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Efeito no curto prazo do tratamento de doenças digitais sobre a locomoção e atividades comportamentais de vacas leiteiras com claudicação severa

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EFEITO NO CURTO PRAZO DO TRATAMENTO DE DOENÇAS DIGITAIS SOBRE A LOCOMOÇÃO E ATIVIDADES COMPORTAMENTAIS DE VACAS LEITEIRAS COM CLAUDICAÇÃO SEVERA[1]

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RESUMO

A claudicação é a expressão clínica de dor ou desconforto e é uma das principais, se não a principal limitação à questão de bem estar animal em pecuária leiteira, mundialmente. Este trabalho objetivou verificar se o tratamento de doenças digitais de vacas com claudicação severa melhora, em curto prazo, a locomoção e o comportamento. Foram utilizadas 34 vacas leiteiras com claudicação severa. Escores de locomoção foram conferidos às vacas, no dia anterior e no 6º dia após o tratamento. As observações comportamentais foram realizadas no dia anterior e um, seis e oito dias após o tratamento de doenças digitais. As vacas foram observadas durante a parte diurna do dia para estimar as proporções de tempo gasto nas atividades ingestivas: ruminação, alimentação, sem atividade mastigatória (descansando) e em atividades posturais: deitadas e em pé. Os dados comportamentais foram submetidos à análise de variância, procedimento Mixed do SAS ®, de acordo com um delineamento experimental inteiramente casualizado com medidas repetidas no tempo, considerando-se no modelo o efeito de dia, raça, tipo de lesão, idade, dias em lactação, posição e número de membros com lesão. O tratamento melhorou o escore de locomoção uma semana após o tratamento, mas não houve alterações nas proporções dos tempos despendidos para as atividades comportamentais observadas. Embora o tratamento tenha melhorado o escore de locomoção, o comportamento animal não foi alterado.

Palavras-chave: claudicação, comportamento, escore de locomoção, vacas leiteiras

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SHORT TERM EFFECT OF TRIMMING ON THE LAMENESS SCORE AND BEHAVIOR OF DAIRY COWS SEVERELY LAME[2]

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ABSTRACT

Lameness can cause discomfort and pain which may impair behavior and welfare. This study aimed to verify if the corrective trimming on cows with severe claudication improves the locomotion and behavior. Thirty-four lactating Jersey and Holstein cows with severe lameness problems were used. Cows were scored for lameness on day -1 (previous) and 6 after trimming according to a 5-point scale, while behavior was evaluated on days -1 (previous) and on days 1, 6 and 8 following trimming. Cows were observed during the diurnal part of the day and the proportions of time spent in ingestive behavior: ruminating, feeding and resting (no chewing activity) as well as in posture lying or standing were registered. Behavior data was submitted to variance analysis using the Mixed procedure of SAS® according to a completely randomized design with repeated measurements, considering in the model the effect of day, breed, type of lesion, age, days in milk, number and position of locomotion members with lesion. Lameness score was reduced one week after treatment. The treatment improved the lameness score a week after treatment, but there was no change in the proportions of time spent in the behavioral activities. Despite the improvement on lameness score, trimming did not change expressively the behavior of lactating cows in a short time period.

Key words: behavior, dairy cows, lameness, locomotion score

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

%	Percentagem
cm	centímetros
FDN	fibra em detergente neutro
h	hora/horas
ITU	índice de temperatura e umidade
Kg	Quilos
mm	Milímetros
MS	matéria seca
NDT	nutrientes digestíveis totais
ºC	graus Celsius
PB	proteína bruta
Tbs	temperatura de bulbo seco
UR	umidade relativa do ar

CAPÍTULO I

1. Introdução Geral

Houve grande intensificação no melhoramento genético e em técnicas de manejo de vacas leiteiras, principalmente nas últimas décadas. Tais avanços foram motivados pelo desejo de aprimoramento da capacidade produtiva dos rebanhos. No entanto, características ligadas à conformação de pés e pernas não foram selecionadas com a mesma intensidade e, aliado a esse fato, muitos produtores passaram a utilizar instalações do tipo *free-stall* ou galpões para arraçoamento, deixando os animais em grande parte do tempo sobre pisos de concreto, aumentando, assim, a ocorrência de problemas locomotores.

A claudicação tem origem multifatorial, podendo ser agravada de acordo com as características do ambiente onde o animal é mantido, como pisos de concreto, excesso de umidade, acúmulos de lama e esterco, ou presença de pedras e espinhos. Há também fatores nutricionais como dietas com baixo teor de fibras e outras práticas inadequadas como falta de casqueamento preventivo do rebanho. A consequência das desordens locomotoras se reflete em perdas econômicas, já que, após problemas reprodutivos e casos de mastite, as doenças digitais ocupam a terceira posição entre as causas de prejuízos associados com inadequações da sanidade dos rebanhos, inclusive abate involuntário e descarte de animais.

Lesões nos dígitos podem causar dor e desconforto, modificando os padrões comportamentais da espécie e comprometendo o bem-estar animal. Muitos estudos comparam animais claudicantes e sadios com o objetivo de entender as diferenças comportamentais causadas por distúrbios locomotores. Atualmente grande parte das doenças digitais que acometem rebanhos leiteiros tem sua etiologia conhecida.

É preciso avaliar o comportamento dos animais com distúrbios na saúde, pois estes apresentam um comportamento caracterizado por menor ingestão de alimento e água, menor tempo de ruminação e tendem a permanecer parados, poupando energia. Estes comportamentos alterados ocorrem em resposta às infecções ou lesões teciduais e são chamados de comportamento em resposta à doença. Necessita-se, portanto, de um entendimento mais aprofundado sobre as alterações comportamentais e/ou dos padrões fisiológicos que ocorrem nos animais claudicantes.

2. Revisão Bibliográfica

2.1 O Casco bovino

O casco dos bovinos é formado por tecido epidérmico queratinizado, rígido, e pode ser divido em regiões de acordo com constituição, localização e função (Figura 1): talão, sola, muralha, pinça e linha branca. O grau de dureza dessas regiões também varia, sendo a muralha aquela de maior dureza, seguida por sola, talão e linha branca (RAVEN, 1989). A queratina, também chamada de tecido cornificado, é composta por diversos aminoácidos, em especial o aminoácido sulfurado metionina, e cerca de 30% de água, 1% de minerais e alguns ácidos graxos. Alterações nessa composição, como por exemplo, a dessecação do tecido, podem levar ao surgimento de fissuras (FEITOSA, 2008).

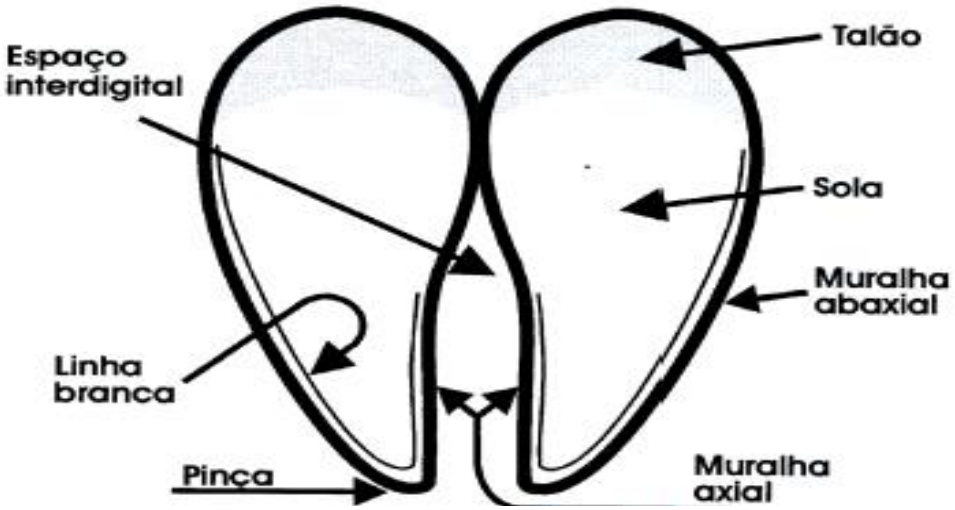

Figura 1. Regiões anatômicas de um casco bovino. Adaptado de Ferreira et al. (2005).

O crescimento do casco ocorre a partir do cório coronário, região próxima à coroa do casco, primeiro na sua porção abaxial e em seguida na sua porção axial (FEITOSA, 2008), e esses queratinócitos recém-formados pela epiderme coronária são empurrados para as porções mais distais do dígito (SAMUELSON, 2007). O casco cresce cerca de 5 mm por mês em condições adequadas de umidade e temperatura, com maior taxa de crescimento durante o verão e menor taxa de crescimento durante o inverno (FEITOSA, 2008).

A sola apresenta uma composição histológica semelhante à da muralha, mas com maior porcentagem de água e menor espessura, o que facilita a ocorrência de perfurações normalmente causadas por objetos pontiagudos presentes nos caminhos e ambientes das vacas, provocando a exposição do cório sensitivo, hemorragia e claudicação (FEITOSA, 2008).

Entre a muralha do casco e a sola há uma estrutura delimitada, de cerca de 2 mm de largura, denominada de linha branca, que representa a união do epitélio laminar da muralha com a sola do casco (FEITOSA, 2008). A linha branca apresenta um nível de compactação e dureza distintas dos tecidos adjacentes (é mais macia), além de uma pigmentação diferente, permitindo,

dessa forma, que a muralha seja visualmente distinguida da sola (SAMUELSON, 2007). Devido à sua consistência mais macia, lesões na região da linha branca são comuns (FEITOSA, 2008).

2.2 Claudicação e Escore de Locomoção

A claudicação é uma alteração na marcha dos animais, geralmente relacionada à presença de lesões, ferimentos ou situações de desconforto. Segundo Rosenberger (1993), para a detecção de quaisquer alterações na marcha dos animais, utiliza-se uma superfície dura e se observa eventuais anormalidades na elevação e no apoio dos membros – quando essas movimentações são encurtadas em algum dos membros, caracteriza-se uma claudicação.

A claudicação não é uma doença, mas um sinal clínico de uma afecção de origem multifatorial (GREENOUGH, 2007) que pode estar relacionada à condição fisiológica e sanitária do animal, ao ambiente, gerando consequências para o rebanho. Vacas no estádio inicial de lactação são mais propensas a desenvolver o problema e a média de perdas pode ser de 360 kg de leite em um período de 305 dias de lactação (GREEN et al., 2002). A ordem de parto também é um fator relacionado à claudicação – vacas de primeiro parto são mais propensas a desenvolver o problema, o que pode ter relação com as mudanças no metabolismo que costumam ocorrer nesse período de alterações fisiológicas importantes (WEBSTER, 2001). Ainda, vacas que já foram claudicantes na primeira lactação têm maiores chances de reapresentar o problema (HIRST et al., 2002). As condições ambientais, como a presença de sujidades e umidade, são fatores de risco para o surgimento de lesões, com importância relevante na sua etiologia (VERMUNT & GREENOUGH, 1994).

A claudicação pode ser classificada em graus conforme a severidade do distúrbio, variando de 1 para vacas sadias a 5 no caso de vacas com claudicação severa (Tabela 1). Na claudicação de grau 5, por exemplo, o animal evita apoiar o peso sobre o membro afetado (ROSENBERGER, 1993). A observação da marcha dos animais e a atribuição de escores de locomoção para cada indivíduo é um método amplamente utilizado para distinguir animais com problemas de locomoção antes mesmo que lesões nos cascos possam ser identificadas através do casqueamento (BICALHO et al., 2007; CHAPINAL et al., 2009). No entanto, para que a utilização de determinada escala possa ser eficiente, é necessário o estudo e o treinamento dos observadores.

Além da observação do escore de locomoção, há outras maneiras para se avaliar a marcha dos animais, como a observação da simetria da caminhada e distribuição do peso sobre os membros em esteiras de pressão (VAN NUFFEL et al., 2013), e a força de reação do solo em três dimensões (THORUP et al., 2014), mas a utilização desses métodos, no entanto, requer maior aparato tecnológico e recursos financeiros. Norring et al. (2014) destacam que a implementação de novas tecnologias aplicadas a um grande número de animais, juntamente com o aumento do conhecimento sobre a etiologia da claudicação, irá facilitar a compreensão, a prevenção e a cura da doença.

Tabela 1. Critérios utilizados para atribuição um grau de claudicação

Escore de Locomoção	Descrição	Critérios de avaliação
1	Movimento suave e fluido	Coluna plana. Cabeça firme. Marcha simétrica. Todas as pernas suportam igualmente o peso.
2	Locomoção imperfeita. Pode mover-se livremente	Coluna plana ou levemente arqueada e cabeça firme. Marcha levemente assimétrica. Todas as pernas suportam igualmente o peso.
3	A capacidade de mover-se livremente está comprometida	Coluna arqueada. Cabeça firme. Leve claudicação pode ser percebida.
4	Capacidade de mover-se livremente está obviamente diminuída	Coluna evidentemente arqueada. Cabeça balança levemente. Marcha assimétrica. Animal relutante em suportar o peso sobre pelo menos um membro, mas ainda usa esse membro para se locomover.
5	Capacidade de locomoção é severamente restrita e deverá ser forçada a se mover.	Coluna extremamente arqueada. Evidente balanço de cabeça. Passos curtos, hesitantes e cuidadosos. Marcha assimétrica e há incapacidade de suportar o peso de um ou mais membros.

Adaptado de Flower & Weary (2006).

2.3 Afecções podais

O aumento da produtividade na bovinocultura associado ao manejo mais intensivo dos meios de produção levou a um aumento na frequência e intensidade das enfermidades do aparelho locomotor (FEITOSA, 2008). A saúde dos membros locomotores é pré-requisito para a manutenção do bem-estar, tendo em vista os prejuízos causados por sinais clínicos, como, por exemplo, a dor: redução da locomoção e da ingestão de alimentos (CRUZ & DRIEMEIER., 2007). Os membros podem ser afetados de diferentes formas: diretamente, como uma fonte originária de doenças independente ou não de outros sistemas, e através de problemas generalizados (nutricionais ou metabólicos) (ROSENBERGER., 1993).

Essas afecções ocorrem principalmente em propriedades com condições ambientais inadequadas, o que não está relacionado, necessariamente, a uma infraestrutura insificiente, mas certamente, a técnicas de manejo equivocadas. As doenças digitais podem levar à queda de produtividade, descarte e infertilidade (CRUZ & DRIEMEIER, 2007). As principais doenças do dígito podem ser classificadas em três grupos, de acordo com a localização anatômica: infecciosas (dermatites digital e interdigital e necrobacilose interdigital), laminite e suas sequelas (úlcera de sola, úlcera de pinça e doenças da linha branca) e doenças digitais de etiologia incerta ou secundárias (hiperplasia interdigital, pododermatite séptica, fissuras horizontal e vertical, pododermatite do paradígito e erosão do talão) (RIET-CORREA et al., 2007).

2.3.1 Doenças infecciosas do dígito

As dermatites digital e interdigital juntamente com a necrobacilose interdigital são as principais doenças digitais infecciosas que afetam os bovinos, com importância econômica ligada aos custos do tratamento e às perdas de desempenho produtivo e reprodutivo (CRUZ & DRIEMEIER, 2007). A primeira descrição da dermatite digital ocorreu na década de 70, na Itália (CHELI & MORTELLARO, 1974), e, atualmente, é descrita como uma doença contagiosa, de distribuição universal e possivelmente causada por sucessivas colonizações por micro-organismos anaeróbicos, dos quais o gênero *Treponema sp* tem sido o mais consistentemente identificado CRUZ & DRIEMEIER, 2007; PRINGLE et al., 2009). Esta lesão geralmente ocorre entre os talões, superfície plantar e pode também atingir a pele da margem coronária (PAVLENKO et al., 2011). Em grande parte dos isolamentos de casos de dermatite interdigital, há a presença dos agentes sinérgicos *Dichelobacter nodosus* e *Fusobacterium necrophorum* (RISCO, 2011), de modo que, este último também é descrito como agente causador da necrobacilose interdigital (GREENOUGH & WEAVER, 1997). Ainda que ocorram em locais distintos e com diferentes formas, lesões causadas por dermatites digital e interdigital compartilham algumas características clínicas, como áreas erosivas e proliferativas, o que pode dificultar sua identificação (Cruz et al., 2005). Algumas medidas como menor tempo de exposição à umidade, lama ou esterco, aliadas ao acesso a locais confortáveis e secos de descanso, redução do tempo de contato com superfícies irregulares e/ou objetos pontiagudos e menor número de animais por lote são descritas como medidas profiláticas eficientes para este grupo de doenças (CRUZ & DRIEMEIER, 2007).

2.3.2 Laminite e sequelas

As causas da laminite asséptica não estão totalmente esclarecidas, sendo descrita como de origem multifatorial, ligada a fatores fisiológicos e também externos ao animal como, por exemplo, nutrição, manejo, higiene, bem-estar animal, interações sociais e rotina de casqueamento. Úlcera de sola, úlcera de pinça e doença da linha branca são as principais sequelas deste distúrbio (RIET-CORREA et al., 2007).

Dentre as sequelas da laminite, a úlcera de sola é a mais comum e de maior importância econômica (DIAS & MARQUES JÚNIOR, 2003). Úlceras de sola são lesões dolorosas e causam desconforto, podendo levar a claudicação e alterações do comportamento natural do animal. O aparecimento dessas lesões pode estar relacionado à distribuição desigual do peso corporal sobre as diferentes regiões do casco bovino, de modo que, com animais em estação, maiores pressões são exercidas sobre as unhas laterais posteriores e mediais anteriores, regiões conhecidas como mais sensíveis à ocorrência de lesões (GREENOUGH & WEAVER, 1997; VAN DER TOL ET AL., 2002). A úlcera de pinça tem etiologia semelhante à úlcera de sola, sendo o Uruguai um dos poucos lugares em que ocorrem maiores casos de úlcera de pinça em relação a úlceras de sola, resultado observado devido aos altos índices de laminite e as médias ou longas distâncias percorridas pelos animais sobre solos duros e ásperos (RIET-CORREA et al., 2007).

Em animais com laminite, pode ocorrer a separação da união entre a sola e muralha do casco, formando uma rachadura. A doença da linha branca surge quando há a penetração de fezes ou corpos estranhos nesse local e infecção dos tecidos da parte interior da unha. Embora tenham diferentes sintomas e etiologias, medidas como a redução da exposição do casco a locais úmidos e com presenças de sujidades, assim como realização de casqueamento corretivo dos animais e remoção dos tecidos descolados ou necrosados (de acordo com a doença) são recomendados como medidas profiláticas e curativas destas enfermidades (DIAS & MARQUES JÚNIOR, 2003; RIET-CORREA et al., 2007).

2.4 Comportamento animal

Grande parte dos trabalhos que descrevem padrões de comportamento foi realizada com animais hígidos, porém, animais enfermos e sadios têm diferentes prioridades, de modo que alterações fisiológicas e comportamentais em animais com infecções agudas ocorrem com o intuito de facilitar a sobrevivência do hospedeiro durante a infecção ou lesão tecidual (HART, 1988), ou seja, a reorganização das prioridades comportamentais em animais doentes é uma estratégia de defesa contra patógenos (JOHNSON, 2002). Animais com lesões teciduais ou processo inflamatório têm um comportamento distinto, chamado de comportamento doentio (em inglês, *sickness behavior*). Essas modificações podem ser representadas por redução no tempo de alimentação e ruminação, além de maior tempo de ócio (ALMEIDA et al., 2008; FOGSGAARD et al., 2012), ou seja, os animais tendem a permanecer maior tempo inativos na tentativa de economizar as reservas de energia necessárias para o aumento dos custos metabólicos da febre (Hart, 1988). Um melhor conhecimento do comportamento doentio em bovinos pode auxiliar a detecção de doenças em fases iniciais, especialmente quando os animais são alojados em grandes grupos (KEYSERLINGK et al., 2009).

No entanto, os reflexos dos problemas locomotores podem se manifestar de diferentes formas, de modo que alterações no comportamento não foram relatadas somente em casos de claudicação (ITO et al., 2010; BLACKIE et al., 2011), mas foram influenciadas devido ao número de partos (BRZOZOWSKA et al., 2014; MIGUEL-PACHECO et al., 2014), idade (PÉREZ-CABAL & ALENDA., 2014), estádio de lactação (PALMER et al., 2012.; NORRING et al., 2014) e estação do ano (JUAREZ et al. 2003; BRZOZOWSKA et al. 2014).

Animais claudicantes podem modificar o comportamento ao longo do dia, evitando conflitos (BLACKIE et al., 2011) e alterando os tempos de permanência em pé *(standing behavior)* e deitado *(lying behavior)*, e esses são parâmetros mais comumente empregadas e como indicadores de conforto e saúde (ZOBEL et al., 2015). Vacas claudicantes apresentam diferentes condutas quando comparadas a animais saudáveis: Hassall et al. (1993) observaram que animais claudicantes foram os últimos a entrar na sala de ordenha devido à menor velocidade da marcha. No estudo de Blackie et al. (2011), houve acréscimo de duas horas no tempo deitado de vacas em lactação com escore de locomoção EL=3 quando comparadas a animais com EL= 1 e 2.

A observação do tempo em que os animais permanecem deitados pode fornecer informações sobre o estado sanitário do plantel, e Juarez et al. (2003) afirmam que, apesar de condições de claudicação grave, no verão as vacas permaneceram deitadas durante apenas 20% do período de observação como uma estratégia para dissipação do calor. Da mesma forma, Ito et al. (2010) alegam que a permanência em pé durante períodos superiores a 14,5 horas/dia está associada à maior chance de ocorrência de claudicações severas. Por outro lado, Blackie et al (2011) demonstram que, quando comparadas a animais saudáveis, vacas claudicantes permaneceram deitadas por maior tempo e com redução significativa do tempo em pé. Já Chapinal et al. (2009) não observaram diferença no tempo deitado de animais com dermatite digital ou hemorragia de sola quando comparados a animais sem lesões. Juarez et al. (2003) afirmam que o ato de permanecer deitada é um mecanismo pelo qual as vacas aliviam a dor causada pela sustentação do peso. No entanto, Ito et al. (2010) afirmam que o tempo deitado, por si só, não é uma medida eficaz para obtenção de diagnósticos de claudicação, mas Yunta et al. (2012) mostram que modificações no tempo deitado em torno do período de fornecimento de alimentação suplementar podem ser eficientes para identificar vacas com claudicação moderada.

Alguns autores também relataram que a presença de claudicação pode provocar a diminuição de ingestão de matéria seca (PALMER et al., 2012) e, ainda, causar a modificação dos indicadores comportamentais relacionados à alimentação desses animais. Miguel-Pacheco et al. (2014) relataram a redução dos tempo de alimentação e ruminação de vacas claudicantes. Norring et al. (2014) relataram que a diminuição do tempo de alimentação observada em vacas com claudicação severa (escores 4 e 5) pode ser uma estratégia para reduzir a dor causada no membro durante a alimentação. Por fim, Blackie et al. (2011), sugerem que, para a detecção de claudicação em gado leiteiro, as diferenças nos tempos de comportamento deitado, de alimentação e ruminação podem ser utilizadas não de forma isolada, mas sim em conjunto com outras medidas.

2.5 Tratamento de doenças digitais

O casqueamento rotineiro de bovinos leiteiros - não somente de vacas lactantes, mas de todas as categorias do rebanho - é um manejo que pode ter fins profiláticos, curativos ou estéticos. Van der Tol et al. (2002) afirmaram que a distribuição da pressão do peso corporal não ocorre de forma uniforme sobre o casco, de modo que pode haver uma relação causal entre as regiões onde há maior pressão e a ocorrência de lesões nos cascos. O casqueamento promove aumento da área de contato do casco com o solo, resultando em melhor distribuição de peso nas patas (ZEINER et al., 2007) e, após a realização deste manejo, há melhora também na simetria da caminhada entre as pernas direita e esquerda, indicando efeito positivo do tratamento (THORUP et al., 2014).

Van Hertem et al. (2014) concluíram ter o casqueamento provocado efeito prolongado de até 70 dias sobre o escore de locomoção. No entanto, no mesmo estudo, houve aumento da pontuação de locomoção logo após o casqueamento, sugerindo ter este manejo causado desconforto ou dor. De

acordo com Dyer et al. (2007), ao se removerem os tecidos lesionados do casco, há um aumento na sensibilidade dos receptores de dor por pressão externa, diminuindo o limiar de dor após o casqueamento. Deste modo, é provável que o apoio desse membro no chão cause dor e, portanto, o animal pode apresentar mudanças comportamentais e na marcha imediatamente após o casqueamento.

O tratamento da doença infecciosa mais comum em rebanhos leiteiros, a dermatite digital, é realizado após a limpeza da região lesionada, com a aplicação local de antibióticos e proteção com bandagem. Este procedimento é reconhecido como o mais eficaz, desde que a retirada da bandagem ocorra em, no máximo, 72 horas após a realização do tratamento. A utilização de pedilúvios com antimicrobianos é uma alternativa de controle para rebanhos com baixa prevalência (CRUZ & DRIEMEIER, 2007).

O controle da úlcera de sola é feito retirando o tecido córneo descolado e casqueamento da unha afetada. Deve-se evitar que o animal apoie o peso corporal sobre a unha lesionada, assim, a utilização de tacos ortopédicos ou botas especiais que impedem o contato da sola lesionada com o terreno é a forma de tratamento eficaz (RIET-CORREA et al., 2007).

3. Hipótese e Objetivos

Hipótese

- A claudicação causa dor e desconforto em vacas lactantes, o que leva à modificação no comportamento animal, com menores tempos de pastejo e ruminação, comportamento observado em animais enfermos. O tratamento das lesões digitais melhora a marcha dos animais, reduz o escore de locomoção e modifica favoravelmente o comportamento dos animais, minimizando os sinais de desconforto.

Objetivo Geral

- Identificar e correlacionar os comportamentos influenciados pelas doenças digitais e verificar se o tratamento dos dígitos afetados altera no curto prazo o comportamento de vacas lactantes.

Objetivos Específicos

- Avaliar se o tratamento dos dígitos afetados melhora a marcha de vacas com problemas severos de claudicação até oito dias após o tratamento;
- Avaliar atividades relacionadas ao comportamento social e ingestivo de vacas claudicantes antes e após o tratamento das lesões digitais.

CAPÍTULO II
EFEITO NO CURTO PRAZO DO TRATAMENTO DE DOENÇAS DIGITAIS SOBRE A LOCOMOÇÃO E COMPORTAMENTO DE VACAS LEITEIRAS COM CLAUDICAÇÃO SEVERA[3]

[3] Artigo a ser enviado ao Journal Animal (Cambridge, online)

Short term effect of trimming on the lameness score and behavior of severely lame dairy cows

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Pre and post trimming behavior of lame dairy cows

Implications

Digital diseases are among the major causes of losses in dairy herds. Digital injuries can cause behavioral and physiological changes and reduction of animal welfare due to associated pain or discomfort. The observation of the animals is a tool used to detect behavioral changes caused by discomfort situations. This study aims to study the short-term effect of treatment of digital lesions on the behavior and locomotion of severely lame dairy cows.

Abstract

Lameness can cause discomfort and pain which may impair behavior and welfare. This study aimed to verify if the corrective trimming on cows with severe claudication improves the locomotion and behavior. Thirty-four lactating Jersey and Holstein cows with severe lameness problems were used. Cows were scored for lameness on day -1 (previous) and 6 after trimming according

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to a 5-point scale, while behavior was evaluated on days -1 (previous) and on days 1, 6 and 8 following trimming. Cows were observed during the diurnal part of the day for the proportions of time spent in the ingestive behavior: ruminating, feeding and resting (no chewing activity) as well as in posture: lying down and standing. Behavior data was submitted to variance analysis using the Mixed procedure of SAS® according to a completely randomized design with repeated measurements, considering in the model the effects of day, breed, type of lesion, age, days in milk, number and position of locomotion members with lesion. Lameness score was reduced one week after treatment. The treatment has improved the lameness score a week after treatment, but there was no change in the proportions of the time spent in the behavioral activities. Despite the improvement on lameness score, trimming did not change expressively the behavior of lactating cows in a short time period.

Key words: behavior, dairy cows, lameness, locomotion score, trimming

Introduction

Lameness is a change in the gait of animals, usually related to the presence of lesions which lead to pain or discomfort. Some conditions such as higher milk production in early lactation (Green *et al.*, 2002.; Bicalho *et al.*, 2008), low body condition score during the periparturient period (Hoedemaker *et al.*, 2009), age (Dippel *et al.*, 2009) and environment (Cook & Nordlund, 2009) may enhance the occurrence of lameness in the herd.

Lameness is not a disease but a clinical sign of various diseases affecting the digits and may impair locomotion at different levels of severity,

affecting animal welfare (Webster, 2005; Greenough, 2007). The presence of inflammation or tissue damage trigger the sickness behavior (Almeida *et al.*, 2008; Fogsgaard *et al.*, 2012), which is characterized by reducing food intake and modification in social behavior (Bareille *et al.*, 2003; Cook & Nordlund, 2009; Palmer *et al.*, 2012). Lesions on the limbs can result in lower milk production (Green *et al.*, 2002; Juarez *et al.*, 2003; Bicalho *et al.*, 2008), and reduced fertility and increased risk of premature culling (Hernandez *et al.*, 2001; Garbarino *et al.*, 2004; Bicalho *et al.*, 2007).

The gait score of animals for each individual is a widely used method to identify animals with mobility problems (Bicalho *et al.*, 2007; Chapinal *et al.*, 2009). There are factors as type of lesion, age, days in milk, number and position of locomotion members with lesion which may influence the behavior after treatment. The objective of this study was to determine whether the treatment of digital lesions of dairy cows improve gait and behavioral activities in a short period of time.

Material and methods

Location and duration of the experiment

This study was approved by the Ethics Committee on Animal Use the Federal University of Rio Grande do Sul, number 25875, within the project "Pain assessment in lame cows and its relationship to behavior, milk production and its physical and chemical characteristics". The experiment was conducted in the Farm VB, dairy farm located at BR390 road, km 135, Eldorado do Sul - RS

(latitude 30º04'22.35 "S and longitude 51º35'47.85" O), from January 2014 to May 2014.

Animals and housing

Thirty-four lactating cows were used: 28 Holstein and six Jersey cows, aged of 4 ± 4 years, presenting average body weight of 616 ± 189 kg, DIM 192 ± 208 and calving number of 2 ± 4 births. Cows were milked twice daily, at 5:00 h and 15:00 (GMT -3: 00), in a herringbone type milking parlor. The animals stayed on a grass paddock measuring approximately 1.6 ha with free access to water, shade and Tifton pasture (*Cynodon* spp). Supplementation was provided in the trough *ad libitum* twice daily immediately after milking, and it was comprised by about 25 kg of corn silage; 1.5 kg of hay, 2.0 kg of soybean hulls, and 9 kg of commercial concentrate and mineral mixture. The composition of the total mixed ration was 38.8% DM; 14.8% CP; 43.6% NDF and 70.4% TDN.

Hoof trimming and locomotion scoring

The identification of lame cows occurred on their way to the milking parlor. The locomotion score was assessed on two occasions: the day before the first behavioral assessment and after a week. Attribution of scores was performed by trained observers, who standed at a distance of approximately 3 meters of animals. Cows walked on a concrete floor with grooves and were gait scored using a 1-to-5 numerical rating system where 1 = perfect gait and 5 = severely lame (Flower & Weary, 2006). Severely lame cows (locomotion scores,

LS, 4 and 5) were identified and allocated to a separate paddock from the other cows while healthy animals or those gait scored with 3 or less were not used. The cows were restrained in a tilt table for claw trimming. The tilt table was tilted from a vertical to a horizontal position, cows lying on their right flank. The veterinarian of the farm diagnosed the lesion and applied the corrective treatment on the injured digits. Animals affected with digital dermatitis were treated with local application of antimicrobial and protection of the lesion with a band, which was removed after seven days. Cows with sole ulcers were treated with the placement of orthopedic shoes.

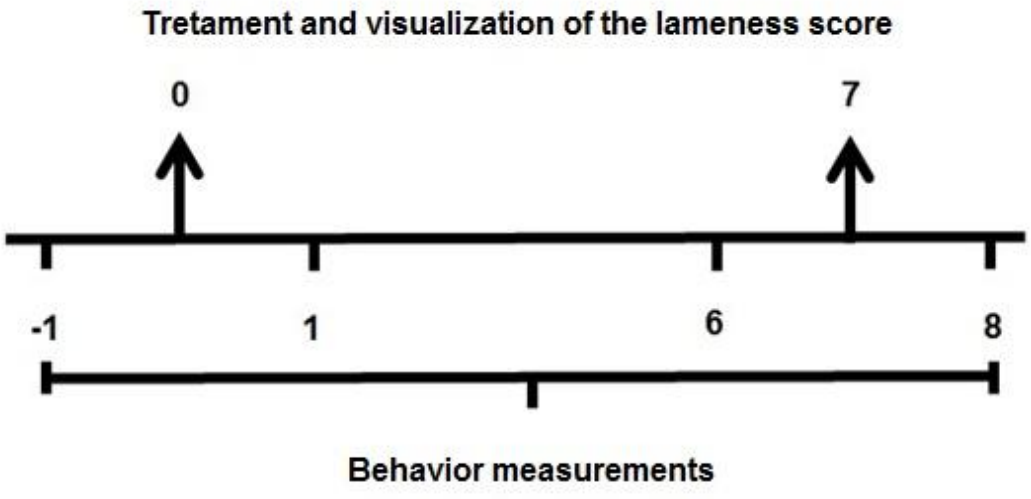

Figure 1. Layout of development activities throughout the experiment for each cow enrolled in the trial

Behavior measurement

Behavioral observations were performed on day -1 (pre-treatment) and on days 1, 6 and 8 after the treatment. The evaluation of time spent standing, lying down, ruminating, resting and feeding were estimated by visual observation at ten minute intervals. Behavior was not registered during periods

of displacement between the paddock and the milking parlor and during milking activities. Behavioral attributes were recorded from sunrise to sunset, with average of 720 minutes a day, starting between 6:30 and 7:30 am and finishing around 6:00 pm and 7:30 pm. As the length of daytime evaluation period changed from the beginning and end of the experiment due to the variation of the photoperiod, the activity times are expressed as a percentage of the total evaluation time.

Table 1. Description of the activities seen in the ethogram

Activity	Description of the activity
Lying	Proportion of time spent lying down with the left or right flank touching the ground
Standing	Proportion of time spent standing time (stand + walking + feeding), ruminanting or not.
Rumination	Proportion of time spent rumination
Repose	Proportion of time with no chewing
Feeding	Proportion of time spent feeding (grazing or feeding on the trough)

Measurement of climate variables

The measurement of temperature and relative humidity were performed at 30 minutes-interval during the daylight period with the use a portable globe thermometer Instrutemp, ITWTG-2000 model, placed at 1.5 meters above ground in the shaded area. The temperature and humidity index (THI) was calculated using the formula proposed by Johnson et al. (1962), wherein the temperature is measured in °C (Tdb) and the relative humidity is measured in % (RH): THI = (1.8 X Tdb + 32) - (0.55 - 0.0055 X RH) X (1.8 X Tdb - 26.8).

Statistical analysis

The design was the completely randomized with repeated measurements. Cows were considered as the experimental units. Descriptive analysis was performed initially to determine the frequencies of the classes of the attributes. In the evaluation of the factors influencing the locomotion score, the statistical model included the effects of: day (n = 2; previous day and sixth day after treatment), breed (n = 2; Holstein and Jersey), age (n = 3; one to three years, three to five years and more than five years), days in milk (n = 3; 1 to 100 days, 101-200 days and more than 200 days lactation), lesion (n = 2; dermatitis and laminitis sequels), the affected limb position (n = 2; front or hind), number of affected members (n = 2; one or two affected limbs). The average of THI at the evaluation day and the number of previous treatments at the time of evaluation were used at covariates. No interactions were evaluated between the main effects because there was not sufficient number of observations in all classes of the effects. The means were separated by Lsmeans option. The significance criterion was taken as $P < 0.05$.

In the evaluation of the factors that influenced the behavioral variables, the statistical model included the effects of: day (n=4; -1, 1, 6 and 8 days after the treatment), breed (n=2; Holstein and Jersey), age (n = 3; one to three years, three to five years and more than five years), days in milk (n = 3; 1 to 100, 101-200 and more than 200 days lactation), lesion (n = 2; dermatitis and laminitis sequels), the affected limb position (n = 2; front or hind), number of affected members (n = 2; one or two affected limbs). The average of THI at the evaluation day and the number of previous treatments at the time of evaluation

were used at covariates. No interactions were evaluated between the main effects because there was not sufficient number of observations in all classes of effects. The means were separated by Lsmeans option. The significance criterion was taken as $P < 0.05$ and tendency was taken as $P < 0.10$.

Results

Descriptive analysis showed that about 20% of the lactating herd presented lameness. Of those presenting LS equal or higher than 4 (n=34), 56% of the animals were in their first and second thirds of lactation, while the remaining 44% were lactating for more than 200 days. The selected group of severely lame cows was composed of 79% of animals with up to five years old and 21% of animals older than 5 years old; 97% of the injuries occurred in the hind limbs, while 3% of the injuries occurred in the forelimbs. Dermatitis was more prevalent lesion, with 74% of occurrence while laminitis sequels represented 26% of the affections. At the beginning of the experiment, due to the experimental protocol, only cow showing LS of four or five were enrolled, representing respectively 59% and 41% of the total. Seven days after treatment of the digital diseases, 65% of animals were scored lower or equal to three, indicating a significant improvement in gait.

Seven days after treatment, the overall mean of LS was reduced from 5 to 3.5 (Table 2). Despite the improvement in the gait score, cows did not show significant changes in their proportions of time spent in ingestive or posture behaviors.

Table 2. Proportion of time spent in behavioral activities of severely lame dairy cows evaluated before and on days 1, 6 and 8 after trimming and treatment of digital diseases

	Days relative to treatment				
Atributes	-1	1	6	8	Significance
LS	5.0[a]	nd	3,5[b]	nd	***
Lying[1]	56.3	54.3	57.2	56.7	ns
Standing[1]	43.7	45.7	42.8	43.3	ns
Rumination[1]	22.5	21.6	22.8	24.0	ns
Resting[1]	47.2	46.9	47.4	46.5	ns
Feeding[1]	30.3	31.5	29.8	29.5	ns

[1]Values are expressed as the proportion of time spent in each activity; *** P<0.0001; Nd = not determined; ns = not significant, [a,b] Different means on the same line differ by F test

Breed affected the proportion of time spent ruminating but it did not influence the LS, the proportions of time spent resting, feeding, standing and lying. Holstein cows showed higher proportion of the time spent ruminating than Jersey cows (Table 3). Age affected LS. Cows under 3-years old (group 1) presented higher LS than cow older than 5-years (group 3), and both were similar to the group of cows aged from 3 to 5 years (group 2).

Table 3. Mean values of the behavioral attributes evaluated in cows of the Holstein and Jersey breeds, with different ages and lactation stages

	Breed[2]			Age[3]				Days in milk[4]			
	H	J	*P*	1	2	3	*P*	1st	2nd	3rd	*P*
Locomotion score	4.5	4.1	ns	4.7[a]	4.3[ab]	3.9[b]	**	4.4	4.3	4.2	ns
Lying[1]	59.1	53.0	ns	69.8[a]	61.0[b]	37.5[c]	***	64.3[a]	52.3[b]	51.7[b]	**
Standing[1]	40.9	47.0	ns	30.2 [a]	39.0 [a]	62.5 [b]	***	35.7 [b]	47.7[a]	48.3[a]	*
Rumination[1]	26.7[a]	18.8[b]	**	24.5	23.1	20.6	ns	23.7[ab]	25.0[a]	19.5[b]	*
Resting[1]	44.2	49.9	ns	45.9	48.0	47.1	ns	49.0[ab]	42.8[b]	49.3[a]	†
Feeding[1]	29.1	31.3	ns	29.6	28.9	32.3	ns	27.3	32.2	32.1	ns

[1]Values represented by percentage of time;[2] Breed: Holstein = H, Jersey = J [3] According to the age groups: 1 (up to three years), 2 (three to five years) and 3 (over five years); [4] Days in milk: 1st (up to 100 days) 2nd (between 101 and 200 days), 3rd (over 200 days).*P<0,05 **P<0,01 ***P<0.0001; ns= not significant; [a,b,c] Different means on the same line differ by F test;

Cow aged of less than 3 years (group 1) and those aged from 3 to 5 years (group 2) lay down during 65% of the evaluation period while remained

standing for 2.5% to 11.6% in this period stations, respectively. On the other hand, the third group of cows remained lying and in station for 37.5% and 31.5% of evaluation period. Age of cows did not affect the proportion of time spent in rumination, resting and feeding.

Animals in the first third of the lactation (group 1) presented higher proportion of the time spent lying down but lower proportion of time spent standing up than cows at the middle or at the end of the lactation. Cows at the last third of the lactation had lower proportion of time spent in rumination but tended to present higher proportion of time without chewing than cows at the second third of the lactation. Stage of lactation did not affect the locomotion score.

The type of lesion, dermatitis or sequels of laminitis, did not affect the LS (Table 4) but tended to change the proportion of time spent lying down and standing, as animals with dermatitis showed lower proportion of time spent lying than animals with laminitis sequels, being the opposite for the proportion of time spent standing. Type of lesion did not change the ingestive behavior.

Table 4. Mean values of the behavioral attributes evaluated in cows of the Holstein and Jersey breeds, according to the type of injury, location and number of affected members

	Type of lesion[2]			Affected member			Number of injuried members		
	D	L	*P*	Front	Hind	*P*	1	2	*P*
Locomotion score	4.2	4.3	ns	5.0[a]	3.5[b]	*	4.1[b]	4.5[a]	*
Lying[1]	52.7[b]	59.4[a]	†	68.8[a]	43.4[b]	*	48.8[b]	63.4[a]	***
Standing[1]	47.3	40.6	†	31.2	56.6[a]	*	51.2[a]	36.6[b]	***
Rumination[1]	22.8	22.7	ns	25.1	20.4	ns	21.7	23.7	ns
Resting[1]	46.2	47.8	ns	40.0	54.0	ns	46.6	47.5	ns
Feeding[1]	31.0	29.5	ns	34.9	25.6	ns	31.7[a]	28.8[b]	**

[1]Values represented by percentage of time; [2] According to the kind of injury: D (Dermatitits) and L (laminitis sequels); † P<0.10 * P<0.05 ** P<0.01 *** P<0.0001; ns= not significant; [a,b,] Different means on the same line differ by F test;

Animals with lesions on the front limbs had higher LS and proportion of time spent lying but lower proportion of time spent feeding compared animals with lesions on the hind limbs. Animals with two limbs injured had higher LS and proportion of time spent lying compared to animals with lesions in just one limb. On the other hand, the number of injured members did not change the proportion of time spent in rumination and resting (no chews) behaviors.

Discussion

The high prevalence of lameness, 20% of the whole herd (about 500 lactating cows) is probably due to a combination of factors such as long time on concrete floor, which was most of the time dirty and wet and medium to high walking distance to and from the milking parlor, as the area of the farm was approximately 1000 ha. Cows spent about 4 to 5 hours per day walking and standing on a concrete floor, if we consider time in the waiting room before milkings and at the food trough after both milkings. The use of concrete floors has been identified as a risk factor for increased claw lesions and lameness (Vanegas *et al.,* 2006).

At the beginning of the trial, all animals used in this study presented lesions in the digits, and they were compared with themselves, pre and post treatment. The decrease in the locomotion score of severely lame cows by 1.5 units observed seven days after treatment confirms that treatment of digital lesions improves gait of the animals, due to healing of the infection and inflammation in the affected digits, which reduce the discomfort or pain (Thorup *et al.*, 2014). However, treatment did not change the proportion of time spent in

ingestive and postural behaviors, despite the expectations that after treatment cows would decrease the high proportion of time spent lying and would increase the proportion of time spent feeding.

We might suppose that one of the reasons we did not detect changes in the feeding behavior was that we have not registered the ingestive behavior during one of the main grazing periods, as our observation were performed from 7 am to 18 pm. Grazing cattle usually present three to five grazing periods distributed during the day, which vary according to the environment, behavioral adaptations and photoperiod (Gregorini, 2012); but, regardless of the frequency, the main grazing events for healthy dairy cows occur in the morning/early morning and late afternoon/early evening (Gibb et al., 1998). In the other side, feeding behavior comprised both time spent grazing and eating at the feed through, which we were able to fully registered. Eating at the trough corresponded to more than 60% of the time spent feeding. When we previously analyzed the eating behavior split into grazing and eating at the trough, none of them were different before and after treatment. We also could speculate that cows did not make much efforts to graze as they were in a ward paddock, with a restricted area (nearly 1.6 hectares), but with no limitation to intake as pasture mass was high, about 3.000 kg/ha and the food supplemented at the trough contributed with the major part of the diet as it comprised about of 18.5 kg of DM/cow/day. This result differs from the work of González *et al.* (2008), where lame cows within 30 days after treatment showed a tendency to increase the daily feeding time and the time for each meal.

The absence of differences for the proportion of time spent lying was unexpected as cows kept lying approximately for 55% of their diurnal time, independently of the treatment and environmental temperature. Maybe the short period (8 days) we used to evaluate the behavior was not enough to detected significant changes in cow's use of time. Other authors reported conflicting results. Lame cows remained lying for a longer time compared with healthy cow (Blackie *et al.*, 2011). In the other side, no differences were detected for lying time of animals with digital dermatitis or sole bleeding when compared to animals without lesions (Chapinal *et al.*, 2009).

The differences for rumination time between Holstein and Jersey cows are likely to be an inherent characteristic of the breed, with no relation with treatment or the LS. Similar results were reported by Aikman *et al.* (2008) and Prendiville *et al.* (2010), when Holstein cows spent more time in rumination than Jersey cows and they attributed their results to higher mouthfuls, lower food selectivity and shorter time of passage of food in the rumen.

Lower LS of older cows are probably related to a tendency for younger cows, especially those at first calving, to have a higher prevalence of lameness problems due to metabolic changes and stress occurring during this period. Besides as severe foot and legs problems are one of the major causes of culling, the probability to have older cows with high LS is low (Alban, 1995). Furthermore, digital dermatitis occurs most often in young cows (Quinn *et al*., 2005), reinforcing the results found by Read & Walker (1998), where 43% of animals diagnosed with digital dermatitis papillomatous aged up to 3 years old. Frequent contact with mud, manure and increased soil moisture due in the rainy

season are factors that predispose the increased occurrence and spread of this disease.

The fact that cows at the first third of lactation spent higher proportions of time lying than cows at the second or the last third of the lactation is probably related to changes in metabolism which occur during the transition period. Cows observed during two consecutive lactations presented the same profile: high incidence of lesions in the first 150 days after calving, followed by a subsequent decrease (Baird *et al*., 2009). Early lactation and first parturition have an important role in the etiology of hoof lesions, being one of the risk factors (Webster, 2001). This problem is worsened by inadequate management when the animals are kept on humid and dirty concrete floor as well animals are fed with high levels of carbohydrates in the diet.

Cows with dermatitis tend to remain standing for longer time compared to cows with laminitis sequels what can be an indicator of greater severity of pain and discomfort caused by laminitis. In other study, cows with digital dermatitis showed more sickness behavior than cows with laminitis. However type of lesion did not altered ingestive behavior or the locomotion score. (Pavlenko *et al*., 2011).

Cows affected by lesions in the forelimbs have higher LS compared with those with lesions in the hind limbs, what may be linked with the transfer of weight to the rear train, as explained by Neveux et al. (2006), who reported that cows with lesions in the forelimbs transfer part of its weight to the hind limbs and thus lameness become most noticeable. Otherwise, when the lesions occur in the hind limbs, lameness is less visible due to reduced ability to transfer the

body weight. In our study, animals with lesions on forepaws lay for a longer period of time and spent time standing for only 31% of the time, which might indicate greater discomfort signals compared with animals with injured hind limbs. Despite of these changes in posture, we could not notice differences in ingestive behavior, perhaps because cows did not depend so much on grazing to achieve their nutrient requirements, as they could eat silage, hay and concentrate in the trough.

The greater negative impact of having two versus one injured limb on locomotion score and proportions of time lying, standing and feeding is due to the reduced possibility of weight transfer from the injured limb to a healthy one, and therefore, the increase of time spent lying and decrease of time spent standing and feeding took part in the strategies to decrease the painful stimulus in these members (Palmer et al., 2012).

Conclusions

Treatment of severe lame cows proved to be an efficient tool to improve the gait of the animals within a short period. Treatment did not change proportions of time in standing/lying or in feeding activities.

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CAPÍTULO III

3.1 Considerações Finais

Desordens locomotoras além de causarem perdas econômicas, resultam em alterações sobre o organismo do animal, pois prejudicam a marcha e modificam vários aspectos comportamentais. O comportamento vem sendo amplamente estudado, com o objetivo de entender o metabolismo dos animais de produção, a ocorrência de fatores prejudiciais e a influência do ambiente onde está inserido.

As variáveis comportamentais observadas nesse estudo foram citadas por vários autores como alteradas quando os animais estão com problemas nos membros, o que foi confirmado também nesse trabalho, pois os tempos despendidos nas atividades estão relacionados à presença de diferentes lesões, localização e número de membros afetados, além de idade, dias em lactação e raça. Infelizmente, não foi possível realizar observações comportamentais durante o período noturno, o que pode ter contribuído para que poucas modificações comportamentais antes e após o tratamento fossem notadas. Vale lembrar também que devido à limitação de espaço do piquete e número de membros da equipe de pesquisa, as observações foram realizadas num período curto de tempo, ou seja, até 8 dias após o casqueamento e é possível que após este período ocorressem outras modificações no comportamento.

O tratamento das lesões digitais de animais com claudicação severa é uma ferramenta eficiente para melhora da marcha dos animais, pois decorrida uma semana após o tratamento foi observada menor pontuação de escore de locomoção. Ao contrário do que era esperado, o tratamento e a melhora do escore de locomoção não refletiram em alterações comportamentais em relação aos tempos de decúbito, em pé, ruminação, ócio e alimentação.

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3.3 Apêndice

Normas da Revista Animal.

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November 2013

Introduction

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animal attracts the best research in animal biology and animal systems from across the spectrum of the agricultural, biomedical, and environmental sciences; it is the central element in a collaboration between the British Society of Animal Science (BSAS), the Institut National de la Recherche Agronomique (INRA) and the European Federation for Animal Science (EAAP) and represents the merger in 2006 of three scientific journals: *Animal Science*; *Animal Research*; *Reproduction, Nutrition, Development*.

Scope

animal publishes original, cutting-edge research and horizon-scanning reviews on animal-related aspects of the life sciences at the molecular, cellular, organ, whole animal and production system levels. Papers will be considered in aspects of both strategic and applied science in the areas of Animal Breeding and Genetics, Nutrition, Physiology and Functional Biology of Systems, Behaviour, Health and Welfare, Livestock Farming Systems and Environment, and Product Quality, Human Health and Well-being. Emphasis is placed on **managed animals** and on the integrative nature of biological systems. The use of laboratory animal models for the benefit of farmed livestock is within the scope. Studies using farm animals with the aim of improving human health are also acceptable but they must also indicate benefits to farmed livestock. Wild animals which are marginally bred in a few countries or which could be bred in the future, and wild animals raised in captivity are not considered as farm animals. Papers dealing with the translation of basic and strategic science into whole animal and system impacts on Productivity, Product Quality, the Environment and Humans (health, nutrition and well being) will be particularly welcome. Papers should also be of **international relevance,** appeal to an international readership and then **not limited to national or regional conditions**. The full **scope of the journal** is available on http://www.animal-journal.eu/scope.htm and should be consulted before submitting a paper.

animal is essential reading for all animal scientists interested in biochemistry, microbiology, nutrition, physiology, modelling, genetics, behaviour, immunology, epidemiology, engineering, economics, sociology, food science and technology, human health, farming systems, and land-use management, environmental impact and climate change.

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animal publishes different types of articles:

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- Short communications will only be accepted in special cases where, in the Editor's judgement, the contents are exceptionally exciting, novel or timely. Partial data or complete studies with a very limited amount of results will not be considered as short communications. The maximum length should not exceed 4 journal pages (approx. 3,000 words) including a maximum of 3 tables and/or figures and a maximum of 10 references. These short communications will be peer-reviewed in the same way as full papers. Very short publications which are not considered as short communications by the editorial board will be handled as full papers.

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margins. Manuscripts that are too long will be sent back to the author. As for full research papers, supplementary material can be proposed.

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The journal will consider for publication the results of original work and critical reviews that are presented at conferences/symposia. Acceptance of such papers will be subject to:

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The responsibility for the preparation of a paper in a form suitable for publication lies in the first place with the author. Authors should consult a free issue or a free article of *animal*, available at http://journals.cambridge.org/anmsample, in order to make themselves broadly familiar with the layout and style of *animal*. The English must be acceptable for publication. If the English is not good enough, editors may ask for a linguistic

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Title

A title needs to be concise and informative. It should:

(*a*) arrest the attention of a potential reader scanning a journal or a list of titles;

(*b*) provide sufficient information to allow the reader to judge the relevance of a paper to his/her interests and whether it will repay the effort of obtaining a copy;

(*c*) incorporate keywords or phrases that can be used in indexing and information retrieval, especially the animal species on which the experiment has been carried out;

(*d*) avoid inessentials such as 'A detailed study of ...', or 'Contribution to ...';

(*e*) not include the name of the country or of the region where the experiment took place; (*f*) be shorter than 170 characters including spaces.

Authors and affiliation

The names and affiliations of the authors should be presented as follows:

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The corresponding author indicated in the manuscript who will be the correspondent for a published paper can be different from the corresponding author who submits and manages the manuscript during the review process; the latter corresponding author will need to be registered on Editorial Manager.

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Authors should propose a running head of no more than 50 characters. If the proposed running head is not appropriate, it could be modified by the Editorial Office, with the author's agreement.

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Every paper should have a **one-paragraph abstract** of not more than 400 words which is complete and understandable without reference to the paper. It should state succinctly the problem, the experimental methods, results and conclusions but should not be overburdened by numerical values or probability values. References to tables and figures, and undefined abbreviations are not acceptable.

Keywords

Up to a maximum of five keywords selected from CAB Thesaurus (1995) or from an equivalent volume should be selected. Keywords are essential in information retrieval and should be indicative of the content of the paper (animal species, etc.). If the proposed keywords are not appropriate, the manuscript will be returned to the authors. The use of non-standard abbreviations in the list of keyword is discouraged.

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Authors must write maximum 100 words explaining the implications of their work. Implications explain the expected importance or economic, environmental and/or social impact. This must be in simple English suitable for non science readers. This section is mandatory and will be peer-reviewed.

Introduction

The Introduction should briefly present the current issues that the authors are addressing while outlining the context of the work, ensuring that the objectives are clearly defined, and that the main features of the experiment or of the work are clear to the reader. Increasing the knowledge on a subject is not an objective *per se*. References in the Introduction should be limited as it should not be a preliminary discussion or a literature review.

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Material and Methods should be described in sufficient detail within this section, so that it is possible for others to repeat the experiment. If the methods are numerous, authors should refer to one of their previous publications in which they are described in detail.

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They can be presented together (Results and Discussion) or in 2 different sections (Results followed by Discussion). Conventions for presenting these sections or the Materials and Methods section (sub- headings, etc.) are presented later in this document.

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A statistical guide for authors is available on the website at http://www.animal-journal.eu/statistical_instructions.htm. The methods and models of statistical analysis must be indicated and sufficient statistical details given to allow replication of the experiment. Where reference is made to statistical significance, the level of significance attained should normally be indicated by using the following conventional standard abbreviations (which need not be defined): $P>0.05$ for non- significance and $P<0.05$, $P<0.01$ and $P<0.001$ for significance at these levels. In tables, levels of significance should be indicated by *, ** and *** respectively. Statistical significance (e.g. $P=0.07$) can also be used in the text or tables. **Treatment means should normally be given without their standard deviation** (i.e. variability in a sample or a population). An indicator of the precision of the measure such as the pooled standard error, the residual standard deviation (RSD) or the root mean square error (RMSE) should be given for each criteria/item/variable/trait in an additional column (or line). Differences between treatments (or comparison of mean values) will be indicated using the following conventional standard: a, b for $P<0.05$; A, B for $P<0.01$; in most cases, the 0.05 level is sufficient.

Tables

These should be as simple and as few as possible. The same material should not normally be presented in tabular and graphical form. When both forms are possible, authors should present tables. Generally, variables are in rows and treatments in columns. In designing tables, authors should refer to the page size and column widths of *animal* as guidance. Each table should be typed, preferably in double spacing, on a page separate from the main body of the text (one table per page) and an indication given in the text where it should be inserted. Tables which are created in Word should not use tabs but should use the table function within the programme. Tables should not be prepared with vertical lines between columns and horizontal lines between rows of data. Tables should be given Arabic numbering and each should have its own explanatory title, footnotes and definitions of abbreviations which are sufficient **to permit the table to be understood without reference to the text** ("Effect of fat source and animal breed on carcass composition in pigs" is preferred to "Carcass composition"). The title should not contain too many details about the protocol, the definition of abbreviations, etc. Such details are preferred as footnotes. All tables must be cited consecutively in the text. Column headings should be concise and units should be clearly stated using standard abbreviations; any non-standard abbreviation should be defined. Only the first letter of the first word is in capitals. Sub-items describing the data in the rows should be indented relative to crossheadings; where they involve printing on more than one line, they should be indented in the second and subsequent lines. Sub-sub-items should also be indented. Footnotes should

be used sparingly and kept brief. They must provide the bases for statistics (levels of significance, statistical model, etc.). The reference symbols used in footnotes are numbers in low cases. The values in the tables should be given with meaningful decimals; practically, the last digit should correspond to about one tenth of the standard error. The number of decimals for mean treatment values and the corresponding indicator of residual variability (RSD, SEM, RMSE, etc.) should be consistent in all the tables, either identical or one more for the variability indicator, but not both possibilities. Values such as efficiencies or digestibilities are preferred as percentages.

Figures

It is recommended that the width of any diagram submitted should be either 175 mm (2 columns) or 83 mm (1 column) including the legend at the side(s). In choosing ornaments, solid symbols should be used before open symbols, and continuous lines before dotted or dashed lines. The size of symbols should be appropriate (neither too small nor too big and clumsy). The use of colour in figures should be avoided, unless it is essential to understanding the figure. Figures are then usually supplied as black and white and as one file per figure. Colour figures are reproduced at no cost to the author for the on- line version. But the authors are liable to cover the **additional costs for printing figures in colour**. Publication charges can be found at http://www.animal-journal.eu/documents/Reprints_cost.pdf. All figures must be numbered consecutively in the text. **Captions for all figures should be typed on a separate page** at the end of the manuscript and should be sufficiently detailed to allow the figure to be understood without reference to the text; figures are submitted without their captions. An indication of where a figure should appear should be given within the text. Diagrams and plates are referred to within the text as Figure 1, etc., and the captions begin with Figure 1, etc. For details of submission requirements, refer to section on ‘Submission and evaluation of the manuscript’.

Acknowledgements

In this section, the authors may acknowledge (briefly) their support staff, their funding sources, their credits to companies or copyrighted material, etc. All papers with a potential conflict of interest must include a description/explanation under the Acknowledgements heading.

References

It is the author’s responsibility to ensure that all references are correct. The references should adhere to the guidelines, be relevant to the text content and they should all be cited in the text. The maximum number of references is 10 for short communications, 35 for original articles and 50 for review papers, except when the editor agrees a higher number. No more than 3 references can be given for the same statement (except for reviews and meta-analyses). Authors should minimise the number of references to conference proceedings, reports, PhD theses, and other references which cannot easily be obtained by the reader. The accuracy of the references is the responsibility of the authors. Authors should carefully check authors’ surnames and first names, article title, journal title, volume and page numbers, book publisher’s

information, proceedings exact description. Literature cited should be listed in alphabetical order of authors and references should not be numbered. For a same first author, the rank of references will be i) publications with one author ranked by year; ii) publications with two authors ranked by year; iii) publications with more than two authors ranked by year then, if necessary, by alphabetical order of the second author.

Typical references are:
Journal article or abstract:
Format: Author(s) surname and Initials Year. Title. Full title of the journal volume, pages. The issue within the volume is not mentioned, except if the numbering is per issue and not per volume (ex: newspapers). The word 'abstract' if applicable is not mentioned. Titles which cannot be written in Latin characters will be translated in English, followed by (in xxx) where xxx is the original language. Examples:
Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351-365.
Morgavi DP, Martin C, Jouany JP and Ranilla MJ 2012. Rumen protozoa and methanogenesis: not a simple cause-effect relationship. British Journal of Nutrition 107, 388-397.
When the article is online but not yet printed, the right format is:
Zamaratskaia G and Squires EJ. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal, doi:10.1017/S1751731108003674, Published online by Cambridge University Press 17 December 2008.

Book:
Format: Author(s) or editor(s) surname and Initials, or institution Year. Title, number of volumes if more than 1, edition if applicable. Name of publisher, place of publication (i.e. city, state (if applicable) and country).
Example: Association of Official Analytical Chemists 2004. Official methods of analysis, 2 vol., 18th edition. AOAC, Arlington, VA, USA.
Book chapter or edited conference proceedings:
Format: Author(s) surname and Initials Year. Title. In Title of the book or of the proceedings (eds followed by the editor(s)), volume number when applicable, pages. Name of publisher, place of publication (i.e. city, state (if applicable) and country).
For edited proceedings, it is not necessary to mention the date and place of the symposium.
Example: Nozière P and Hoch T 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In Nutrient digestion and utilization in farm animals (eds E Kebreab, J Dijkstra, A Bannink, WJJ Gerrits and J France), pp. 40–47. CABI Publishing, Wallingford, UK.
Report at an event (conference, meeting, etc) not included in a book or edited proceedings:
Format: Author(s) surname and Initials Year. Title. Nature of the event, date of the event (i.e. day month year), place of the event (i.e. city, state (if applicable) and country), pages or poster/article number (if applicable).
Examples:

Martuzzi F, Summer A, Malacarne M and Mariani P 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, Budapest, 26-29 August 2001, Budapest, Hungary
Bispo E, Franco D, Monserrat L, González L, Pérez N and Moreno T 2007. Economic considerations of cull dairy cows fattened for a special market. In Proceedings of 53rd International Congress of Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.

Thesis:
Format: Author surname and Initials Year. Title. Type of thesis, University with English name, location of the University (i.e. city, state (if applicable) and country).
Example: Vlaeminck B 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. Thesis PhD, Ghent University, Ghent, Belgium.

Website addresses can be used when no other reference is available. They should be presented as for standard references but, in addition, they should include the date when the document was retrieved: Bryant P 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from http://darwin.bio.uci.edu/~sustain/bio65/Titlpage.htm

Citation of references
In the text, references with three or more authors should be cited on all occasions with the first author followed by *et al*. (**in italics;** e.g. Smith *et al*.). References with two authors should be cited in full on all occasions. Names of organizations used as authors (e.g. Agricultural and Food Research Council) should be written out in full in the list of references and on first mention in the text. Subsequent mentions may be abbreviated (e.g. AFRC). Ampersands (&) are not to be used. Multiple references should be as follows: Wright *et al*., 1993 and 1994; Wright *et al*., 1993a and 1993b. When several references are cited simultaneously, they should be ranked by chronological order (e.g. Smith *et al.,* 1995; Fabre *et al.,* 1996; Schmidt *et al.,* 1998; Fabre *et al.,* 1999).
'Personal communication' or 'unpublished results' should follow the name of the author in the text where appropriate. The author's initials but not his title should be included, and such citations are not needed in the reference list. Check that all of the references in the text are in the list of references and *vice versa*.
Bibliographic database softwares can be used. The output styles for Endnote, Procite and Reference Manager may be found on the journal website http://animal-journal.eu/instructions_to_authors.htm.

Supplementary material
Authors can include supplementary material in any type of text (full research paper, review paper, short communication, etc.). Supplementary material will appear only in the electronic version, and is not limited in length. It will be peer-

reviewed along with the rest of the manuscript, but will not be copy- edited. **Authors are entirely responsible for its content and must check carefully the format and styles.** This supplementary material could contain original *modus operandi*, tables or figures which are not necessary for understanding the text within the main body of the paper, mathematical models, references of publications which are used, for example, in a meta-analysis and which do not appear in the text, or pictures improving the understanding of the text. The manuscript must stand alone without the supplementary material for those readers who will be reading the hard copy only. This should be submitted with the main manuscript in a **separate file** and identified as "Supplementary File – for Online Publication Only". Each figure should have its own title embedded in the figure (below). Supplementary material should be identified and mentioned in the main text as Supplementary Table S1, Supplementary Table S2, etc. for tables or Supplementary Figure S1, Supplementary Figure S2, etc. for figures or Supplementary Material S1, Supplementary Material S2, etc. for other material). For example: “The list of references used for the meta-analysis is given in Supplementary Material S1”. A link to this on-line supplementary material will be included by the Production Editor at the proof preparation stage.

Typographical conventions and consistencies

Headings

As illustrated and detailed above and in the style sheet (see website), the *animal* convention is as follows.

(*a*) *Title* of the paper is in bold with only the first letter in capitals. Authors’ names are in lower case with initial capitals and their addresses are in italics.

(*b*) *Main section headings* (Abstract, Introduction, Implications, Material and methods, Results, Discussion, Acknowledgement(s), References) are printed in bold throughout and placed by the left margin.

(*c*) *Subheadings* are italicized and only the initial letter is in capitals. The two classes are: (i) side italics unpunctuated (shoulder headings); (ii) italics, punctuated and text run-on side headings). The sequence is always (i) to (ii).

The names of the chemicals do not need to be written out in full; chemical symbols are sufficient. Fatty acids are abbreviated using the following rules: cis-18:1 for the sum of cis octadecenoic acids. When isomers are described, the double bond positions are identified by numbering from the carboxylic acid end: c9,t11-18:2; iso-15:0. The terms “omega 3” and “omega 6” are banned and replaced by “n-3” and “n-6”, e.g. 18:3n-3. Trivial names can be used for the most known fatty acids (myristic, palmitic, oleic, linoleic, linolenic) and abbreviations in some cases: CLA for conjugated linoleic acids, EPA for eicosapentaenoic acid, DHA for docosahexaenoic acid. Chemical names and trivial names cannot be mixed in a same table.

Capitals

(*a*) Initial capitals are used for proper nouns, for adjectives formed from proper names, for generic names and for names of classes, orders and families.

(*b*) Names of diseases are not normally capitalized.

Italics
Use italics for:
(*a*) titles of books and names of periodicals in the text; (*b*) authors' addresses;
(*c*) subheadings (see above);
(*d*) titles for tables (but not captions for figures);
(*e*) most foreign words, especially Latin words, e.g. *ad hoc, ad libitum, et al., in situ, inter alia, inter se, in vitro, per se, post mortem, post partum* but *no italics* for c.f., corpus luteum, e.g., etc., i.e., N.B., via
(*f*) mathematical unknowns and constants; (*g*) generic and specific names;
(*h*) letters or numbers in the text which refer to corresponding letters or numbers in an illustration;
(*i*) letters used as symbols for genes or alleles e.g. *HbA, Tf D* (but not chromosomes or phenotypes of blood groups, transferrins or haemoglobins, e.g. HbAA, TfDD);
(*j*) first occurrence of a special term;
(*k*) repeated emphasis of a special term (use cautiously);
(*l*) Latin names of muscles (but not of bones), e.g. *m. biceps femoris*.

Spelling
All papers must be written in English. Spelling may be in British or American English but must be consistent throughout the paper. Please refer to standard dictionaries e.g. Webster's, Collins, Concise Oxford for the correct spelling of words and to Fowler's Modern English Usage (3rd edition, edited by R.W. Burchfield, Oxford University Press) for usage. Care should be exercised in the use of agricultural terminology that is ill-defined or of local familiarity only.
Numerals
(*a*) In text, use words for numbers zero to nine and figures for higher numbers. In a series of two or more numbers, use figures throughout irrespective of their magnitude.
(*b*) Sentences should not, however, begin with figures.
(*c*) For values less than unity, 0 should be inserted before the decimal point.
(*d*) For large numbers in the text substitute 10^n for part of a number (e.g. 1.6 10^6 for 1 600 000).
(*e*) To facilitate the reading of long numbers in tables, the digits should be grouped in three about the decimal sign but no point or comma should be used.
(*f*) The multiplication sign between numbers should be a cross (x).
(*g*) Division of one number by another should be indicated as follows: 136/273.
(*h*) Use figures whenever a number is followed by a standard unit of measurement (e.g. 100 g, 6 days, 4th week).
(*i*) Use figures for dates, page numbers, class designations, fractions, expressions of time, e.g. 1 January 2007; type 2.
(*j*) Dates should be given with the month written out in full in the text and with the day in figures (i.e. 12 January *not* 12th January). Single non-calendar years should be written 2006/07; periods of two calendar years as 2006-07.

(*k*) For time use 24-h clock, e.g. 0905 h, 1320 h.

Units of measurement
The International System of Units (SI) should be used. A list of units is found for example at http://physics.nist.gov/cuu/Units/units.html. Recommendations for conversions and nomenclature appeared in *Proceedings of the Nutrition Society,* **31:** 239-247, 1972. Some frequently used units which are not in the SI system are accepted: l for litre, ha for hectare, eV for electron-volt, Ci for curie. Day, week, month and year are not abbreviated.

Concentration or composition
Composition expressed as mass per unit mass or mass per unit volume should have as denominator the unit of mass, the kilogram, or the unit of volume, the litre. Values should thus be expressed as nanograms, micrograms, milligrams or grams per kilogram or per litre. The term *content* should not be used for concentration or proportion.

Statistical terms
chi square χ^2
coefficient of determination R^2
coefficient of variation CV
correlation
multiple *R*
sample coefficient *r*
degrees of freedom d.f.
expectation of mean square e.m.s.
least significant difference LSD
mean square m.s.
non-significant $P>0.05$
probability *P*
$P<0.05$, in tables use *
$P<0.01$, in tables use **
$P<0.001$, in tables use ***
regression coefficient *b*
root mean square error r.m.s.e.
standard deviation s.d.
standard error of difference s.e.d.
standard error of mean s.e.m. standard error of estimate *Sy.x*
residual standard deviation r.s.d.

Tabela 1. Escore de locomoção dos animais antes e após o tratamento e percentual de tempo que as vacas permaneceram deitadas nos dias observados.

	Escore Locomoção		% Permanência deitada			
Vaca	Dia -1	Dia 6	Dia -1	Dia 1	Dia 6	Dia 8
1	4	2	8,64	16,05	17,28	34,57
2	5	3	37,04	34,57	28,40	41,98
3	5	2	56,79	43,21	54,32	60,49
5	4	5	28,40	13,58	23,46	40,74
6	4	5	80,25	66,67	69,14	69,14
7	4	4	44,44	29,63	59,26	66,67
8	5	3	41,03	32,05	38,27	33,33
9	4	3	35,90	60,26	54,32	51,85
10	5	5	52,56	34,62	39,51	4,94
11	4	5	74,36	62,82	45,68	72,84
12	5	1	41,03	53,85	45,68	45,68
13	5	5	55,13	66,67	60,49	75,31
14	4	2	74,36	68,00	65,75	69,23
15	4	2	20,51	26,67	16,44	23,08
16	5	5	60,26	60,00	76,71	57,69
17	5	3	67,95	52,00	64,38	61,54
20	4	2	5,13	44,00	1,37	14,10
21	4	1	37,18	18,67	39,73	35,90
22	4	1	23,08	24,00	27,40	14,10
7	5	4	58,67	34,67	61,11	50,00
14	5	3	88,00	73,33	76,39	79,17
23	4	1	37,33	28,00	30,56	41,67
27	5	3	61,33	68,00	62,50	37,50
28	4	1	30,67	29,33	43,06	38,89
3	5	4	57,14	55,07	66,67	59,68
5	4	1	30,00	31,88	39,68	45,16
29	5	4	45,71	56,52	69,84	72,58
30	5	2	74,29	40,58	68,25	64,52
33	5	4	60,00	46,38	49,21	50,00
34	4	1	21,43	11,59	25,40	11,29
35	5	4	47,54	54,84	55,00	48,33
36	5	4	75,41	70,97	66,67	75,00
37	4	4	54,10	80,65	53,33	46,67
38	4	3	63,93	54,84	61,67	40,00
39	4	3	54,10	16,13	35,00	41,67
41	4	3	49,18	41,94	41,67	45,00
21	4	3	3,33	36,67	9,09	5,56
43	4	3	63,33	58,33	56,36	51,85
44	4	4	66,67	60,00	74,55	59,26

Tabela 2. Percentual de tempo de permanência em estação de acordo com os dias observados.

Vaca	Dia -1 (%)	Dia 1 (%)	Dia 6 (%)	Dia 8 (%)
1	61,73	49,38	56,79	51,85
2	53,09	34,57	46,91	39,51
3	25,93	23,46	23,46	19,75
5	39,51	61,73	53,09	30,86
6	9,88	12,35	11,11	17,28
7	28,40	30,86	19,75	16,05
8	38,46	51,28	51,85	56,79
9	37,18	20,51	32,10	39,51
10	38,46	46,15	55,56	77,78
11	7,69	23,08	16,05	19,75
12	37,18	20,51	32,10	41,98
13	29,49	16,67	17,28	19,75
14	6,41	13,33	10,96	8,97
15	51,28	44,00	54,79	48,72
16	16,67	18,67	5,48	20,51
17	11,54	18,67	13,70	11,54
20	51,28	33,33	56,16	39,74
21	29,49	56,00	32,88	29,49
22	44,87	46,67	30,14	48,72
7	8,00	34,67	12,50	26,39
14	1,33	6,67	8,33	8,33
23	36,00	49,33	23,61	26,39
27	12,00	10,67	12,50	40,28
28	28,00	29,33	34,72	33,33
3	21,43	31,88	17,46	14,52
5	45,71	40,58	30,16	30,65
29	28,57	20,29	14,29	11,29
30	15,71	31,88	12,70	20,97
33	25,71	26,09	20,63	20,97
34	50,00	59,42	38,10	45,16
35	11,48	16,13	16,67	16,67
36	1,64	6,45	15,00	3,33
37	14,75	6,45	25,00	18,33
38	13,11	22,58	18,33	33,33
39	18,03	51,61	41,67	30,00
41	14,75	12,90	33,33	25,00
21	65,00	36,67	70,91	62,96
43	13,33	16,67	20,00	27,78
44	20,00	16,67	9,09	14,81

Tabela 3. Percentual de tempo despendido nas atividades de ruminação e ócio de acordo com os dias de observação.

	Ruminação (%)				Ócio(%)			
Vaca	Dia-1	Dia 1	Dia 6	Dia 8	Dia-1	Dia 1	Dia 6	Dia 8
1	18,52	24,69	19,75	28,40	51,85	40,74	54,32	55,56
2	18,52	18,52	12,35	12,35	70,37	48,15	64,20	69,14
3	7,41	14,81	11,11	20,99	71,60	51,85	64,20	58,02
5	27,16	14,81	20,99	24,69	39,51	56,79	55,56	51,85
6	16,05	16,05	13,58	20,99	72,84	65,43	66,67	65,43
7	16,05	20,99	7,41	18,52	58,02	38,27	71,60	62,96
8	11,54	14,10	0,00	11,11	67,95	69,23	87,65	80,25
9	17,95	28,21	11,11	30,86	58,97	50,00	76,54	60,49
10	12,82	12,82	1,23	6,17	78,21	67,95	93,83	75,31
11	8,97	16,67	6,17	7,41	73,08	69,23	74,07	83,95
12	21,79	20,51	6,17	12,35	55,13	52,56	71,60	75,31
13	8,97	8,97	12,35	2,47	70,51	73,08	77,78	92,59
14	19,23	6,67	39,73	26,92	61,54	74,67	36,99	51,28
15	28,21	28,00	30,14	30,77	44,87	41,33	41,10	39,74
16	30,77	38,67	47,95	35,90	46,15	41,33	34,25	41,03
17	25,64	21,33	46,58	26,92	52,56	49,33	31,51	46,15
20	30,77	21,33	28,77	23,08	26,92	54,67	28,77	30,77
21	10,26	10,67	24,66	6,41	56,41	64,00	47,95	57,69
22	23,08	29,33	35,62	30,77	44,87	40,00	21,92	32,05
7	36,00	24,00	23,61	18,06	30,67	45,33	48,61	58,33
14	33,33	20,00	16,67	27,78	54,67	60,00	68,06	58,33
23	17,33	25,33	16,67	16,67	56,00	52,00	34,72	51,39
27	32,00	34,67	34,72	45,83	41,33	44,00	37,50	31,94
28	18,67	21,33	26,39	22,22	41,33	37,33	51,39	50,00
3	15,71	5,80	15,87	33,87	62,86	81,16	68,25	40,32
5	37,14	26,09	34,92	37,10	38,57	44,93	34,92	38,71
29	20,00	10,14	26,98	14,52	54,29	66,67	41,27	69,35
30	25,71	27,54	33,33	43,55	64,29	44,93	47,62	38,71
33	20,00	11,59	34,92	35,48	64,29	60,87	34,92	37,10
34	22,86	11,59	28,57	24,19	48,57	59,42	34,92	32,26
35	21,31	16,13	18,33	20,00	39,34	45,16	51,67	45,00
36	26,23	38,71	35,00	38,33	50,82	38,71	46,67	40,00
37	29,51	48,39	20,00	28,33	40,98	38,71	58,33	36,67
38	40,98	32,26	23,33	25,00	36,07	45,16	56,67	46,67
39	29,51	25,81	31,67	20,00	42,62	41,94	45,00	51,67
41	27,87	25,81	28,33	25,00	36,07	32,26	46,67	45,00
21	20,00	23,33	9,09	12,96	48,33	50,00	70,91	55,56
43	21,67	21,67	1,82	24,07	55,00	51,67	74,55	55,56
44	16,67	20,00	21,82	16,67	70,00	56,67	58,18	55,56

Tabela 4. Percentual de tempo de caminhada de acordo com os dias de observação.

Vaca	Dia -1 (%)	Dia 1 (%)	Dia 6 (%)	Dia 8 (%)
1	6,17	6,17	2,47	2,47
2	0,00	1,23	1,23	1,23
3	0,00	3,70	2,47	4,94
5	4,94	6,17	2,47	1,23
6	1,23	3,70	2,47	1,23
7	1,23	1,23	2,47	2,47
8	1,28	2,56	2,47	0,00
9	0,00	0,00	2,47	0,00
10	3,85	2,56	1,23	0,00
11	2,56	1,28	1,23	0,00
12	3,85	6,41	6,17	1,23
13	3,85	3,85	0,00	0,00
14	1,28	5,33	1,37	1,28
15	1,28	4,00	0,00	0,00
16	1,28	0,00	1,37	0,00
17	1,28	1,33	0,00	2,56
20	6,41	2,67	5,48	0,00
21	3,85	1,33	2,74	6,41
22	1,28	0,00	4,11	2,56
7	0,00	0,00	0,42	0,14
14	0,13	0,27	0,00	0,28
23	0,00	0,13	0,14	0,00
27	0,00	0,00	0,00	0,00
28	0,00	0,13	0,00	0,14
3	2,86	0,00	0,00	1,61
5	1,43	0,00	4,76	1,61
29	1,43	1,45	0,00	0,00
30	1,43	0,00	0,00	1,61
33	0,00	1,45	6,35	1,61
34	0,00	1,45	3,17	3,23
35	9,84	0,00	5,00	0,00
36	3,28	3,23	6,67	0,00
37	1,64	0,00	0,00	0,33
38	3,28	3,23	0,00	0,00
39	3,28	3,23	0,00	3,33
41	4,92	3,23	0,00	0,00
21	1,67	0,00	0,00	3,70
43	3,33	3,33	3,64	0,00
44	1,67	1,67	0,00	3,70

Tabela 5. Percentual de tempo de pastejo e alimentação no cocho de acordo com os dias de observação

	Pastejo (%)				Alimentação no cocho (%)			
Vaca	Dia -1	Dia 1	Dia 8	Dia 8	Dia -1	Dia 1	Dia 6	Dia 8
1	14,81	22,22	9,88	6,17	9,88	7,41	9,88	6,17
2	0,00	22,22	1,23	7,41	7,41	4,94	18,52	11,11
3	0,37	19,75	2,47	4,94	13,58	8,64	14,81	9,88
5	12,35	16,05	0,00	1,23	13,58	12,35	13,58	19,75
6	0,00	8,64	2,47	3,70	7,41	4,94	11,11	7,41
7	4,94	23,46	1,23	0,00	14,81	7,41	13,58	13,58
8	2,56	0,00	0,00	6,17	16,67	16,67	7,41	3,70
9	1,28	2,56	2,47	6,17	20,51	19,23	9,88	3,70
10	1,28	5,13	1,23	0,00	2,56	12,82	3,70	6,17
11	1,28	0,00	6,17	6,17	10,26	14,10	11,11	1,23
12	1,28	5,13	0,00	2,47	16,67	15,38	17,28	8,64
13	6,41	17,95	11,11	4,94	7,69	0,00	0,00	0,00
14	10,26	10,67	8,22	11,54	8,97	5,33	16,44	10,26
15	10,26	12,00	9,59	15,38	17,95	14,67	21,92	14,10
16	2,56	10,67	8,22	6,41	20,51	12,00	10,96	17,95
17	6,41	14,67	6,85	7,69	15,38	14,67	17,81	16,67
20	17,95	5,33	8,22	12,82	20,51	17,33	32,88	33,33
21	14,10	5,33	5,48	10,26	14,10	21,33	23,29	23,08
22	12,82	13,33	6,85	8,97	17,95	20,00	35,62	26,92
7	10,67	14,67	11,11	11,11	24,00	18,67	12,50	13,89
14	0,00	10,67	4,17	0,00	10,67	9,33	11,11	11,11
23	10,67	10,67	26,39	16,67	18,67	13,33	20,83	15,28
27	5,33	6,67	9,72	9,72	22,67	18,67	16,67	13,89
28	10,67	13,33	9,72	11,11	30,67	29,33	15,28	15,28
3	7,14	0,14	1,59	3,23	14,29	13,04	15,87	22,58
5	2,86	0,29	0,00	6,45	20,00	27,54	30,16	17,74
29	1,43	0,00	0,00	1,61	24,29	23,19	17,46	16,13
30	1,43	8,70	7,94	9,68	4,29	20,29	14,29	6,45
33	1,43	7,25	1,59	1,61	15,71	20,29	23,81	25,81
34	1,43	5,80	0,00	3,23	25,71	23,19	34,92	38,71
35	11,48	6,45	8,33	15,00	19,67	35,48	16,67	21,67
36	0,00	0,00	5,00	0,00	19,67	22,58	11,67	23,33
37	8,20	0,00	11,67	8,33	13,11	16,13	11,67	25,00
38	9,84	0,00	11,67	6,67	8,20	22,58	10,00	21,67
39	3,28	3,23	10,00	5,00	13,11	22,58	15,00	23,33
41	0,00	6,45	5,00	5,00	32,79	35,48	21,67	26,67
21	25,00	8,33	3,64	1,85	6,67	20,00	18,18	29,63
43	16,67	11,67	7,27	9,26	5,00	11,67	14,55	14,81
44	10,00	10,00	3,64	0,00	3,33	13,33	16,36	22,22

Tabela 6. Índice de temperatura e umidade (ITU) máximo, médio, mínimo e média da temperatura ambiente (TA) e umidade relativa do ar (UR) nos dias de observações comportamentais.

Data	ITU Máximo	ITU médio	ITU mínimo	TA (ºC) média	UR (%) média
22/01/2014	88,44	81,44	72,16	30,0	71,6
24/01/2014	87,67	83,40	71,96	31,1	69,3
29/01/2014	88,99	83,06	69,08	30,5	75,5
31/01/2014	86,07	80,89	69,34	28,2	81,6
05/02/2014	88,36	84,66	73,03	33,5	59,4
07/02/2014	89,06	86,27	71,95	33,2	66,7
20/02/2014	76,50	73,40	71,23	23,5	90,6
22/02/2014	89,40	79,64	69,07	28,3	80,6
26/02/2014	79,87	72,94	69,61	23,0	99,9
28/02/2014	78,61	72,65	62,96	22,6	83,6
12/03/2014	76,79	73,39	64,02	23,8	74,0
14/03/2014	82,77	77,21	66,20	26,1	81,9
19/03/2014	77,12	71,59	68,17	22,2	98,9
21/03/2014	81,81	73,34	64,04	23,6	86,5
02/04/2014	80,88	77,11	69,61	25,4	91,0
04/04/2014	80,42	77,31	66,92	25,9	82,5
09/04/2014	80,30	76,58	66,56	25,3	83,9
11/04/2014	77,42	74,47	71,95	23,7	99,3
23/04/2014	74,08	69,15	56,12	21,8	67,3
25/04/2014	70,51	68,12	59,31	20,7	63,1
29/04/2014	74,33	71,01	57,02	21,7	78,1
01/05/2014	72,52	67,06	63,32	20,0	92,9
07/05/2014	81,49	73,36	67,64	23,3	91,5
09/05/2014	70,42	64,88	53,60	18,6	82,5

3.4 Vita

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