadas como matéria-prima na indústria de cigarros e especiarias, além do óleo e a oleorresina, destilados de flores ou folhas, que podem ser usados como medicamentos (BERMAWIE, 2008).

Os compostos bioativos do cravo-da-índia (*Syzygium aromaticum*), extraídos do botão floral seco, possuem atividades antimicrobianas e antioxidantes pela presença de eugenol e outros compostos fenólicos. Atua como bactericida contra: *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes* e *Salmonella typhimurium*, tendo atividade antioxidante e quelante de metais (CHAIEB et al., 2007). Por possuírem o eugenol como composto bioativo (HOSSAIN et al, 2012) e atividade antioxidante (SHAN

et al, 2005), os óleos e derivados do cravo vêm sendo usados como antibacteriano, antifúngico, antisséptico e antiviral (BHOWMIK et al, 2012). Entretanto, a característica de odor intenso, volatilidade e instabilidade em condições ambientais (temperatura, luz e oxigênio), torna seu uso na indústria limitado (BERMAWIE, 2008).

1.1.2. Lactoferrina bovina

A Lactoferrina bovina é uma glicoproteína encontrada no soro do leite e faz parte de um grupo de proteínas chamadas transferrinas (SGARBIERI, 2004; STEIJNS e VAN HOOIJDONK, 2000). Presente em secreções como a saliva, lágrima, sêmen e secreção vaginal, é predominantemente encontrada em produtos de glândulas exócrinas localizadas na entrada dos aparelhos digestivo, respiratório e reprodutivo. Pode ser encontrada no sangue e plasma, derivada de neutrófilos - em resposta a um estímulo inflamatório (STEIJNS e VAN HOOIJDONK, 2000).

As propriedades funcionais como: modulação metabólica e inibição ou retardamento de processos patológicos ou do envelhecimento precoce em animais de experimentação e, na espécie humana (SGARBIERI, 2004), são característica da sequência de aminoácidos cuja cadeias polipeptídicas simples têm habilidade de se ligarem a íons Fe^{3+} e sinergicamente a íons carbonato ou bicarbonato (STEIJNS e HOOIJDONK, 2000). As estruturas peptídicas catiônicas antipáticas em alfa-hélice são relacionadas com a atividade antimicrobiana, e se dá pela formação dos canais iônicos através da membrana, alterando a permeabilidade dos microrganismos (MOITA, 2011). A ação antimicrobiana da lactoferrina parte da instabilidade da membrana citoplasmática da bactéria e inibição da proliferação e do crescimento de gram-positivas e gram-negativas (SGARBIERI, 2004).

Atualmente, a lactoferrina é utilizada em alimentos como fórmulas infantis, comprimidos de suplementação alimentar, iogurtes, leite desnatado, bebidas, em alimentos para animais de aquicultura, também para cães e gatos (BAKER, 2009). O efeito esperado nesses produtos é que haja diminuição de infecções, melhoramento da microflora intestinal, imunomodulação, alívio em inflamações e atividade antioxidantes (TOMITA et al., 2002; WAKABAYASHI, 2006).

1.1.3. Gel Carbopol 940®

Os géis hidrofílicos são polímeros amplamente utilizados como base para produtos dermatológicos veiculando princípios ativos ou medicamentos (CORRÊA, 2005). Possuem uma conformação viscosa à preparação semissólida, sendo composto por partículas que ficam dispersas (coloidais) (MAIA-CAMPOS, 1999). Os polímeros caracterizados como ácidos carboxivinílicos (Carbopol 940®) são de caráter aniônico e pH dependentes, estáveis em pH neutro ou próximo da neutralidade (MAIA-CAMPOS, 1999). Apresentam viscosidade constante – tixotropia – dificultando a separação dos constituintes da fórmula e propiciam maior vida de prateleira (“shelf-life”) (MARTIN, 1993). Formulações que usam como base o Carbopol 940®, são vantajosas devido a propriedade tixotrópica que permite que o gel se deforme durante a aplicação, facilitando o espalhamento do princípio ativo pela fluidez proporcionada pelo gel e, recuperando a viscosidade ao fim da aplicação (MAIA-CAMPOS, 2003; CORRÊA, 2005).

Os géis carboxivinílicos são materiais sem toxicidade e sem evidências de hipersensibilidade quando administrados topicamente, sendo usados em seres humanos (CARNALI & NASSER, 1992). Diferentes Carbopois® apresentam diferentes propriedades reológicas dependendo do seu tamanho de partícula, peso molecular entre ligações cruzadas, distribuições de ligações e fração das unidades totais que aparecem

como terminais, isto é, extremidades de cadeia livre. O peso molecular para o gel Carbopol 940® foi relatado como 1450 unidades monoméricas (ou $1450 \times 72 = 104.400$ g / mol) (CHAWLA & SARAF, 2012). Com peso molecular alto, não penetram na pele durante a aplicação e são boas alternativas como veículo de princípios ativos lipo e hidrossolúveis.

1.2. Produção e manejo de coelhos no Brasil

A criação intensiva de coelhos no Brasil teve início em meados da década de 1970, quando foi criada a Associação Nacional dos Criadores de Coelhos. Anteriormente a produção era pequena e voltada ao setor farmacêutico, produzindo animais para testes de vacinas (KLINGER & TOLEDO, 2017). O avanço das pesquisas e as vacinas sendo testadas e produzidas a partir de ovos de galinha (KLINGER & TOLEDO, 2017), a cunicultura se voltou para a produção de carne e, desde então vem crescendo como forma de renda para propriedades maiores e subsistência, na agricultura familiar.

O custo de produção nacional é mais elevado que nos países europeus, pela falta de técnica como a inseminação artificial, ração de preço elevado e estudos genéticos ainda emergentes. O incentivo à produção vem crescendo desde então e, apresentando crescimento sólido e altos reajustes em coelhos comercializados vivos (KAC, 2015). A atividade atrai a atenção dos produtores pelas vantagens na forma de criação: utilização de espaços pequenos, animais de fácil manejo, utilização de fibra bruta como fonte de energia, carne de alta qualidade e produção durante todo o ano pela alta fertilidade dos animais (KLINGER & TOLEDO, 2017; FERREIRA et al, 2012).

A cunicultura é relativamente recente quando comparada com outros animais (FERREIRA et al, 2012), mas há estímulo em aumentar a produção de forma eficaz. Estudos estão sendo desenvolvidos com o intuito de corrigir os problemas nessa cadeia

produtiva, incrementar novas técnicas e promover aumento da produção e sustentabilidade.

1.2.1. Afecções podais em coelhos

Os coelhos são animais sensíveis e susceptíveis a alterações de manejo e de temperatura climática (FERREIRA, 2003). Fatores no manejo dos animais contribuem para a formação de lesões, feridas e disfunções. As lesões de pele, possuem etiologia variada, e parte dessas feridas se deve à posição anatômica, ao peso corporal, tipo de piso das gaiolas de manejo, umidade ambiental, limpeza e principalmente predisposição fisiológica (KOSIAK, 1959; NIITSUMA et al., 2003). Quando a pele sadia está sujeita ao contato direto ou fricção – apoio dos membros nas gaiolas/ descanso de patas – uma lesão intradérmica abaixo do extrato granuloso, começa a se formar, dando início a dermatite de decúbito (WITKOWSKI & PARISH, 1982). As úlceras de decúbito possuem espectros de desenvolvimento: eritemas, dermatite, úlceras propriamente ditas/escaras e, gangrena (WITKOWSKI & PARISH, 1982). Frequentemente, infecções bacterianas e fúngicas estão entre as complicações mais comuns e com maior gravidade (NIITSUMA et al., 2003).

As inflamações cutâneas causadas pelas lesões de descanso causam danos à qualidade de vida de animais confinados para fins de produção, podendo causar disfunção na coluna vertebral e lesões nos membros (NIITSUMA et al., 2003), levando a dificuldades reprodutivas, diminuição da performance e queda na produção.

1.3. Manejo e produção de caprinos no Brasil

A caprinocultura se estende por todos os continentes do planeta, porém, a prevalência de caprinos está nos países em desenvolvimento. No Brasil, essa atividade está presente desde sua colonização, quando se deu início a construção do rebanho

nacional: animais sem raça definida, que produziam leite para sua prole (FONSECA et al., 2012). Nos últimos anos, a criação de cabras para a produção de leite se tornou uma alternativa viável para a geração de emprego e renda - via Programa de Fortalecimento da Agropecuária Familiar (PRONAF), favorecendo a produção e mantendo o homem no campo (SIMPLÍCIO et al., 2004; DAL MONTE et al., 2010).

A concentração de caprinos no Brasil é estimada em 9,3 milhões de cabeças, e 92,7% destas estão alocadas na região Nordeste (FEITOSA et al., 2020). A região é responsável por 70% da produção nacional de leite de cabra (ANUALPEC, 2017), há fácil adaptação desses animais às condições climáticas do Nordeste (IBGE, 2014), além da facilidade de manejo.

A atividade de produção de leite de cabra foi impulsionada quando se iniciou a comercialização do leite produzido por agricultores familiares para o Programa de Aquisição de Alimentos (PAA) (BATISTA et al., 2012). Apesar do aumento considerável, nem sempre a produtividade corresponde às expectativas de produção, tornando-se imprescindível encontrar formas que otimizem o sistema com o objetivo de alcançar resultados melhores para o produtor (FEITOSA et al., 2020) e garantir o bem-estar animal.

1.3.1. Manejo e mastites em cabras de leite

A mastite é o problema de maior ocorrência na produção leiteira no Brasil e está entre os principais responsáveis pela queda de produção e descarte dos animais. A prevalência é influenciada por diversos fatores, sendo de etiologia ampla, destacando-se a maior susceptibilidade do animal durante o período de lactação, enquanto no período seco, observa-se maior frequência da mastite ambiental (PRESTES et al. 2002). No momento da colostrogênese - 14-21 dias antes do parto - ocorre o crescimento de novas

células mamárias, início da produção de colostro e, como as células se encontram em alta atividade de síntese, aumentando o risco de novas infecções pelo aumento da pressão intramamária, disfunção imune e alterações metabólicas associadas (SANTOS & FONSECA, 2019), que aliadas à contaminação ambiental dão início às infecções: mastite clínica (sintomática) ou subclínica.

A mastite em cabras não tem caráter sazonal, podendo ocorrer durante todo o ano. Entretanto, há maior prevalência em períodos úmidos ou em propriedades com maior produção leiteira (PINHEIRO et al. 2000, ALBIZU & BASELGA 2002). A mastite do tipo subclínica é predominante nos rebanhos de pequenos ruminantes. Em contrapartida, a mastite clínica – sintomática -, apresenta-se em níveis abaixo de 5%, podendo alcançar maiores taxas em determinadas situações de manejo (CONTRERAS et al. 2007).

Uma das características dessa afecção, condiz à diversidade de microrganismos patogênicos, com relação à espécie caprina, pesquisas demonstram maior ocorrência de *Staphylococcus caprae* (BERGONIER et al. 2003). A frequência de mastite causada por *Escherichia coli* é relevante, mesmo sendo esporádica, os sinais clínicos podem ser localizados ou resultarem em sintomas clínicos severos com episódios fatais (SANTOS, 2006).

Novos métodos de controle e erradicação da doença dentro dos rebanhos visam os cuidados com o animal durante o período seco. O período de desmame proporciona uma série de mudanças no tecido mamário, dando início ao período de involução mamária, ao passo que pesquisas demonstram a importância do tratamento preventivo no período seco e pré-parto (SANTOS, 2019). Visando essa finalidade, a "terapia da vaca seca", método desenvolvido em 1950, podendo ser aplicado em pequenos ruminantes e tem como objetivo principal o controle de infecções recorrentes durante o período seco, por meio da administração de antimicrobianos de longa duração (CHAFFER et al. 2003; SANTOS,

2019). O manejo adequado do rebanho é a chave para prevenir e controlar a incidência de mastite, especialmente antes do parto e nos dias que se seguem, buscando menores índices da doença, sem a necessidade de utilização dos medicamentos antimicrobianos (SHWIMMER et al. 2008).

Os próximos anos indicam projeções de crescimento populacional, aumento do consumo de alimentos per capita, expansão dos grandes centros urbanos e restrições no uso da terra (SAATH, 2018). Esses fatores aliados à disponibilidade de renda, devem aumentar o consumo de produtos de origem animal, trazendo preocupações quanto à quantidade de alimentos, sistemas de produção, sustentabilidade e bem-estar animal. Esse conjunto de informações levam às pesquisas atuais com o objetivo de alavancar a produção e, se preocupando com o meio ambiente, com a melhora na qualidade de vida animal e o equilíbrio entre o homem e a natureza.

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II. OBJETIVOS GERAIS

Determinação *in vivo* da ação antimicrobiana e inflamatória do cravo-da-índia e da lactoferrina, como alternativa no tratamento e prevenção de injúrias inflamatórias decorrentes do manejo animal.

III. OBJETIVOS ESPECÍFICOS

Tratar lesões podais em coelhos com o uso tópico de gel à base de cravo-da-índia; caracterizar a atividade antioxidante do cravo; verificar atividade antimicrobiana do cravo sobre *Escherichia coli* e *Pseudomonas spp.*; caracterizar as alterações estruturais, físico-químicas, moleculares e histológicas do processo de cicatrização das lesões.

Prevenir mastite em cabras no período seco com administração de gel intramamário veiculador da lactoferrina bovina; caracterizar a atividade antimicrobiana da lactoferrina bovina; analisar aumento dos sólidos totais do leite; diminuir a incidência de infecções no canal do teto.

1 **Original Article**
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3 (Modelo Revista: Research in Veterinary Science – Elsevier)
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7 **Cloves (*Syzygium aromaticum*) fluid gel on healing of pododermatitis in rabbits**
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Abstract

Wounds are damaging to the quality of life of confined animals, causing dysfunction in spinal, members injuries, and reduction in productive performance. This research investigated the clove antimicrobial and antioxidant activity on the healing of decubitus wounds (pododermatitis) of rabbits (*Oryctolagus cuniculus).* Adult animals were treated for 21 days every three days with a fluid gel spray in the wound region: control fluid gel without the addition of clove (FGC0), fluid gel with the addition of 1% clove powder (FGC1), and fluid gel with 2% clove powder (FGC2). Microbiological analyses for *Escherichia coli* and *Pseudomonas spp.* were performed during the 21 days of the experimental period. After this period, samples from treated skin were evaluated for histological analysis and evaluation of the healing process by spectroscopy (FTIR-ATR). Rabbits treated with FGC2 showed advanced healing and decreased tissue inflammation similar to healthy rabbits, while FGC0 rabbits showed a decrease in bacterial contamination without signs of healing. Both FGC1 and FGC2 rabbits demonstrated antimicrobial and antioxidant action against both bacteria tested, favoring the wound healing process. Considering the results, we indicate the use of fluid gel with 2% of clove powder (*Syzigium aromaticus*) based on the best antimicrobial, antioxidant e anti-inflammatory activities on the healing of decubitus wounds (pododermatitis) of rabbits in the commercial farming system.

Keywords: epidermis, infections, inflammation, injuries, skin, wounds.

41 **Introduction**
42
43 Decubitus ulcers in feet (pododermatitis) are damaging to the quality of life of
44 breeders and confined animals by production purposes causing spinal dysfunction and
45 members wounds (Niitsuma et al., 2003). Several factors of handling contribute to the
46 formation of wounds on the skin, mainly in the feet (heel region). A breach of the skin
47 barrier is the major contributing factor to the development of skin ulcerations
48 (Vercauteren et al., 2019) It presents a varied etiology since most of these ulcers are due
49 to body weight, type of floor, management cages, environmental humidity, cleaning and
50 physiological predisposition (Kosiak, 1959; Lindan, 1961; Niitsuma et al., 2003).
51 Bacterial infections are among the serious problems that threaten animal health
52 (Cui et al., 2013). Different species and biotypes of *Pseudomonas spp.* are characterized
53 by resistance to routine cleaning of surfaces and tools, and the ability to form biofilms
54 (Giaouris et al., 2014), these bacteria survive and grow with the *Escherichia coli* present
55 in the environment are a serious cause of infections in preexisting wounds (Jang et al.,
56 2017). To avoid prolonged use of chemical drugs and antibiotics, the industry and
57 researchers are looking for viable alternatives for the treatment of this type of wound.
58 Hydrophilic gels as a dermatological base have been widely used, due to easy
59 propagation and non-greasy compounds, and may contain water-soluble active principles.
60 Anticancer drugs for melanoma skin therapy have been administered using hydrophilic
61 gels as a vehicle (Xu et al., 2020). Among the raw materials used in the preparation of the
62 gels, the carboxyvinyl acids (Carbopois®) stand out (Corrêa et al., 2005).
63 The use of natural products rich in bioactive substances has promoted the growing
64 interest of pharmaceutical industries (Nassar et al., 2007). Species of cloves (*Syzygium*
65 *aromaticum*) have been reported to possess antibacterial, antiviral (Shafi et al., 2002) and
66 anti-inflammatory activity (Muruganandan et al., 2001). The antimicrobial activity of

67 cloves essential oil has been studied against multi-resistant *Staphylococcus epidermidis*
68 (Chaieb et al., 2007). In addition, the clove has been defined against *E. coli* (Fu et al.,
69 2007). The mechanisms of action of the antibacterial activity of the clove are not yet fully
70 understood, but the cloves have many compounds to possess growth inhibitory activity
71 against oral pathogens namely: biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid,
72 ellagic acid and oleanolic acid (Cai & Wu, 1996). The use of natural compounds that
73 exploit their therapeutic properties has been an alternative to replace the use of chemical
74 drugs that cause resistance and need a shortage of time.
75 The clove has therapeutic compounds (Shafi et al., 2002) and this research
76 evaluated the antimicrobial, antioxidant e anti-inflammatory activities of clove on the
77 healing of decubitus wounds (pododermatitis) of rabbits (*Oryctolagus cuniculus)* in the
78 commercial farming system.
79
80 **Material and methods**
81 *Material*
82 The cloves and Carbopol 940 Gel were purchased from a local supplier. Methyl
83 alcohol, 2.2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2.2-Diphenyl-
84 1-picrylhydrazyl (DPPH), potassium persulfate, ethanol, and paraplast was purchased
85 from Sigma-Aldrich (São Paulo, Brazil). Violet Red Bile Agar (Neogen Corporation -
86 Acumedia 7165A), peptone bacteriological solution, hematoxylin, and eosin (H&E) were
87 purchased from a local supplier.
88
89 *Fluid gel preparation*
90 The formulation was carried out using Carbopol 940 Gel – gel basis (0.3%
91 concentration); pH 7.02 at 25° C; neutralized with trietanolamina. The Carbopol 940 Gel

92 added of clove powder (60-mesh) was in a water bath for 10 min at 37° C, which was
93 mixed until the solution formation. Three treatments were prepared: control fluid gel
94 without the addition of clove (FGC0), fluid gel with 1% of clove powder (FGC1), and
95 fluid gel with 2% of clove powder (FGC2). The fluid gels were used by spray application.

96

97 *Animals and experimental design*

98 This project was approved by the Animal Ethics Committee with protocol number
99 9332180620. The experiment was conducted at the commercial farm system, which has
100 a herd of 130 breeding rabbits. An assay was conducted using 60 New Zealand rabbits
101 (*Oryctolagus cuniculus*), with 35 male rabbits and 25 females between 3 and 5 years old,
102 weighing approximately 5 kilos and showing pododermatitis (wounds) in the calcaneal
103 region. The wounds in the calcaneal region with skin lesions measuring between 1 and 3
104 cm in diameter and were classified: soft, mean and severe: soft wounds (without scars);
105 mean wounds (presence of blood and deep scarification); and severe wounds (deep
106 scarification and signs of infection). Healthy rabbit of the herd that were neither treated
107 nor had pododermatitis was selected for comparative analysis.

108 The rabbits were raised in individual cages and fed a commercial diet. The first
109 procedure was document foot plantar face of both right and left foot of all animals with
110 digital images. This procedure was repeated at the end of experimental period at day 21.
111 The second procedure was collected samples from rabbit's feet to microbiology analysis.

112 The experimental design was completely randomized in a factorial scheme 3×5,
113 with three compositions of fluid gels (0, 1, and 2% of clove powder) and five period of
114 analysis (0, 1, 7, 14, and 21 days) and 10 experimental units. The study was randomized
115 and the animals were separated into groups: rabbits that received FGC0 (fluid gel at 0%),
116 FGC1 (fluid gel added 1% powder clove) and FGC2 (fluid gel added 2% powder clove).

117 The fluid gel was sprayed directly on the wound surface every 3 days, during the 21 days
118 of the experiment period.
119
120 *Clove and fluid gel characterization*
121 The clove was acquired from a local market and standardized at 60 mesh. The
122 bioactive compounds were extracted by methanol (100%) (9 mL) added to cloves (1 g),
123 homogenized for 10 min, and centrifuged at 3000 rpm for 10 min. The supernatant was
124 recovered and diluted in methanol (1:1000; v/v) for posterior analysis of antioxidant
125 activity. The same was done for fluid gel FGC0, FGC1, and FGC2. The pH was
126 determined using a previously calibrated digital pHmeter (Testo 205), for fluid gels.
127
128 *Antioxidant activity of clove and fluid gels*

129 The antioxidant activity was determined by ABTS (2,2'-azino-bis(3-
130 ethylbenzothiazoline-6-sulfonic acid)) radical cation assay with some modifications
131 (Brand-Williams et al., 1995). The $ABTS^+$ cation was formed by incubating ABTS
132 (7 mM) with potassium persulfate (140 mM) for 16 h at room temperature in dark
133 conditions. The $ABTS^+$ activated radical was diluted with ethanol until an absorbance of
134 0.70 ± 0.02 was achieved, and 1960 µL was mixed with 40 µL of extract. The absorbance
135 at 734 nm was measured after 6 min and the radical scavenging activity (%) was
136 calculated using Eq. 1:

137 $$ABTS(\%)=(1-(A_{samplet}/\ A_{samplet}=0))\times 100 \quad (1)$$

138 Where A_{sample} = absorbance of the sample at 6 min, and $A_{samplet}=0$ = absorbance of
139 the sample at time zero.
140 The antioxidant activity was determined by DPPH (2,2-diphenyl-1-picryl-

141 hydrazyl-hydrate) assay with some modifications (Re et al., 1999; Li et al., 2009). The
142 extract (150 μL) was mixed with DPPH solution (2.85 mL) (60 μM) for 10 s, was
143 incubated for 30 min in dark conditions, and the absorbance measured at 515 nm. The
144 antioxidant activity was calculated using Eq. 2:

145
$$DPPH(\%)=(1-(A_{samplet}/A_{samplet}=0))\times 100 \quad (2)$$

146 Where $A_{samplet}$ = absorbance of the samples at 30 min, and $A_{samplet}=0$ = absorbance
147 of the sample at time zero.

148 The antioxidant activity DPPH and ABTS assay of the clove powder was
149 performed at dilutions: 1:10; 1:100; 1:1000 (v/v) and found the line equation to express
150 the results by the minimum concentration of compound required to inhibit 50% (IC50)
151 activity. The DPPH and $ABTS^+$ assay for fluid gels were conducted in 1, 7, 14, and 21
152 days of storage.

153

154 *Microbiology analysis*

155 The samples from rabbits woods (n = 20) were collected at days 0, 1, 7, 14, and
156 21 days with swabs. Each sample was diluted in 5 mL of peptone bacteriological solution
157 (1 g/L deionized water) and incubated aerobically in Violet Red Bile Agar prepared
158 according to directions of title and presented final pH 7.4 at 25° C, stored in a
159 bacteriological incubator for 26 h at 31° C.

160

161 *Tissue collection and histological and Evaluation of the healing process by infrared*
162 *spectroscopy by attenuated total reflectance (FTIR-ATR) analysis*

163 At the end of experimental period, the animals were sent to a commercial
164 slaughtering. During the slaughter procedures the feet from 03 animals/treatment (n = 03)
165 were collected. Additionally, one healthy rabbit (n = 1) considered normal (without any

166 treatment) and one injured rabbit (n=1) with wound also without treatment were collected
167 as negative and positive controls. Samples from calcaneus skin were collected to
168 histological and to infrared spectroscopy by attenuated total reflectance (FTIR-ATR)
169 analysis.

170 Samples with 0.5 cm from plantar surface of skin of normal and injured (wound)
171 treated or not, were dissected macroscopically and fixed by immersion in 10%
172 formaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). After that, samples were
173 dehydrated in an increasing series of ethanol solutions (70% to 100%) and routinely
174 embedded in paraffin blocks. The embedded tissue samples were cut (5 μm) and stained
175 with hematoxylin and eosin. The slides were analyzed qualitatively in a light microscopy
176 and digital images were obtained using the Motic Images Pro-Plus software 2.0 to
177 illustrate the results.

178 Skin samples with and without wounds from the same animals collected in the
179 commercial slaughter were collected. Samples were collected from 3 rabbits per treatment
180 group (FGC0, FGC1 and FCG2) in addition to the healthy rabbit (without any treatment)
181 and the wounded rabbit (injuries) that were not in the treatment protocol, for comparative
182 analysis between healthy and injured animals who received or not the fluid gel treatments.
183 A 2 mm puch and from these samples/ animal were selected. The samples were
184 considered as experimental units (n = 15).

185 The skin spectra of the rabbits' foot were obtained using a Fourier transform
186 infrared spectrometer (FTIR) with full attenuated reflectance accessory (Vertex 70v,
187 Bruker Optik GmbH, Ettlingen, DEU), with diamond ATR crystal. The spectral range
188 studied was 4000 - 400 cm^{-1}, with 128 scans and a resolution of 4 cm^{-1}. The data were
189 corrected by the baseline and normalized by the total spectrum area. The multivariate
190 method was applied in the first derivative of all spectra, using Principal Component

191 Analysis (PCA), through computer code developed in Mathematica 7.0 software
192 (Wolfram Research, Illinois, USA).

193

194 *Statistical methods*

195 Each experiment was performed in triplicate. Analysis of variance (ANOVA) was
196 performed using the general linear model with SPSS (v.19.0) (IBM SPSS Statistics, SPSS
197 Inc., Chicago, USA) for Windows. Means and standard deviations were calculated for
198 each variable. Concentrations of clove and storage time were considered fixed factors in
199 the factorial design. Differences were considered significant at $P < 0.05$ using the Tukey
200 test.

201

202 **Results**

203 *Antioxidant activity and pH of fluid gel*

204 The $ABTS^{+}$ assay revealed the antioxidant properties of clove powder extract,
205 since the concentration of clove extract 0.19 µg/ mL was able to scavenge 50% of the free
206 radical and of 0.03 µg/ mL of clove extract was able to scavenge 50% of the DPPH free
207 radical. The pH that was measured to assess the stability of the antioxidant action of the
208 gel shows constant values and close to neutrality (7.04±0.01) for all fluid gels at 21 days
209 of storage.

210 The data (Table 1) demonstrated that the FGC0 had less antioxidant activity than
211 the other fluid gels. The FGC2 showed greater antioxidant activity. The results for the
212 analysis of DPPH antioxidant activity (Table 1) showed a significant difference between
213 the FGC0, FGC1, and FGC2. The fluid gel FGC2 showed greater DPPH antioxidant
214 activity (56.98±2.48%) and $ABTS^{+}$ antioxidant activity (81.06±2.44%) than the others.
215 The results for FGC1 were intermediate. The fluid gel without the addition of clove

216 (FGC0) did not demonstrate an efficient antioxidant activity, showing a maximum value
217 of 7.76±0.18% for DPPH on day 21 and a maximum value of 13.75±0.92% for $ABTS^+$
218 on day 1.
219
220 *Microbiological analysis on the wounds*
221 The microbiological results - *Escherichia coli* and *Pseudomonas spp* - of day 0
222 correspond to the wounds of the rabbits before the experiment with the use of the fluid
223 gel. After the first application of the spray fluid gel (1 day), a decrease in the bacterial
224 count over 21 days was noted (Table 2).
225
226 *Evaluation of the healing process by infrared spectroscopy by attenuated total reflectance*
227 *(FTIR-ATR)*
228 The FTIR-ATR spectroscopy was applied to assess the physicochemicalchanges
229 in the skin samples after treatment of the lesion. The spectra of the dermis and healthy
230 epidermis were observed with the assignments of the most important absorption bands of
231 both layers (Fig. 1A). The data from FTIR-ATR technique allowed to differentiate the
232 dermis from the epidermis (Fig. 1B). The bands that present a great contribution to
233 differentiate the spectra in the groups being studied' are centered on 2920 and 2850 cm^{-1}
234 attributed to vibrations of symmetrical CH_3 and symmetrical CH_2 stretching, related to
235 proteins and lipids (Fig. 1C). The absorption band of the band centered on 1747 cm^{-1} is
236 attributed to C = O bands, related to lipids. The bands in 1634, 1555, and 1241 cm^{-1} are
237 assigned to amide I, amide II, and amide III, respectively, related to proteins. The band
238 in 1455 is attributed to vibrations of CH_2 deformation related to proteins. The band at
239 1080 cm^{-1} is attributed to vibrations of CC stretching, related to lipids (Movasaghi et al.,
240 2007; Greve et al., 2008).

241 The spectra of animals with wounds without treatment (injuries rabbits) and
242 treated with FGC0, FGC1, and FGC2 show spectral differences when compared to a
243 healthy group (Fig. 2A and 2B). It was observed that the greatest contributions to
244 differentiate the spectra in the studied groups are also the bands centered on 2920 and
245 2850 cm^{-1} related to proteins and lipids (Fig. 2E and 2F). Rabbits that received the FGC2
246 was grouped with healthy rabbits for both layers, dermis, and epidermis, showing to be
247 more effective when compared to the FGC1 (Fig. 2C and 2D).
248
249 *Histological analysis*
250 The wound histological analysis showed the difference between treated rabbits
251 (FGC0, FGC1, and FGC2) and compared to the rabbit with healthy calcaneal skin
252 (healthy rabbits). Histology of healthy rabbits (Fig. 3) was the negative control for the
253 evaluation of histology. The presence of exudate with the presence of polymorphonuclear
254 cells is noted and shows areas of tissue inflammation of the dermis and epidermis (Fig.
255 4). The rabbits that received FGC1 and FGC2 showed collagen proliferation,
256 characterizing wound resistance. Those who received FGC2 (Fig. 5) showed greater
257 accumulation of collagen and fibroblasts and addition to newly formed blood vessels than
258 those who received FGC1 but with greater relevance in FGC2 (Fig. 6).
259
260 **Discussion**
261 *Evaluation of fluid gels*
262 The antimicrobial, anti-inflammatory, and antioxidant activity of clove
263 (Muruganandan et al., 2001; Shafi et al., 2002; Chaieb et al., 2007; Nassar et al., 2007) in
264 the treatment of skin wounds in the fluid gel demonstrated antioxidant activity and
265 needed 0.03 μg/mL for scavenging 50% of the DPPH free radical. This result indicates

266 that antioxidants in the clove are efficient (Anita, 2015). There were no relevant variations
267 in antioxidant activity over the 21 days of storage. This is due to Carbopol 940® Gel
268 thixotropy maintaining colloidal gel characteristic and antioxidant activity of the clove
269 (Maia-Campos, 1999). Besides, the fluid gel has an anionic character and is pH-
270 dependent, which is stable in neutral pH or close to neutrality (Maia-Campos, 1999)
271 helping to maintain "shelf life" during the 21 days of storage. That explains why the gel
272 showed pH values close to neutrality even when adding cloves.
273
274 *Evaluation of the results in vivo*
275 The stability of pH and antioxidant activity of the gel is important to modify the
276 inflammatory conditions of the wound to promote healing. The antioxidant compounds
277 can modulate the Nrf2 / Keap1 pathway - nuclear transcription factor, acting indirectly
278 on oxidative stress, on the expression of the transcription factor NFκB (Soares et al.,
279 2015), and in the inflammatory response. The action of antioxidants disfavors the
280 formation of disulfide bridges between proteins, preventing oxidative stress (Ferreira &
281 Matsubara, 1997). These antioxidant mechanisms of the fluid gels prepare the wound for
282 tissue repair and make the clove's antimicrobial action remarkable.
283 The swabs analysis for microbiological of *Escherichia coli* and *Pseudomonas*
284 *spp*. showed that a decrease in the count of colony-forming units (CFU) was seen for both
285 bacteria (Table 3 and 4). This indicates that the use of fluid gel on the wound prevented
286 or controlled bacterial proliferation due to the antimicrobial activity of the clove
287 (Muruganandan et al., 2001; Shafi et al., 2002). The contamination of the wound by *E.*
288 *coli* decreased with the use of fluid gel with the addition of cloves (Table 3). The oil clove
289 and infusion clove has antimicrobial activity *in vitro* against gram-negative bacteria such
290 as *E. coli, Yersinia enterocolitica, Salmonella choleraesuis* and *Pseudomonas aeruginosa*

291 (Lopez et al., 2005). For CFU of *Pseudomonas spp* there was a significant difference after
292 treatment with cloves powder (FGC1 and FGC2 (Table 4). This action of cloves on the
293 *Pseudomonas spp*. can be attributed to eugenol and other active constituents (biflorin,
294 kaempferol, rhamnocitrin, myricetin, gallic acid and ellagic acid) (Cai & Wu, 1996).
295 These compounds disrupt the bacterial cytoplasmic membrane causing an increase in
296 permeability that leads to cell death (Devi et al., 2010).
297
298 *Evaluation of the results post mortem*
299 The wound healing is characterized by the presence of the healing cascade
300 (Broughton et al., 2006) that is noted in the bands centered on 2920 and 2850 cm^{-1} related
301 to the proteins and lipids (Fig. 2E and 2F) characterizing the presence of collagen and
302 keratin that can be seen in histology (Fig. 5 and 6). Although the regeneration is different
303 between the organs, there is a harmonized interaction of different types of cells, signaling
304 systems, growth production, cell-matrix molecules, and different classes of proteases
305 (Broughton et al., 2006). It demonstrates that from the moment of the wound multiple
306 cellular and extra-cellular pathways are activated to restore skin integrity (Fig. 6). This
307 process of wound healing is divided into four phases: hemostasis, inflammation,
308 proliferation, and tissue remodeling (Ueno et al., 2006; Singh et al., 2017). The rabbits
309 that received FGC0 (Fig. 4) showed more inflammatory infiltrate (exudate) than rabbits
310 that received FGC1 and FGC2 (Fig. 5 and 6), which is characterized by the presence of
311 polymorphonuclear cells that are mediated by chemotaxis, mechanisms that include the
312 complement cascade - proteins and cellular components, activation and TGF-B signaling
313 (Broughton et al., 2006). The rabbits that received FGC2 presented the bands centered at
314 2920 and 2850 cm^{-1} related to the most relevant of proteins (Fig. 6) being mainly
315 characterized by the presence of collagen and keratin. In histology the presence of healing

316 components was demonstrated, as collagen and complement cascade and fibroblasts (Fig.
317 6), indicating the healing process after administration of the FGC2. The fibroblasts are
318 stimulated by growth factors released during coagulation and the wound becomes rich in
319 fibroblasts that deposit proteins in the extracellular matrix, which in turn produce collagen
320 and are the key component in providing strength to tissues (Gantwerker et al., 2011;
321 Mayrand et al., 2012), which is noted in Fig. 5 and 6. This process revitalizes the epithelial
322 cells that migrate from the edge of the wound until a complete layer of cells collect the
323 wound and attaches itself to the matrix below (Hinz, 2006) explaining why rabbits treated
324 with FGC2 showed spectra close to healthy rabbits (Fig. 3), which is also seen in the
325 histological section (Fig. 6) where the epithelial conformation is normal. Wounds begin
326 to contract about seven days after injury (Hinz, 2006). The physiological processes of
327 wound healing corroborate the results of rabbits that received treatments with the addition
328 of clove, in which rabbits treated with FGC2 presented the best healing process due to the
329 greater antimicrobial and antioxidant activity than the others, which indicates that higher
330 concentrations of clove powder are necessary for better efficiency of the fluid gel.
331 Antimicrobial activity against Gram-negative bacteria - *E. coli* and *Pseudomonas spp* -
332 has been testify. However, studies are still lacking that demonstrate the true mechanism
333 of action of the active compounds of the clove on the bacteria, in addition to the rupture
334 of the cytoplasmic membrane. The pH neutrality from the fluid gel and antioxidant
335 activity of the clove made it possible to rescue free radicals present in the wounds and
336 consequently acted in the repair of tissue damage and reduction of wound contamination,
337 which led to the efficient, physiologically expected healing process.

338 The use of Carbopol 940 Gel as a vehicle of the active ingredient (clove) made
339 it possible to extend the shelf life (storage) of the fluid gels. The cloves presented an
340 efficient choice in the control of the studied bacteria (*E. coli* and *Pseudomonas spp*.).

341 However, studies are still lacking that demonstrate the true mechanism of action of the
342 active compounds of the clove on the bacteria.
343

344 **Conclusions**

345

346 The present study demonstrated that the use of fluid gel with 2% of clove
347 powder (*Syzigium aromaticum*) resulted in the best antimicrobial, antioxidant e anti-
348 inflammatory activities on the healing of decubitus wounds (pododermatitis) of rabbits
349 (*Oryctolagus cuniculus)* in the commercial farming system.
350

351 **Conflict of interest statement**

352 None of the authors has any financial or personal relationships that could
353 inappropriately influence or bias the content of this paper.
354

355 **Acknowledgements**

356 We thank National Council for Scientific and Technological Development
357 (CNPq) for scholarship.
358

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Table 1

Unfolding the interactions between fluid gel and days of evaluation for DPPH (%) and ABTS (%) free radical scavenging of fluid gel stored at 25°C during 21 days.

	Days of storage				
	1	7	14	21	*P*
DPPH					
Fluid gel					
FGC0[1]	6.90±1.36^{C}	7.62±0.32^{C}	7.55±0.18^{C}	7.76±0.18^{C}	0.196
FGC1	30.22±2.94^{aB}	30.86±1.73^{aB}	26.96±0.72^{bB}	21.57±1.91^{cB}	<0.001
FGC2	56.98±2.48^{aA}	57.17±3.36^{aA}	54.79±0.67^{abA}	53.27±0.30^{bA}	0.013
P	<0.001	<0.001	<0.001	<0.001	
ABTS^{+}					
Fluid gel					
FGC0	13.75±0.92^{aC}	13.40±0.64^{aC}	12.85±0.53^{abC}	12.60±0.43^{bC}	0.028
FGC1	53.20±5.48^{B}	55.46±1.84^{B}	53.54±0.75^{B}	51,37±0.34^{B}	0.150
FGC2	81.06±2.44^{aA}	78.20±3.03^{aA}	74.38±0.72^{bA}	73.90±0.47^{bA}	<0.001
P	<0.001	<0.001	<0.001	<0.001	

Different lower case letters on the same line indicate a significant difference between days ($P < 0.05$). Different capital letters in the same column indicate a significant difference between treatments ($P < 0.05$).

[1]FGC0: fluid gel without clove powder; FGC1: fuid gel with 1% of clove powder; and FGC2: fluid gel with 2% of clove powder.

Table 2

Unfolding the interaction between fluid gels and days of the counting of *E. coli* and *Pseudomonas spp.* colony forming units (CFU) from rabbits' breeders skin swab (n=20) after 21 days of treatment.

E. coli (CFU)						
	Days of treatment					
Fluid gel	0	1	7	14	21	*P*
FGC0[1]	32.0±0.11	29.5±4.50^{A}	25.5±4.05^{A}	21.5±6.05^{A}	23.5±8.45^{A}	0.504
FGC1	31.1±0.88^{a}	17.5±0.50^{aC}	15.0±0.00^{aB}	12.5±1.25^{abB}	6.0±2.00^{bB}	0.001
FGC2	31.2±1.12^{a}	19.5±0.50^{aB}	12.5±9.50^{bC}	9.0±0.80^{abC}	4.0±2.00^{cC}	0.001
P	0.089	0.009	0.008	0.001	0.001	
Pseudomonas spp. (CFU)						
FGC0	8.5±0.87	8.5±0.50^{A}	9.0±0.00^{A}	10.0±2.00^{A}	8.0±2.00^{A}	0.400
FGC1	8.6±1.11	6.5±1.50^{B}	7.0±0.00^{B}	5.5±0.50^{B}	4.5±1.05^{B}	0.520
FGC2	8.5±1.02^{a}	8.0±2.00^{aA}	8.5±0.50^{aA}	4.0±2.00^{abC}	2.0±2.00^{bC}	0.001
P	0.390	0.001	0.001	0.001	0.001	

Different lower case letters on the same line indicate a significant difference between days (P <0.05). Different capital letters in the same column indicate a significant difference between treatments (P <0.05).

[1]FGC0: fluid gel without clove powder; FGC1: fluid gel with 1% of clove powder; FGC2: fluid gel with 2% of clove powder.

474 **Figure legends:**

475

476 Fig. 1. (A) Spectra obtained by FTIR-ATR of the epidermis and dermis of the healthy
477 animal. (B) Principal Component Analysis (PCA) obtained from FTIR-ATR spectra. (C)
478 Loading spectrum of the first principal component (PC1).

479

480 Fig. 2. Spectra obtained by FTIR-ATR of the epidermis (A) and dermis (B) of the healthy
481 animal (Healthy), an animal with injury without treatment (Injury), an animal with injury
482 treated without addition cloves (FGC0), an animal with injury treated at 0,1% addition of
483 clove (FGC1) and animal with lesion treated at 0.2% addition of clove (FGC2). Principal
484 Component Analysis (PCA) obtained from FTIR-ATR spectra for the epidermis (C) and
485 dermis (D), the dashed square shows the spectra grouped to the healthy group. Loading
486 spectrum of the first principal component (PC1) for the epidermis (E) and dermis (F).

487

488 Fig. 3. A) Macroscopical view of the foot plantar face with healthy aspect in a rabbit
489 breeder. B-C) Histological view of the normal skin in the plantar face of the feet. The skin
490 is filled with hair follicles (fol). In C details of the epidermis composed by and stratified
491 keratinized epithelium (ep) covered by a thin keratin layer (ke). The dermis has dermic
492 papillae (dp) and loose connective tissue characterized by collagens fibers and cells. The
493 great number of vessels in dermic area is also observed (ves). Paraffin sections, HE. Scale
494 bars: B) 200 µm, C) 100 µm

495

496 Fig. 4. A-B) Macroscopical view of the plantar face in a rabbit breeder in wounds foot
497 condition. Arrow – calcaneal wounds. In A note the wound on day 0 and in B note the
498 wound 21 days after administration of FGC0 fluid gel. C-E) Histological view of skin.
499 In C, the presence of exudate (*) with the presence of polymorphonuclear cells are noted
500 and without hair follicles presence. In D-E close view of the wound edges (arrows) with
501 the presence of epithelialization (ep). Paraffin sections, HE. Scale bars: C) 200 µm, D-E)
502 100 µm.

503

504 Fig. 5. A-B) Macroscopical view of a rabbit breeder in wounds foot condition (plantar
505 face). Arrow – calcaneal wounds. In A note the wound on day 0 and in B note the wound
506 21 days after administration of FGC1 fluid gel. C-E) Close view of wound healing. In C
507 shows the presence of collagen (*) marking the transition area between healing and

508 healthy tissue and histological view of skin filled with hair follicles (fol) and details of
509 the epidermis composed by and stratified keratinized epithelium (ep) covered by a thin
510 keratin layer (ke). In D close view of the and details of the epidermis composed by and
511 stratified keratinized epithelium (ep) covered by a thin keratin layer (ke) and presence of
512 polymorphonuclear cells (arrows). In E it is noted the presence of collagen (*) and
513 vascularization of the tissue (ve) in the dermic area. Paraffin sections, HE. Scale bars: C)
514 200 μm, D-E) 100 μm.

515

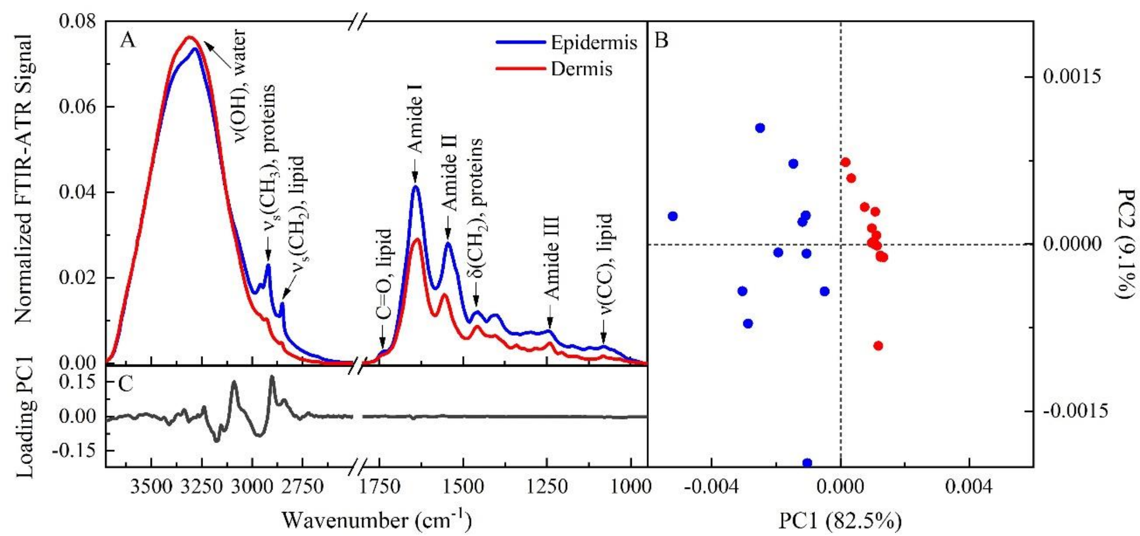
516 Fig. 6. A-B) Macroscopical view of a rabbit breeder in wounds foot condition (plantar
517 face). Arrow – calcaneal wounds. In A note the wound on day 0 and in B note the wound
518 21 days after administration of FGC2 fluid gel. C-E) Close view of wound healing. In C
519 the edges of the wound (arrows) delimited by the presence of collagen (*),
520 epithelialization (ep) and hair follicles (fol) are noted. In D-E close view of the and details
521 of the epidermis composed by and stratified keratinized epithelium (ep) covered by a thin
522 keratin layer (ke) and presence of vascularization of the tissue (ve) in the dermic area and
523 presence of collagen (*) demonstrating the integrity of the epithelial tissue. Paraffin
524 sections, HE. Scale bars: C) 200 μm, D-E) 100 μm.

525

526 **Figures**

527

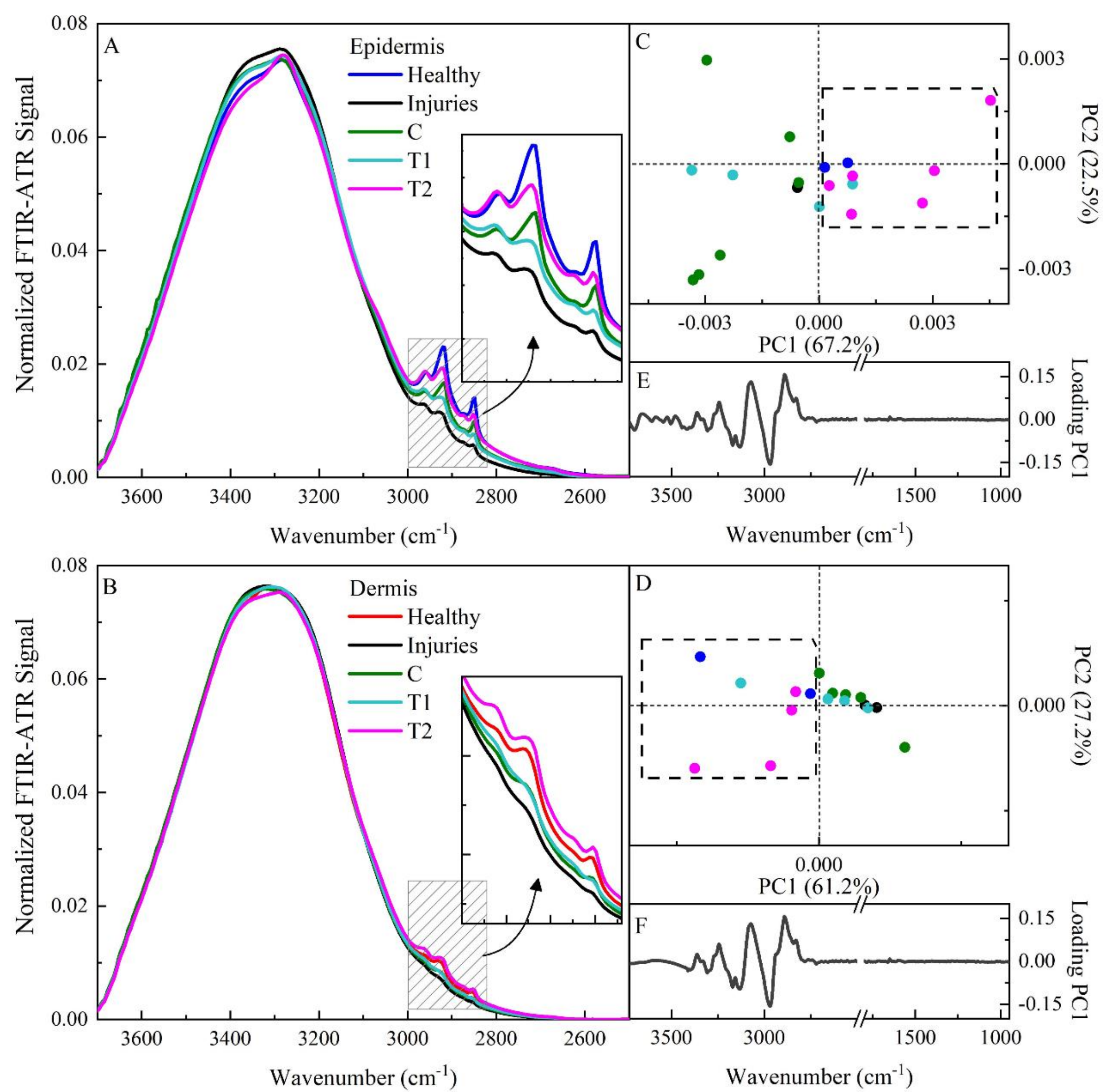
528 Fig. 1



529

530 Fig, 2

531



532

533 Fig. 3

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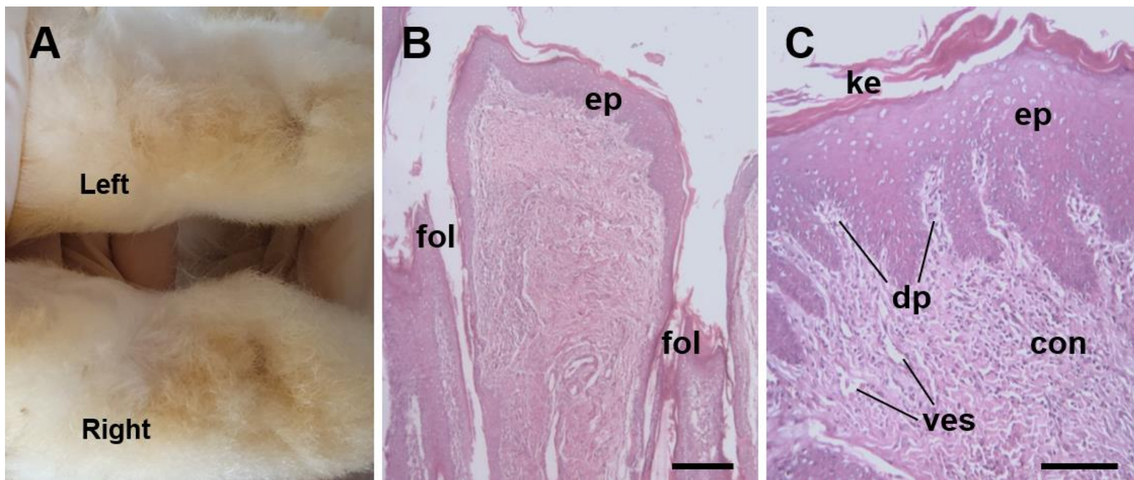
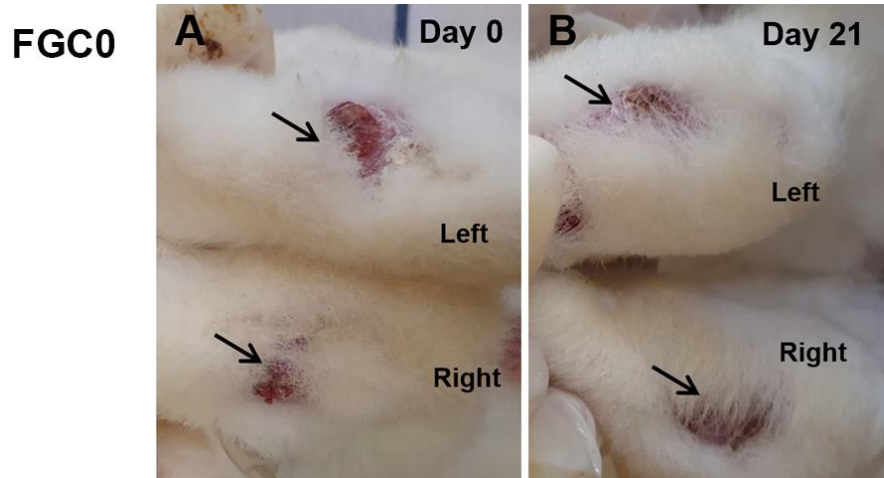


535

536 Fig. 4

537



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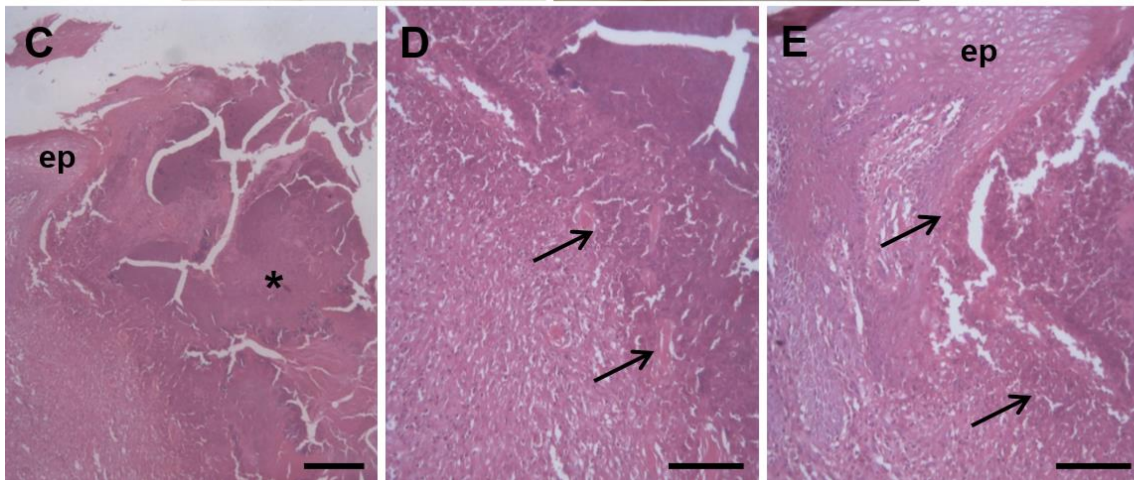
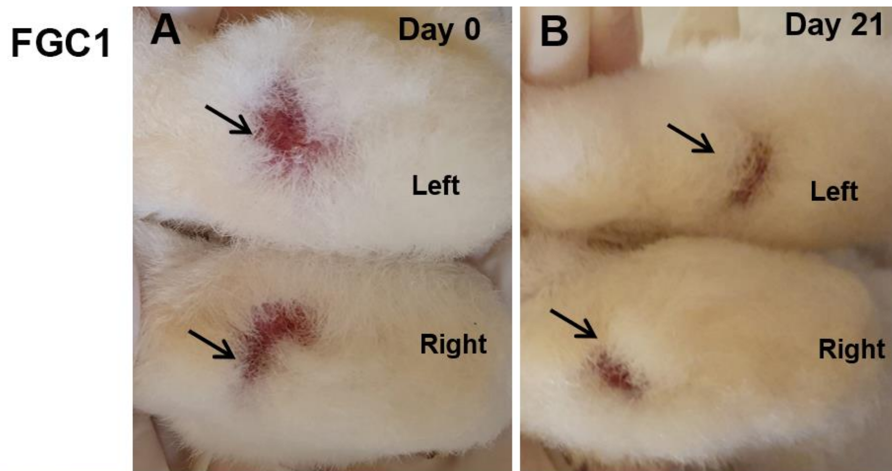


539 Fig. 5

540



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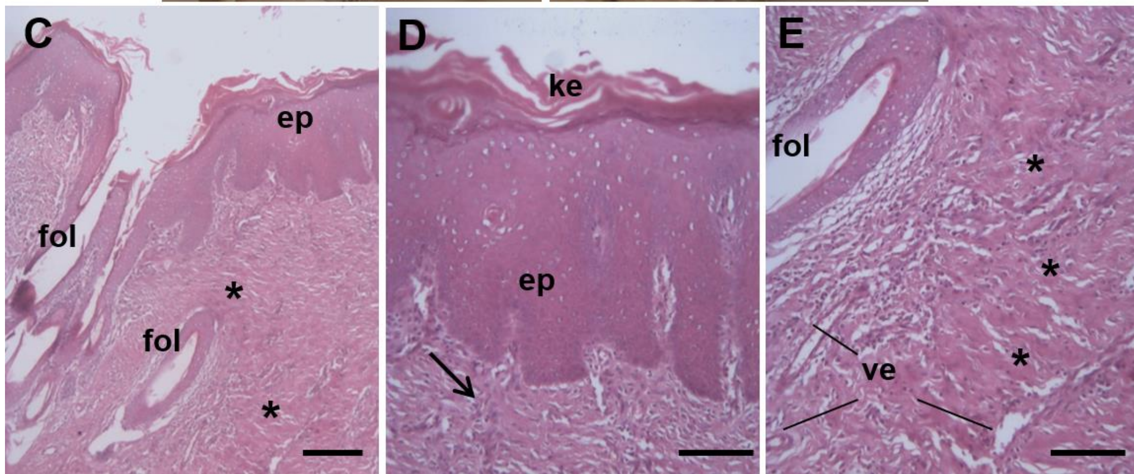

542 Fig. 6

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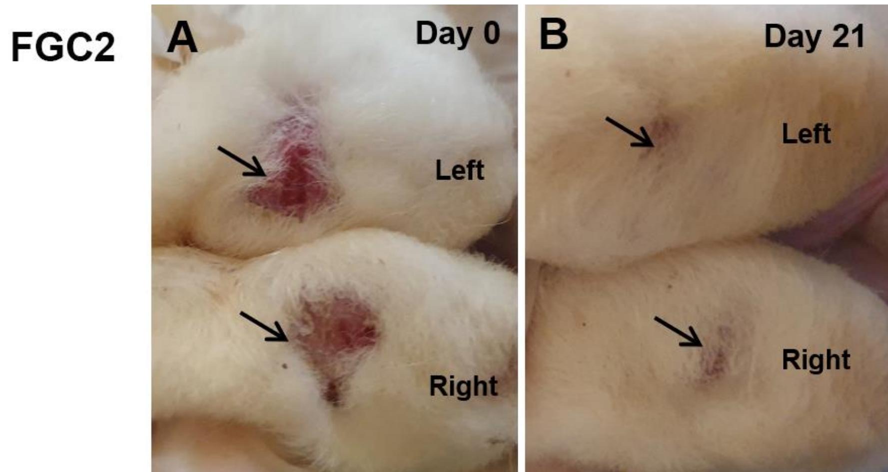

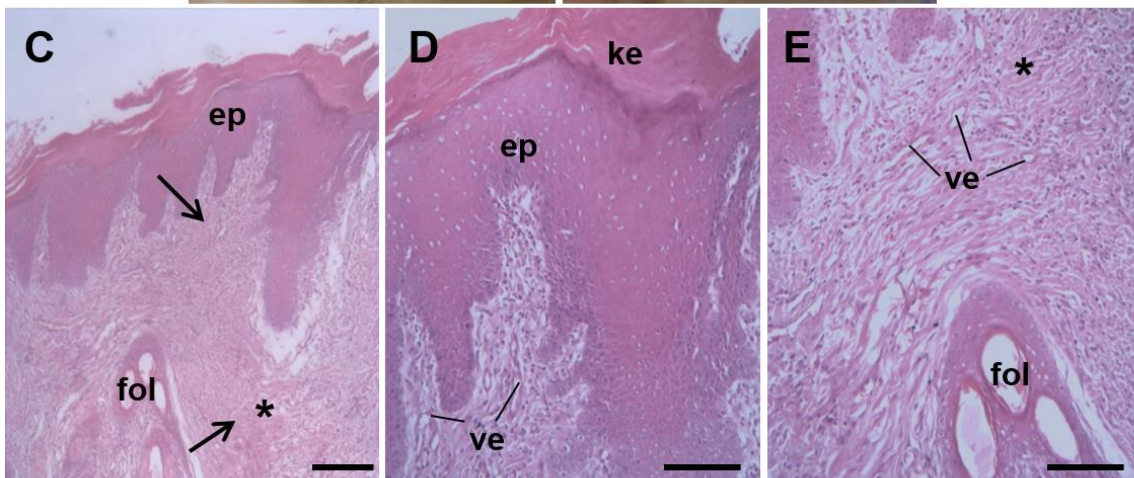

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1 **Original Article**
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3
4 **Lactoferrin bovine as an active in intramammary gel in goats**
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6 (Modelo Revista: Small Ruminant Research – Elsevier)
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Abstract

Goats in the prepartum period and in colostrogenesis are more susceptible to mastitis that lead to a drop in the quality of colostrum and milk. The study aimed to decrease the recurrence of mastitis and improve the quality of colostrum and goat's milk. An intramammary treatment was performed: a group of nine Saanen goats in the prepartum period received the non-lactoferrin Carbopol 940® Gel (NLG) and another group nine Saanen goats added with lactoferrin 0,5% (LG05). Samples of microbiological swabs were collected for the analysis of *Escherichia coli* and *Pseudomonas spp*. After the parturition the colostrum and milk samples were collected for quality analysis. Goats that were treated with lactoferrin (LG05) showed decreased bacterial contamination, increased immunoglobulins in colostrum and improved milk quality.

Keywords: colostrum, immunoglobulins, immunity, mastitis, Saanen.

34 **Introduction**

35 The mastitis is the most common problem in dairy production being of broad
36 etiology (Contreras et al., 2007) and can affect reproductive efficiency in animals (Kumar
37 et al., 2017). Goat mastitis has no seasonal character and can occur throughout the year
38 with higher prevalence in wet periods or in farms with higher milk production (Pinheiro
39 et al., 2000). Subclinical mastitis is prevalent in goat herds (Contreras et al. 2007). The
40 greater susceptibility of the animal during the lactation period is the main cause, because
41 in the dry period there is a higher frequency of environmental mastitis (Prestes et al.,
42 2002).

43 Bacteriological examination and microbial identification, is considered the gold
44 standard diagnosis of mastitis in goats (Paterna et al., 2014). The diversity of pathogenic
45 microorganisms with higher occurrence as *Pseudomonas aeruginosa* is mainly associated
46 with diseases of the urinary tract, chronic pyodermia, and dermatitis (Schauer et al., 2021)
47 and sporadic mastitis. The frequency of mastitis caused by *Escherichia coli* is sporadic
48 and clinical signs can be localized or result in severe clinical symptoms with fatal
49 episodes (Santos, 2006). The animal care starts in the dry and prepartum period in search
50 of lower rates of mastitis, without the need to use antimicrobials (Shwimmer et al., 2008).

51 Animal products and their derivatives are efficient in the treatment of
52 inflammatory and cancerous lesions obtaining satisfactory results approximately sixty
53 percent of anticancer drugs come from this source (Orangi et al., 2016). Lactoferrin (Lf)
54 is part of a group of proteins called transferrins (Steijns & Hooijdonk, 2000) and it is
55 found mainly in milk and has the properties: metabolic modulation, retardation of
56 pathological processes, antiviral, antibacterial, antitumor and anticancer activity
57 (Sgarbieri, 2004). The antimicrobial action of lactoferrin and its derived peptides is
58 related to the positive net charge of these peptides (Moita, 2011) and the recent approval

59 of Lf as an active depends the molecular structure integrity considered when health
60 benefits are proposed (Rosa et al., 2020).

61 The present study aimed to evaluate the antimicrobial and antioxidant action of
62 lactoferrin in the preventive treatment of mastitis in goats in the prepartum period.

63

64 **Material and methods**

65 *Material and reagents*

66 The Lactoferrin bovine (Lf) 10% (Nature's First Imunne Defense™) and
67 Carbopol 940® Gel were purchased from a local supplier. Methyl alcohol, 2,2′-Azino-bis
68 (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-picrylhydrazyl
69 (DPPH), potassium persulfate and ethanol was purchased from Sigma Aldrich (São
70 Paulo, Brazil). Violet Red Bile Agar (Neogen Comporation - Acumedia® 7165A) and
71 peptone bacteriological solution.

72

73 *Intramammary lactoferrin gel (LG) preparation*

74 The formulation was carried out using Carbopol 940® Gel – gel basis (0,3%
75 concentration); pH 7,09 at 25°C; neutralized with trietanolamina. The Carbopol 940® Gel
76 with Lf was in water bath for 10 min at 30°C, that mixed until the solution is formed.
77 Two treatments were prepared: control intramammary gel - Carbopol 940® Gel non-
78 lactoferrin added (NLG) and intramammary gel with the addition of 0.50% Lf (LG05).

79 The NGL and LG05 were stored in individual 5 ml syringes at 10° C during the
80 28 days of experiment.

81

82 *Experimental design and application of LG*

83 The experiment was carried out using 18 Saanen goats in 60 days' period

84 prepartum. The animals were raised in individual stalls and fed with habitual feed. First
85 procedure was the collecting of material from teat for microbiology analysis and after LG
86 administration. The distribution of treatments was randomized: NLG and LG05. The
87 administration of the LG was performed intramammary with the use of syringe - 3 mL
88 per teat.

89

90 *Antioxidant activity of Lf and intramammary LG*

91 Methanol (100%) (9 mL) was added to Lf (1 g), homogenized for 10 min and
92 centrifuged at 3000 rpm for 10 min. The supernatant was recovered and diluted in
93 methanol (1:1000; v/v) to use for analysis of antioxidant activity of the Lf. The NLG and
94 LG05 were homogenized for 15 min and centrifuged for 10 min at 3000 rpm. The
95 supernatant was recovered and diluted in methanol (1:1000; v/v) to use for analysis of
96 antioxidant activity.

97 The antioxidant activity was determined by ABTS (radical cation assay with some
98 modifications (Brand-Williams et al., 1995). The ABTS+ was formed by incubating
99 ABTS (7 mM) with potassium persulfate (140 mM) for 16 h at room temperature in dark
100 conditions. The ABTS activated radical was diluted with ethanol until an absorbance of
101 0.70 ± 0.02 was achieved, and 1960 μL of the resulting solution was mixed with 40 μL
102 of extract. The absorbance at 734 nm was measured after 6 min and the radical scavenging
103 activity (%) was calculated using Eq. 1:

104

105 $$ABTS(\%)=(1-(Asamplet/\ Asamplet=0))\times 100$$

106 (1)

107 Where Asample = absorbance of the sample at 6 min, and Asamplet=0
108 = absorbance of the sample at time zero.

109

110 The antioxidant activity was determined by DPPH assay with some modifications
111 (Re et al., 1999; Li et al., 2009) The extract (150 μL) was mixed with DPPH solution
112 (2.85 mL) (60 μM) for 10 s, was incubated for 30 min in dark conditions, and the
113 absorbance measured at 515 nm. The antioxidant activity was calculated using Eq. 2:

114

115 $$DPPH(\%)=(1-(Asamplet/Asamplet{=}0))\times 100$$
116 (2)

117 Where Asamplet = absorbance of the samples at 30 min, and Asamplet=0
118 = absorbance of the sample at time zero.

119

120 The pH was determined using a previously calibrated digital pHmeter (Testo 205),
121 which was measured at three timein LG sample.

122

123 *Microbiology analysis*

124 The procedure was carried collecting material from the teat and ubber for
125 microbiology analysis in 0, 1, 7, 14 and 28 days. The samples were colleted with swabs.
126 Each sample was diluted in 5 mL peptone bacteriological solution (1 g/L of deionized
127 water). The samples were incubated aerobically in Violet Red Bile Agar (Neogen
128 Comporation - Acumedia® 7165A) prepared according directions of title and presented
129 final pH 7,4 at 25°C, in a bacteriological incubator for 26 h at 31°C. The bacteria analysed
130 were *E. coli* and *Pseudomonas spp.*

131

132 *Analysis of quality of colostrum and milk*

133 The colostrum composition for the protein content by Kjeldahl method was
134 determined according to AOAC (1990). Fat content was performed by extraction with

135 chloroform, methanol and water (2:2:2) (Bligh & Dyer, 1959). The immunoglobulin
136 analysis was determined by estimating the percentage of total solids present by the Brix
137 Refractometer (Nagyová, 2017).
138 The milk composition for the fat, protein content, non-fat solids and lactose was
139 determined by EkoMilk®.
140
141 **Results**
142 *Intramammary gel stability and antioxidant activity*
143 The pH of LG showed stable values of 7.21±1.02. There was no significant
144 difference in the pH values between the NLG and LG05 over the 28 days of storage.
145 The results for the analysis of DPPH and ABTS antioxidant activity showed significant
146 difference between the NLG and LG05. The LG05 - with the addition of Lf - showed
147 antioxidant activity (Table 1).
148
149 *Intramammary gel antimicrobial activity in teats*
150 The animals were tested for the presence of *E. coli* and *Pseudomonas spp.* in the
151 teats on day 0 - without receiving any intramammary gel. Only 11.12% of the goats in the
152 experiment showed the presence of 22.12±0.83 CFU of *Pseudomonas spp* – and 87.22%
153 CFU decrease at the end of the experiment.
154 The microbiological analysis for bacterial count to *E. coli* demonstrated a significant
155 difference between animals treated with NLG and LG05 (Table 2).
156
157 *Quality colostrum and milk*
158 The quality of colostrum from first 12 hours postpartum was analyzed to protein,
159 fat content and immunoglobulins (Ig) (Table 3). The quality of milk from 7 days

160 postpartum was analyzed. The analysis - colostrum and milk - was made by studying
161 means in triplicate.

162 The effects of using LG05 on milk composition (Table 4) from 7 days postpartum
163 were more relevant in relation to animals treated with NLG.

164

165 **Discussion**

166 The pH of NLG and LG05 showed stable values demonstrated that the Carbopol
167 940 Gel used as a basis for intramammary treatment (Maia-Campos et al., 1999) which
168 explains the neutral pH over 28 days of storage.

169 The antioxidant capacity of powdered bovine lactoferrin produced by freeze-
170 drying varies around 46% and 52% DPPH inhibition (Wang et al., 2016). This reduced
171 scavenging capacity can be attributed to the reduction of Fe^{3+} by oxygen (Gallagher,
172 2009) during the antioxidant process in the inflammation. The Lf when added to the
173 Carbopol 940 Gel – LG05, had its antioxidant activity decreased due to the inert and
174 anionic character of the gel (Maia-Campos et al., 1999).

175 The stability of pH and antioxidant activity of the LG05 are important to modify
176 the possible inflammatory conditions. This action of antioxidants activity disfavors
177 disulfide bridges formation between proteins, preventing oxidative stress (Ferreira &
178 Matsubara, 1997). In addition, the thixotropy of the gel and constant viscosity made it
179 difficult to separate the constituents of the formulation (Martim, 1993) and kept the
180 antioxidant and antimicrobial properties of the Lf added during the 28 days of storage at
181 10° C.

182 The increase in *E. coli* colony forming units (CFU) was noticed and indicates that
183 there is environmental contamination and can be a cause of recurrent environmental
184 mastitis (Prestes et al., 2002; Santos, 2006). The animals that received NLG, did not show

185 a decrease in the CFU for *E. coli*, demonstrating that Carbopol 940® Gel alone does not
186 have efficient antimicrobial activity. This antimicrobial activity is due to the presence of
187 Lf that makes direct interaction with the bacterial cell membrane (Rodríguez-Franco et
188 al., 2005). The Lf interacted with the bacterial surface causing the release of
189 lipopolysaccharides: increased cell permeability and release of cytoplasmic content
190 leading to the death of the bacteria (Yamauchi et al., 1993; Rodríguez-Franco et al., 2005).
191 The lipopolysaccharides is the endotoxic component present in the bacterial cytoplasmic
192 membrane that allows it to adhere to the host cell (Reitschel et al., 1994) and the presence
193 of Lf in intramammary gel (LG05) altered the structure of lipopolysaccharides bacterial
194 and prevented bacterial adhesion in the teats.

195 The animals treated with LG05 demonstrated a colostrum formation richer in
196 protein and fat content than the animals treated with NLG. The same was observed for
197 immunoglobulins (Ig) analysis. The total protein content of goat colostrum was
198 5.75%±0.12 for goats treated with NLG and 9.12%±0.09 for those treated with LG05
199 after the first 12 hours,. In the literature, the values of protein in colostrum vary between
200 9.24% and 13.99% in the first milking after parturition and 4.37% and 7.16% after the
201 first 24 hours, variations in protein content occur due to analysis of colostrum of different
202 types of goat breeds (Vilar et al., 2008; Sánzhez-Macías et al., 2014; Kessler et al., 2019).
203 The protein fractions correspond to caseins (κ-, β-, αs1-, αs2-, γ-) and whey proteins
204 (immunoglobulins, β-lactoglobulin, α- lactoalbumin, albumin and lactoferrin) (Vargas et
205 al., 2008).

206 The analysis of Ig by Brix Refractometer indicates the percentage estimate of total
207 solids present in colostrum. Brix values greater than 21% and 31% indicate that colostrum
208 is good and high quality, respectively. Values below 21% indicate poor quality (Nagyová,
209 2017).

210 The amount of immunoglobulins, which represent the largest portion of the
211 protein fraction from colostrum (Costa et al., 2019). Immunoglobulins (Ig) are present in
212 goat colostrum:Immunoglobulin G (IgG), Immunoglobulin M (IgM) and
213 Immunoglobulin A (IgA), however IgG represents the largest protein constituent (Castro
214 el al., 2009; Costa et al., 2019). The goats treated with LG05 showed higher Ig values
215 which indicates that the presence of Lf in the treatment was efficient in modulating Ig.
216 This action benefits the udder and the quality of colostrum due to the increase in total
217 solids. The immunoglobulins present have the function of binding with other defense cells
218 acting as a complement preventing the adhesion of pathogenic microorganisms, blocking
219 bacterial enzymes and neutralizing viruses and toxins (Doan et al., 2008).

220 The goats that received intramammary treatment with LG05 showed a decrease in
221 the *Escherichia coli* count; cause the permeability and release of cytoplasmic content
222 leading to the death of the bacteria, which indicates that the presence of Lf in the gel was
223 efficient to control environmental contamination in the teat. There was no significant
224 count of *Pseudomonas spp.* to perform comparative statistical analysis between
225 treatments. The immunological modulation caused by Lf (LG05) in the udder led to an
226 increase in Ig in colostrum, which was important in combating *Escherichia coli*.. The Lf
227 provided to increased colostrum immunity and improved milk quality, analyzed by the
228 amount of protein, fat and lactose which were shown to be elevated.

229 In addition, the presence of Lf in the prepartum treatment enabled the process of
230 colostrogenesis of the 14 and 21 days prepartum - cellular renewal of mammary tissue
231 occurs and the high synthesis activity makes the udder susceptible to infections and
232 inflammations (Santos & Fonseca, 2007). It is possible that the antioxidant activity
233 present in the Lf components has modulated the Nrf2 / Keap1 pathway - nuclear
234 transcription factor, acting indirectly on oxidative stress and on the expression of the

235 transcription factor NFκB (Soares et al., 2015) acting on the tissue inflammation and
236 preparing the udder for colostrogenesis.

237 The animals that received LG05 presented the milk with high values of content of
238 total solids with an increase in all analyzed components.The composition of healthy
239 goat's milk varies on average: 3.07% fat (2.5% and 4.4%), 11.95% total solids (10.71%
240 and 12.44%), 9, 12% conferir non-fat solids (8.11% and 9.78%), 3.51% protein (2.97%
241 and 4.26%) (Dozet, 1973; Ramos & Juaréz, 1981).The chemical composition of milk
242 varies during the period lactation (Guo, 2001), at the end of lactation the fat, protein and
243 minerals increase while the lactose content decreases (Haenlein, 2004). The percentage
244 of lactose in the milk can indicate indicates that the treatment was able to eliminate
245 pathogenic microorganisms, since lactose is the component most consumed during
246 microbial action, turning into lactic acid.

247

248 **Conclusions**

249 The use of intramammary gel with 0.5% of lactoferrin bovine (Lf) resulted in
250 antimicrobial activity, antioxidant activities during colostrogenesis and lactogenesis, and
251 promoted immunity active in colostrum and increased total milk solids of Saanen goats.

252

253 **Declaration of Competing Interest**

254 The authors report no declarations of competing interest.

255

256 **Acknowledgements**

257 We thank National Council for Scientific and Technological Development
258 (CNPq) for scholarship.

259

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381

382 **Table 1**

383 Unfolding the interactions between intramammary lactoferrin gel and DPPH radical
384 scavenging (%) and ABTS radical scavenging (%)stored during 28 days at 10°C.

385

Gel	Days of storage				*P*
	1	7	14	28	
DPPH					
NLG	7.08±1.06^{B}	7.12±0.11^{B}	7.12±0.15^{B}	7.09±0.10^{B}	0.099
LG05	21.39±2.14^{A}	21.33±1.00^{A}	22.06±0.88^{A}	21.13±1.14^{A}	0.193
P	<0.001	<0.001	<0.001	<0.001	
ABTS					
NLG	10.52±0.12^{cB}	10.55±1.04^{abB}	11,02±0.33^{aB}	10.87±0.13^{bB}	0.033
LG05	31.10±1.48^{A}	31.06±1.04^{A}	32.04±0.95^{A}	31.13±0.04^{A}	0.190
P	<0.001	<0.001	<0.001	<0.001	

386 Different lowercase letters on the same line indicate a significant difference between days
387 (*P* <0.05). Different capital letters in the same column indicate a significant difference
388 between treatments (*P* <0.05). NLG: non lactoferrin added in gel; LG05: 0.50%
389 lactoferrin added in gel.
390

391 **Table 2**

392

393 Unfolding the interaction between intramammary lactoferrin gel and days of the

394 *Escherichia. coli* colony forming units (CFU) from goats' teats swab (n=18)

395 analaysis during 28 days of experiment..

396

	Escherichia coli (CFU)					
	Days of treatment					
Gel	0	1	7	14	28	*P*
NLG	70.2±1.08	65.8±2.10^{A}	65.9±0.05^{A}	64.8±2.11^{A}	63.4±2.25^{A}	0.070
LG05	68.9±1.22	47.3±1.90^{aB}	45.8±0.66^{bB}	42.7±1.05^{abB}	36.0±1.50^{cB}	0.001
P	0.082	<0.001	<0.001	<0.001	<0.001	

397 Different lower case letters on the same line indicate a significant difference between
398 days (*P* <0.05). Different capital letters in the same column indicate a significant
399 difference between treatments (*P* <0.05). NLG: non-Lacteferrin added in gel; LG05:
400 0.50% Lactoferrin added in gel.

401 **Table 3**

402

403 Intramammary lactoferrin gel effect in the goats' teats on the colostrum composition from

404 12 hours postpartum.

405

	NLG	LG05	*P*
Fat content (%)	4.97±0.88	6.08±0.13	0.036
Protein content (%)	5.75±0.12	9.12±0.09	<0.001
Immunoglobulins (%)	18.16±0.01	25.01±0.01	0.034

406 ($P<0.05$) indicate a significant difference between treatments. NLG: non-Lactoferri
407 added in gel; LG05: 0.50% Lactoferrin added in gel.

408 **Table 4**

409

410 Effect of the intramammary lactoferrin gel in goats' teats on the milk composition from

411 7 days postpartum.

412

	NLG	LG05	*P*
Fat content	2.57±0.35	3.68±0.53	0.044
Protein content	2.22±0.09	3.41±0.20	0.002
Non-fat solids	8.08±1.03	9.02±0.94	0.039
Lactose	4.47±0.84	4.53±0.44	0.082
Total solids	9.65±1.22	11.7±1.03	0.009

413 The results of the milk composition by EkoMilk are expressed as a percentage (%). ($P<0.05$)
414 indicate a significant difference between treatments. NLG: non lactoferrin added in gel;
415 LG05: 0.50% Lactoferrin added in gel.

VI. CONSIDERAÇÕES FINAIS

Os experimentos propostos demonstraram resultados positivos, comprovando a eficiência dos compostos bioativos na prevenção e cura de lesões de manejo e enfermidades atuando no controle de microrganismos patógenos, cicatrização de lesões e melhoria na qualidade de vida e bem-estar animal.

VII. APÊNDICES

a. Guide for authors – Research in Veterinary Science

b. Guide for authors – Small Ruminant Research

RESEARCH IN VETERINARY SCIENCE

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AUTHOR INFORMATION PACK

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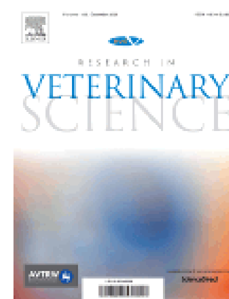

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SMALL RUMINANT RESEARCH

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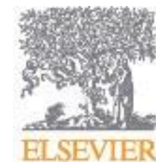

AUTHOR INFORMATION PACK

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Small Ruminant Research publishes original, basic and applied research articles, technical notes, and review articles on research relating to **goats**, **sheep**, **deer**, the **New World camelids llama**, **alpaca**, **vicuna** and **guanaco**, and the **Old World camels**.

Topics covered include nutrition, physiology, anatomy, genetics, microbiology, ethology, product technology, socio-economics, management, sustainability and environment, veterinary medicine and husbandry engineering.

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