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Alteração nos dígitos bovinos relacionadas à dieta e a à própria anatomia

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“Eu agradeço pela vida e a
coragem. Ao Universo pela
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vida eu dedico com amor.
Ao sonho vivo da nossa
humanidade” (Autor: Lucy,
sem fronteiras)

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¹Alteração nos dígitos bovinos relacionadas às características da dieta e dos animais

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RESUMO GERAL

Problemas de locomoção em bovinos estão entre as principais causas de desconforto por afetar diretamente o bem-estar animal, são comumente relacionados a perda de produtividade, acarretando em prejuízos. É uma das principais causas de descarte em bovinos leiteiros, destaca-se que doenças de origem não infecciosa possuem maior impacto no descarte de animais, em virtude das alterações na conformação dos dígitos, que são em sua maioria irreversíveis. Diante deste contexto, está tese de doutorado visou o estudo dos fatores envolvidos nas enfermidades não infecciosas, especificamente o fator nutrição e as características anatômicas. O fator nutrição, foi abordado no primeiro estudo, no qual buscou-se entender a relação causal entre acidose ruminal e ocorrência de laminite por meio de uma revisão sistemática em quatro bases de dados e o banco de teses Capes. Neste primeiro estudo encontramos resultados que demonstraram parcialmente a relação entre acidose e laminite aguda, porém o mecanismo de desenvolvimento continua a ser indefinido. O segundo estudo visou descrever as características da conformação do dígito externo com as estruturas internas consideradas importantes para a saúde do dígito. Foram realizadas mensurações das características externas e internas em 296 dígitos oriundos de vacas Jerseys que vieram a óbito em fazendas leiteiras no período de janeiro a junho de 2018. A avaliação *pos mortem* dos dígitos revelou que o comprimento da parede dorsal foi a principal característica externa associada a espessura de sola, foram encontradas alta frequência (44%) de patas com espessura de sola inadequadas (<7 mm) em vacas primíparas. Unhas com inadequada espessura de sola e rotação de terceira de falange representaram 22% dos resultados.

Palavras chaves: saúde dos dígitos, anatomia do dígito, nutrição e acidose ruminal

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²Changes in bovine digits related to the characteristics of the diet and of the animals²

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ABSTRACT

Locomotion problems in cattle are among the main causes of discomfort because they directly affect animal welfare, are commonly related to loss of productivity, leading to losses. It is one of the main causes of culling rates in dairy cattle. It is worth noting that diseases of non-infectious origin have a greater impact on the culling rates, due to the alterations in the conformation of the digits, which are mostly irreversible. Faced with this context, this doctoral thesis focused on the study of the factors involved in non-infectious diseases, specifically the nutrition factor and the anatomical characteristics. The nutrition factor was addressed in the first study, in which the aim was to understand the causal relationship between ruminal acidosis and the occurrence of laminitis by means of a systematic review in four databases and the bank of thesis (Capes). In this first study we found results that partially demonstrated the relationship between acidosis and acute laminitis, but the mechanism of development continues to be undefined. The second study aimed to describe the characteristics of the external digit conformation with the internal structures considered important for the health of the claw. Measurements of external and internal characteristics were carried out on 296 claws originating from cows that died in dairy farms from January to June 2018. *Post-mortem* evaluation of the hooves revealed that the length of the dorsal wall was the main associated external characteristic (44%) of feet with inadequate sole thickness (<7 mm) were found in primiparous cows. Claws with inadequate sole thickness and third phalangeal rotation represented 22% of the results.

Keywords: health of the claws, claw anatomy, nutrition and ruminal acidosis

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LISTA DE ABREVIATURAS

SARA	Acidose ruminal subaguda
CHLD ⁺²	Claw horn lesions desruptions
DWL	Dorsal wall length
HH	Heel height
IDWL	Internal dorsal wall length
S1	Sole thickness at S1
S2	Sole thickness at S2
S3	Sole thickness at S3
SUB1	Subcutenous layer at local 1
SUB2	Subcutenous layer at local 2

CAPÍTULO I

1. Introdução geral

Claudicação não infecciosa é um problema comum em bovinos leiteiros, associado a prejuízos como a redução da produção leiteira e perdas reprodutivas (Garbarino et al., 2004; Archer et al., 2010; Reader et al., 2011). Impacta de forma direta o bem-estar animal, devido a dor e ao desconforto muitas vezes envolvidas (Whay et al., 1998; Dyer et al., 2007; Tadich et al., 2013; Passos et al., 2017). Animais afetados caminham com dificuldade e são desafiados na realização das atividades diárias, desde caminhar para a sala de ordenha até se locomover para a busca de alimentos e água (Ito et al., 2010; Miguel-Pacheco et al., 2014; Norrington et al., 2014).

A busca pelo aumento da eficiência produtiva de bovinos aumentou o número de confinamentos, possibilitando o uso de dietas mais controladas e a expressão do potencial genético dos animais. No entanto, o confinamento ao utilizar dietas com elevada proporção de concentrados e restringir a movimentação e o espaço dos animais pode causar problemas de claudicação em bovinos de corte e leite. A simultaneidade é atribuída ao elevado consumo de carboidratos facilmente digestíveis, sendo este o mais reconhecido fator causador de laminite (Nocek, 1997). O elevado consumo de grãos facilmente fermentáveis no rúmen aumenta a produção de ácidos graxos de cadeia curta, levando à diminuição do pH ruminal (Metzler-Zebeli et al., 2013). No ambiente ruminal acidificado ocorre a morte de bactérias e a liberação de endotoxinas, como os lipopolissacarídeos (Zebeli e Metzler-Zebeli, 2012), que atingem a circulação sistêmica e, ao chegar nos vasos periféricos, causam lesões e isquemia, que hipoteticamente causariam a laminite.

Apesar de alguns estudos descreverem sinais que podem ser interpretados como laminite em protocolos de indução de acidose ruminal, ainda não está totalmente clara a relação entre acidose ruminal (aguda e subaguda) e laminite, sobretudo em casos subclínicos e crônicos (Momcilovic et al., 2000; Donovan et al., 2004; Danscher et al., 2009). A principal razão é que a nutrição não é o único fator envolvido, pois uma série de outros fatores como as características dos animais, o tipo de piso, alterações metabólicas e hormonais no pós-parto atuam juntamente ao contexto de dieta com alto nível energético (Tarlton et al., 2002; Bicalho et al., 2009, Solano et al., 2015). Além dos fatores relacionados ao ambiente e características dos animais, a maneira como os animais são manejados é um relevante fator, dentro do manejo pode se citar que as práticas de casqueamento preventivo são adotadas no intuito de prevenir problemas podais, no entanto a adoção dessa medida requer um funcionário habilitado com um mínimo de conhecimentos sobre anatomia podal.

O objetivo principal do casqueamento preventivo é reestabelecer o equilíbrio do peso animal nas úngulas mediais e laterais, de forma a evitar a sobrecarga peso sobre apenas um dígito. Busca-se uma reestruturação anatômica e para isso é necessário o entendimento a respeito de que padrão anatômico corresponde a forma saudável. A literatura científica para isso tem se baseado em um guia proposto por Toussaint-Raven (1985), o qual descreve um valor mínimo de comprimento da parede dorsal de 75 mm para vacas da raça

holandês, supostamente os profissionais que baseiam se neste método devem cortar a pinça, atingindo um valor de 75mm que estaria associado a uma espessura de sola de 5 a 7 mm, o qual exerce uma função protetiva do estojo córneo.

Entretanto estudos mais recentes (Tsuka et al.,2014; Archer et al.,2015) encontraram evidências de que o comprimento de 75 mm para parede dorsal nem sempre está associado a uma espessura mínima adequada de sola, o que torna os animais mais suscetíveis a desenvolver doenças na pinça. Não está claro na literatura o comprimento ideal para pinça, pouco conhecimento validado cientificamente suporta as atividades técnicas a campo.

Delinear estudos que envolvam esses diferentes fatores relativos ao ambiente, manejo e mudanças fisiológicas do parto, é um grande desafio para o avanço desta área de pesquisa. A revisão sistemática e a meta-análise podem ser importantes ferramentas para esclarecer dúvidas da etiopatogenia da laminite bovina. A primeira baseia-se em uma revisão fundamentada em uma questão claramente formulada, que faz uso de métodos sistemáticos e explícitos para identificar, selecionar e avaliar, de maneira crítica, pesquisas relevantes, além de coletar e analisar dados de estudos que estejam incluídos na revisão. O método estatístico, que é a meta-análise, auxilia na análise e sumarização dos dados coletados, permitindo uma revisão quantitativa dos resultados de estudos independentes, com aumento do tamanho da amostra e, portanto, melhorando o poder da análise estatística (Normand, 1999; Lovatto et al., 2007; Moher et al., 2009). Dessa maneira, permite transcender o resultado de análises anteriores, sendo uma reflexão crítica sobre elas e, por isso, considerada “a análise das análises” (Luiz, 2002).

Maiores esclarecimentos sobre a relação causal da nutrição e problemas de laminite se fazem necessários para um maior entendimento do processo, que possibilita uma prevenção eficaz das desordens e ao mesmo tempo garantir que sistemas intensivos atuem com segurança ao alimentar os animais sem que ocorra queda em produtividade.

2. Revisão de bibliográfica

2.1. Conceitos anatômicos relevantes

A extremidade do membro bovino é constituída externamente por quatro dígitos: dois principais que se apoiam no chão (III e IV), também denominados dígitos lateral e medial e dois vestigiais (II e V) conhecidos popularmente como unhas acessórias. Morfologicamente a parte exterior de um membro possui as subáreas denominadas de banda coronária, perioplo, pinça, espaço interdigital, linha branca, parede ou muralha, sola, bulbo do talão, talão. A parte interna de cada dígito é composta por três falanges, a proximal, média e distal, falanges distais e médias estão inseridas no interior do estojo córneo ou casco. Internamente é possível observar também tendões, articulações, ligamentos, coxim digital nervos e vasos sanguíneos (Nicoletti, 2004; Monte, 2006).

A parte mais externa e não vascularizada é denominada estrato córneo ou epiderme, formada por filamentos alinhados e estabilizados, os quais garantem flexibilidade, elasticidade e resistência ao estrato córneo (Tomlinson et al.,2004; Bragulla and Homberger,2009). Seguindo da epiderme, encontra-se a camada derme também denominada por cório, o qual corresponde a parte vascularizada e inervada; nesta camada estão localizadas as papilas ou lâminas na junção derme-epiderme. O cório também possui em sua composição fibras colágenas que formam o aparato suspensório do dígito responsáveis pela estabilidade da falange distal no interior do casco (Shearer and Amstel., 2013). Existe ainda uma camada denominada de subcutâneo, porém é uma camada presente apenas em alguns segmentos do casco, como por exemplo, no bulbo ou talão. Imerso ao tecido subcutâneo localiza-se o coxim digital formado de almofadas de gordura e tecido conjuntivo, os quais servem como um amortecedor de choques sob a terceira falange e no calcanhar (Räber et al., 2004).

A integridade das estruturas anatômicas garante o bom funcionamento da locomoção de bovinos, de forma que o impacto do peso do animal durante a caminhada sobre os dígitos é internamente recebido pelo eixo de sustentação do osso das falanges (Shearer and Amstel.,2013a), o qual tem o impacto amortecido pelo coxim digital, em especial esta estrutura de almofadas de gordura minimiza o impacto da pressão exercida pela falange distal e osso navicular na derme (cório tecidual) (Räber et al., 2004, Bicalho et al.,2009). O aparato suspensório fixa a terceira falange através de fibras de colágeno firmemente aderidas, as quais evitam que esta estrutura óssea afunde ou rotacione, contribuindo na diminuição do impacto, o que evita as contusões internas ao estojo córneo (Maierl et al., 2002) Externamente o dígito bovino também necessita de estruturas saudáveis e resistentes, e neste sentido a sola é o componente anatômico que possui maior contato com o solo, necessitando de uma espessura mínima para manter as funções protetivas de barreira entre o ambiente e as estruturas anatômicas internas (Van Amstel et al.,2003; Sanders et al.,2009). A sola esta aderida à muralha por meio da linha branca, que é

composta por um tecido córneo macio e sensível. A sola e a muralha suportam juntas o maior peso do animal (Mulling and Budras, 2003).

As estruturas externas que constituem o estojo córneo (sola, muralha, linha branca, talão e pinça) são dependentes de um sistema regular e funcional de queratinização, o qual necessita de um eficaz sistema de irrigação que garanta o aporte de oxigênio e nutrientes no perioplo, local onde são gerados novos queratinócitos. Porém a queratinização apenas inicia o processo na região coronária, e, à medida que estes queratinócitos amadurecem, eles são movidos em direção à pinça. Assim o processo depende também da integridade de todos os outros estratos da epiderme que necessitam de otimizado suprimento sanguíneo, afim de que tornem viáveis a finalização do processo de queratinização (Vermunt and Greenough, 1995).

Qualquer evento ou insulto que possam alterar a irrigação sanguínea, o processo de queratinização ou a absorção do impacto da terceira falange tendem a predispor o aparecimento de lesões em todas as estruturas dos dígitos (Vermunt and Greenough, 1995). A manutenção das características anatômicas do casco bovino é fundamental para garantir a saúde podal. O dígito bovino é duplamente desafiado por fatores inerentes ao metabolismo animal e também por fatores ambientais. Desafios que aumentaram com a intensificação da produção que, por sua vez, trouxe mudanças no ambiente e na dieta do animal, e cujas as consequências se refletirão no comum problema podal, denominado laminite.

2.2 Laminite (Definição e Patogênese)

Primeiramente definida por Nilson (1963) o qual a descreveu como “uma pododermatite asséptica difusa, aguda, subaguda ou crônica, geralmente envolvendo os tecidos de várias partes do casco e levando a sinais clínicos locais e principalmente sistêmicos”. Ossent et al. (1997) caracterizaram a laminite como o estado que apresenta um rápido início de dor nos dígitos e claudicação. Greenough et al. (2007) ampliou a definição para “uma doença sistêmica com manifestações locais na nos dígitos”.

Patogênese da Laminite

O desenvolvimento da laminite compreende 4 fases. A primeira fase se inicia quando ocorre a liberação na corrente sanguínea de uma substância vasoativa (endotoxinas, histaminas), que ao chegar na microcirculação do casco pode causar lesões endoteliais. A fase 2 é uma continuação da injúria mecânica nos vasos ocorrida anteriormente, uma vez que é instalado o edema, uma isquemia local também é desencadeada, conseqüentemente poucos nutrientes e menos oxigênio chegam até as células da epiderme. A fase 3 é caracterizada pela separação do extrato germinativo e epiderme (junção derme-epiderme), em função da diminuição de nutrientes ocorre também a degradação do cório. A fase 4 é marcada pela separação do extrato germinativo e cório, essas mudanças na camada lamilar resultam em alteração da terceira falange com relação a posição ao cório e parede dorsal, devido a esta mudança de posição do osso, ocorre uma maior compressão sobre os tecidos moles entre o osso e a sola. Esta situação

pode resultar em hemorragia, trombose, edema, isquemia com riscos de ocorrer áreas de necrose (Nocek, 1997; Ossent and Lischer, 1998; Blowey and Weaver, 2011).

2.3. Acidose ruminal

A acidose ruminal é comumente apresentada em duas diferentes formas; acidose ruminal subaguda (ARS) e acidose láctica ruminal aguda (ALRA) (Danscher et al., 2015). As causas principais são associadas ao consumo excessivo de carboidratos facilmente fermentáveis, reduzido tamanho da partícula e baixa quantidade e qualidade de fibra efetiva, os quais provocam aumento na produção de ácidos graxos cadeia curta no interior do rúmen, reduzindo o pH ruminal. Um desequilíbrio na microbiota é gerado, em função do aumento progressivo do número de bactérias produtoras de ácido láctico, como *Streptococcus bovis* e *Lactobacillus* sp., além disso ocorre também morte progressiva de bactérias fermentadores de ácido láctico, como *Megasphaera eldesnii* e *Selenomonas ruminantium* (Nagaraja; Lechtenberg, 2007). Devido ao declínio da população que utiliza ácido láctico, o gênero *Lactobacillus* passa a predominar na microbiota ruminal, alterando a simbiose dos microrganismos (Zebeli e Metzler-Zebeli, 2012), com conseqüente incremento na produção de ácido láctico e reduzindo ainda mais o pH ruminal.

Diagnósticos ARS são baseados em sinais clínicos e também valores de pH ruminal. Valores de pH ruminal entre 5.1 a 5.8 por um período mínimo de 3 horas consecutivas caracterizam o quadro (Danscher et al., 2015), que são vistos com frequência em sistemas de produção que fornecem dietas com alta proporção de concentrados e baixa fibra efetiva. Tipicamente isto ocorre no início da lactação devido ao menor desenvolvimento das papilas ruminais provocado pelo uso de dietas com menor digestibilidade durante o período seco. Este quadro é denominado acidose ruminal subaguda (SARA), comum em confinamentos, sistemas pastoris que utilizam aveia e azevém (Noro and Noro, 2015) pastagens tropicais que utilizam suplementação e ainda em situações de estresse calórico. Por sua vez a ALRA representa um quadro de desafio para a saúde animal, devido a comum alteração clínica dos animais com manifestação de desidratação, diarreia, apatia, frequência cardíaca e respiratória aumentadas. O valor de pH ruminal característico é inferior a 5.0, valores inferiores a 4.5 propiciam o crescimento de *Lactobacillus*, grande quantidade de ácido láctico é gerado nas formas L e D.

2.4. Relação entre Acidose e Laminite

O rápido declínio do pH ruminal gera respostas sistêmicas no indivíduo (Nocek, 1997), a principal causa disso se dá a partir da morte de bactérias ruminais (Gram-negativas), através dessas ocorre a liberação de endotoxinas lipopolissacarídicas da parede celular para o ambiente ruminal (Andersen, 2003). A hipótese mais aceita para a absorção dessa endotoxina é a intestinal, uma vez que o rúmen é revestido por camadas celulares de forte aderência, entretanto em casos de lesões no epitélio ruminal torna se viável a chegada desta substancia na corrente sanguínea. Após a absorção, as endotoxinas chegam a circulação porta, passando pelo fígado e neste podem ser eliminadas do

organismo, variando conforme o funcionamento da função hepática do animal (Andersen, 2003; Khafipour et al., 2009).

Ao atingir a circulação sanguínea, as endotoxinas podem ativar mecanismos da imunidade inata, assim os macrófagos passam a produzir e liberar de diversas citocinas pró-inflamatórias (interleucinas 1 e 6 e fator de necrose tumoral) e proteínas de fase aguda como amiloide sérica A e haptoglobina (Zebeli and Metzler-Zebeli, 2012) A elevada quantidade de endotoxinas no organismo, determina quadros de endotoxemia o qual possui o efeito na circulação devido a característica vasoconstritora que pode ser estendida até a microcirculação. Alguns estudos (Boosman et al., 1991; Mortensen., 1986) conseguiram observar alterações na microcirculação levando a laminite após a injeção de endotoxina na artéria digital.

Histamina, tiramina, ácido láctico, serotonina e endotoxinas são exemplos de substâncias vasoativas produzidas no trato gastro-intestinal. Algumas dessas toxinas podem afetar a perfusão vascular no dígito bovino e causar variados efeitos na microvasculatura digital, prejudicando a perfusão tecidual da derme e epiderme (Greenough, 2007). Um trabalho desenvolvido por Christmann et al., 2002 observou um aumento na pressão capilar e resistência pós-capilar após uma sobrecarga induzida de concentrado, esses autores encontraram transudação de líquido e aumento de pressão no interior do dígito. Segundo Singh & Murray (1994) a constrição venosa é o fator iniciante nas alterações vasculares da laminite, esses autores associaram coagulação intravascular disseminada à ação das endotoxinas, também encontraram que a constrição foi o principal achado em uma avaliação arteriográfica de dígitos de bovinos com e sem lesões na sola. Essas alterações vasculares podem causar congestão, edema e trombose, com conseqüente hipóxia e necrose do cório, tecido conjuntivo, membrana basal e epiderme viva (Van Amstel, 2009).

A histamina é conhecida pelo seu potencial vasodilatador e arterial constritor, sabe-se que esta substância é gerada após a alimentação e pode ser acumulada em condições de acidose ruminal. No entanto, a origem e formação da histamina é um ponto controverso entre autores, segundo Rodwell (1953) a histamina pode ser formada a partir da descarboxilação da histidina em várias espécies de lactobacilos. Já Sanford (1963) sugeriu que a formação de histamina é resultado da mudança da população microbiana ruminal.

Das pesquisas que estudaram a relação entre laminite e histamina, foram encontrados alguns indícios desta relação, em um trabalho foi feita a identificação dos primeiros sinais clínicos de alterações circulatórias condizente ao quadro de laminite, após essa constatação, os animais receberam tratamento de anti-histamínico, e foi observado que os sinais hemodinâmicos foram revertidos, sugerindo o possível mecanismo de ação da histamina. Outras evidências desta relação foi encontrada em um estudo feito por Macclean (1970), o qual relacionou elevada concentração de histamina em casos agudos e crônicos de laminite. As possíveis explicações da elevada concentração de histamina nesses dois casos são distintas segundo o autor, a primeira estaria diretamente relacionada ao insulto metabólico causado pela acidose ruminal, já

no segundo caso, pode ser em virtude do processo de separação tecidual do cório, estresse e degradação de proteínas que podem resultar em liberação de histamina.

Apesar de estudos científicos e recomendações práticas relacionarem condições de acidose ruminal, concentração de histamina e ocorrência de laminite, o mecanismo de ação deste processo não é totalmente conhecido. Estudos prévios apontaram fatores limitantes desta teoria como por exemplo a não direta responsabilidade pelos danos epiteliais (Ahrens,1967), a baixa energia de absorção da histamina, sendo que esta absorção aumenta em casos de pH ácido do rúmen, uma vez absorvida torna-se inativa (Goth,1974). Sendo este um dos principais elos da relação entre acidose e laminite, compreende-lo como atua, traria luz também para a compreensão das diversas formas de laminite descritas na literatura.

2.5. Diferentes formas da Laminite

A literatura clássica costuma dividir casos de laminite em diferentes abordagens (Nocek,1997; Greenough, 2007). Casos na forma aguda, são considerados raros na literatura, nesta forma de laminite os animais adoecem de forma sistêmica, pode –se observar respiração acelerada, aumento dos batimentos cardíacos, fezes líquidas, papilas ruminais paradas, escore de locomoção alterado, animal pode andar com os “cotovelos”. Ocorre também alterações locais, em função da inflamação do cório muitas vezes pode ser visto o ingurgitamento superficial de veias, intensa dor, vermelhidão na região coronária, acompanhado de aumento de volume e temperatura na região. (Nocek,1997; Shearer and Amstel., 2013b). Baseado nessas características, conceitua-se laminite aguda como uma doença sistêmica que apresenta manifestações locais (Thoefner et al.,2004).

Forma subaguda é definida como uma forma mais branda de casos agudos, quando os sinais observados são desconforto moderado ao caminhar, animais andam com cuidado, inchaços da borda coronária desaparecem espontaneamente, linhas de injúria costumam aparecer sobre a parede dorsal, o que pode causar fissura (Nocek,1997, Greenough, 2007).

Na laminite subclínica não ocorrem manifestações de sinais sistêmicos, porém pode haver deformações no formato e crescimento do casco. Animais não alteram escore de locomoção, e ao exame clínico dos dígitos é possível observar em alguns casos o casco macio e descolorido, além de solas amareladas ou hemorrágicas. Estima-se que as alterações ocorram em média após 2-3 meses após o evento da acidose (Nocek,1997, Greenough, 2007).

Laminite crônica é caracterizada por alteração no formato do dígito, pois, os cascos apresentam crescimento excessivo, e se assemelham a chinelos, ou ainda apresentam deformações no formato de tesoura ou saca-rolhas. Outra característica relevante são as linhas horizontais na parede dorsal, quando são profundas tornam a muralha com um formato côncavo (Nocek,1997, Greenough, 2007).

2.6. Estudos de indução da laminite

Laminite aguda foi a única forma induzida experimentalmente com êxito (Thoefner et al.,2004; Danscher et al.,2009), nesses estudos foram realizados a indução de acidose por meio da oligofrutose (Sousa, 2017; Noronha, 2017; Danscher et al.,2009; Thoefner et al.,2004). Os animais apresentaram sinais de acidose clínica, com baixos valores de pH nas primeiras 48 h de indução e foram considerados condizentes com o quadro de laminite aguda, no qual os animais manifestaram alterações sistêmicas (escore de locomoção) e reações locais (teste de sensibilidade nos dígitos). Em média as respostas sistêmicas e locais iniciaram após 24h da indução, e as reações de sensibilidade nos dígitos foram observadas até 216 horas após a indução por Danscher et al. (2009). Tais reações de sensibilidade nos dígitos foram interpretadas pelos autores como presença de dor, o que estaria condizente com a fase 1 da patogênese da laminite em que após a lesão vascular da microcirculação dos dígitos ocorre o aumento da permeabilidade e vazamento de exudatos, que resultam em edemas internos, hemorragias no cório, responsáveis por intensa dor.

Ao delinear um estudo com intenção de induzir laminite, é importante definir qual forma de laminite se objetiva avaliar, para escolher a melhor maneira de induzir acidose, assim como também escolher os métodos diagnósticos que serão usados como indicadores de que os animais de fato estiveram em um processo acidótico ruminal agudo ou subclínico, o qual resultou em laminite. Ressalta-se que cada forma de laminite (aguda, subclínica, crônica) pode requerer diferentes meios diagnósticos, devido à complexidade da patogênese da doença com suas diferentes fases (Ossent and Lischer, 1998; Blowey and Weaver, 2011).

Até o presente momento, estudos que de alguma forma objetivaram avaliar os efeitos subclínicos e crônicos da laminite (Momcilovic et al., 2000; Donovan et al., 2004) fizeram uso da indução via alimentação com altas doses de concentrado na dieta. Momcilovic et al. (2000) induziram acidose através de diferentes dietas (níveis de energia e proteína) em bezerras leiteiras, e nas primeiras 48h observaram sinais clínicos de acidose como letargia, diarreia e alteração de coloração e odor do líquido ruminal, com redução do pH. Apesar do sucesso em induzir acidose, não foram observados sinais agudos de laminite com os métodos diagnósticos utilizados pelos autores nas primeiras 48 h (radiografia e termografia). Possivelmente os autores esperavam pelo aumento significativo da temperatura nos membros, como uma forma de prever a presença de inflamação esperada no curso da doença e na radiografia buscava-se pela movimentação da terceira falange, porém não obtiveram nenhuma relação significativa.

A rotação de terceira falange é comumente explicada pelo insulto ao aparato suspensório que mediante a situação de acidose tem suas fibras de colágeno degradadas devido à ação das enzimas metaloproteinases de matriz, as quais são ativadas pela presença endotoxinas, lactato e citocinas, interleucina 1 e fator de necrose tumoral alfa (Mulling & Greenough, 2006). Afim de testar o modo de ação das metaloproteinases, Mulling et al. (2004) expuseram colágeno dermal de diferentes regiões do casco a dois tipos de metaloproteinases (tipos 2

e 9). Os autores, neste estudo *in vitro*, verificaram a degradação das fibras colágenas. Um estudo *in vivo* feito por Danscher et al. (2010), avaliou a biomecânica do aparato suspensório em novilhas induzidas à acidose ruminal, porém não foi encontrada diferenças significativas de que o insulto da acidose provocaria mudanças no aparato suspensório nas primeiras 72 h. Estes resultados sugeriram que, durante a fase aguda do processo de laminite, ainda não são perceptíveis mudanças com relação à movimentação da terceira falange, possivelmente a degradação das fibras de colágeno requer um tempo, para assim que ocorrer e iniciar o processo de movimentação da falange.

A teoria clássica da laminite aponta que a rotação de terceira falange é uma mudança estrutural mais comum em casos subclínicos, a qual tem forte influência sobre os casos de doenças do estôjo córneo. Pesquisas realizadas no âmbito de relacionar a laminite subclínica com alteração ruminal (Momcilovic et al., 2000; Donovan et al., 2004) não conseguiram elucidar se esta relação possui efeito causal, sobretudo pela subjetividade na avaliação do desenvolvimento da doença e interpretação dos sinais clínicos. Estima-se que este processo se desenvolva em torno de 2-3 meses após a exposição de alta carga de carboidratos facilmente digestíveis (Greenough, 2007) O estudo de Momcilovic et al. (2000) realizou inspeção dos dígitos em dois momentos após a indução de acidose via alimentação, primeiramente depois 3 meses e posteriormente após 7 meses. A primeira inspeção encontrou características que podem ser consideradas como sinais de laminite subclínica, quando foram diagnosticadas alterações de presença de linhas horizontais fundas, erosão de sola e talão. Um fato que chama atenção nestes resultados, é de que a maioria dos animais que foram alimentados com dieta de baixo nível de energia, também apresentaram tais alterações. No entanto os autores não justificaram possíveis razões para este fato, nem elencaram a possibilidade de outros fatores estarem envolvidos.

Outro estudo sobre a relação entre dieta e laminite subclínica foi desenvolvido por Donovan et al. (2004). Os autores buscavam pela relação entre o uso de dietas de transição (pré e pós-parto) com níveis altos e baixos energia com a ocorrência de laminite subclínica. O indicador principal usado para avaliar a condição clínica neste estudo, foi o método de classificação das solas proposto por Greenough & Vermunt (1991), que consiste em numerar as zonas da sola e classifica-las conforme a presença de pontos hemorrágicos, áreas de descoloração, presença de hemorragias, desintegração do tecido córneo e úlceras de sola. Os autores encontraram uma interação entre dias em lactação, pontuação das solas e diferentes grupos de dieta. E observaram que animais que receberam dietas de baixa energia no pré-parto e dieta com alta energia no pós-parto tiveram pontuações da sola superiores aos demais grupos. No entanto os autores relatam não terem encontrado a direta relação entre acidose ruminal e laminite subclínica. Devido ao tempo relativamente longo (2-3 meses), outros fatores também passam a interferir como por exemplo o ambiente e tipo de manejo. Além disso, os estudos randomizados encontram dificuldades em elaborar um delineamento que reproduza de forma estatisticamente correta a natureza multifatorial da laminite subclínica e crônica.

2.7. Fatores não nutricionais que influenciam doenças não infecciosas

É unânime entre os autores, a natureza multifatorial da claudicação bovina. Há mais de 20 anos (Greenough and Vermunt, 1991) é reconhecido que além da nutrição, o manejo dos animais em confinamento, o período de transição para vacas leiteiras, a idade, a genética, a conformação e o comportamento estão entre os fatores que predispõe à laminite. A criação de gado de maneira intensiva tem sido apontada como a grande influenciadora de doenças não infecciosas, uma vez que a maioria das vacas confinadas manifesta algum grau de claudicação (Shearer and Van Amstel, 2017).

Influência do ambiente na laminite

O tipo de piso é fator relevante no desenvolvimento de lesões associadas à laminite, especialmente em interação com outros fatores de risco (Holzhauer et al., 2008; Cook and Nordlund, 2009). Pisos muito ásperos, como concreto, empregado nas instalações de grandes propriedades leiteiras, desgastam excessivamente o tecido córneo do casco, que pode superar o seu crescimento (Cook et al., 2004; Mulling et al., 2006). Como consequência, ocorre a produção de tecido córneo de qualidade inferior que, quando submetido ao maior desgaste provocado pelo concreto, resulta em sola de pouca espessura. Esse fato predispõe o desenvolvimento da laminite, promovendo maior incidência de lesões como úlcera de sola e lesões na linha branca (Mulling, 2002, Mulling and Greenough, 2006).

Outro aspecto importante é o ambiente para o repouso em decúbito. Um ambiente confortável para a vaca significa espaço suficiente para deitar e cobertura adequada de piso, como areia ou palha. Ressalta-se que em um ambiente confortável, as vacas podem passar de 12 a 15 horas diárias deitadas (Bergsten, 2003). O repouso em decúbito favorece a ruminação e a circulação sanguínea nas extremidades distais dos membros, considerados fatores benéficos para a saúde dos dígitos (Mulling et al., 2006; Greenough, 2007). Galpões de estabulação livre dimensionados de forma inadequada não favorecem o decúbito e são associados à maior incidência de lesões digitais (Bergsten, 2003; Norlund et al., 2004).

Parto

Ao longo dos anos foi permitido reconhecer que esta etapa exerce grande influência na ocorrência de doenças não infecciosas, sobretudo quando associadas à uma mudança brusca na dieta dos animais que não recebem uma dieta de transição com introdução moderada de energia (Vermunt and Greenough, 1994). Dentre as mudanças fisiológicas que ocorrem durante o parto, a principal delas é o aumento nos níveis de estrógeno e relaxina, os quais estimulam a presença de enzimas associadas ao remodelamento fisiológico e patológico de colágeno na região do cório laminar de fêmeas. Ocorre o aumento da atividade de enzimas metaloproteinases de matriz tipo 2 que atuam na degradação de colágeno. (Vermunt and Greenough, 1994; Bergstein, 2003; Knott et al., 2007). Como consequência pode haver um aumento na frouxidão

do tecido conjuntivo que suporta a terceira falange no interior do casco. Uma vez que a terceira falange se encontra instável, ocorre uma maior mobilidade do osso falangiano no interior do estojo córneo, conseqüente em função disso essa estrutura anatômica terá maior impacto sobre o cório laminar, predispondo a doenças na sola (Tarlton et al.,2002).

Após as mudanças fisiológicas que acontecem no parto, algumas alterações se estendem no pós-parto, de forma que, durante o primeiro terço da lactação, os animais se encontram em balanço energético negativo, o que acarreta em perda de condição corporal, devido à mobilização de reservas energéticas. A perda de gordura corporal também tem reflexo na redução da espessura da almofada coxim digital, que tem forma complexa na espessura e tamanho, e é composto de almofadas de gordura e tecido conjuntivo, os quais servem como um amortecedor de choques sob a terceira falange e no calcanhar (Raber et al.,2004). Bicalho et al (2009) observaram, com uma avaliação ultrassonográfica, que a espessura do coxim diminuiu de forma constante após o parto e associaram com o aumento do risco de lesões de tecido córneo (doença da linha branca e úlcera de sola). Esses autores concluíram que a prevalência de sola de úlceras e doença da linha branca foi significativamente associada à espessura do coxim digital, e este foi positivamente associado ao escore de condição corporal. Nesse caso se pode observar que problemas anteriormente apenas relacionados às sequelas da laminite (úlceras e doença de linha branca) aparecem desvinculados da teoria clássica de consequência de acidose ruminal subclínica.

Importante ressaltar que anteriormente a claudicação era reconhecida por afetar o bem estar e comportamento animal, conseqüentemente levaria à menor ingestão de alimentos e perda de escore de condição corporal. Bicalho et al. (2009) mostraram em seus estudos que esta relação também pode ser vista da forma inversa, assim o baixo ECC pode também levar um animal a claudicar. O emagrecimento, que ocasiona menor função protetora do coxim, combinado ao maior impacto da terceira falange no cório somado ao desafio das instalações inadequadas, desafio metabólico que animais de alta produção são submetidos compreendem as peças chaves no entendimento da etiologia da claudicação.

Existem diversos fatores envolvidos no desenvolvimento da laminite e suas sequelas. Apesar da interação entre os fatores envolvidos, foi utilizada outra denominação para designar problemas não infecciosos, denominada por "rompimento de tecido córneo do casco" (*claw horn disruption*) para se referir à laminite subclínica, em que os autores questionam o caráter inflamatório das lesões que caracterizam o quadro (Knott et al.,2007; Vermunt, 2007). Baseado nestas dúvidas, um estudo de revisão sistemática dos trabalhos que avaliaram a relação entre acidose ruminal e ocorrência de laminite pode auxiliar a compreender melhor a etiologia da doença para os diferentes sistemas de produção da espécie bovina de leite e corte. Uma vez entendida a etiologia, a prevenção se torna mais eficaz, além de direcionar novas pesquisas na área.

3. Hipóteses

1. Existe relação causal entre acidose decorrente do consumo elevado de carboidratos facilmente fermentáveis e a ocorrência de laminite nas formas aguda, subclínica e crônica.
2. A conformação anatômica externa dos dígitos se correlaciona com a saúde das estruturas internas. Dessa forma os valores recomendados para comprimento da parede dorsal, ângulo e altura de talão na prática de casqueamento estão relacionados com a saúde interna do dígito para a manutenção das funções protetivas.
3. Um valor mínimo para o comprimento de parede dorsal do dígito bovino é necessário para a manutenção da proteção mecânica da espessura de sola.

Objetivos

1. Revisar sistematicamente os estudos científicos primários que abordem a relação causa-efeito entre acidose ruminal causada por excesso de carboidratos solúveis na dieta e a ocorrência de laminite nas suas diversas formas em bovinos.
2. Descrever as características morfológicas anatômicas externa e interna dos dígitos de vacas da raça Jersey.
3. Avaliar a correlação entre as estruturas anatômicas internas e estruturas externas.

CAPÍTULO II
A SYSTEMATIC REVIEW OF THE RELATIONSHIP BETWEEN RUMEN
ACIDOSIS AND LAMINITIS ³

³ Article to be sent to Plos one Journal

A systematic review of the relationship between rumen acidosis and laminitis

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Introduction

Throughout history, the term laminitis has had several definitions. The first definition was done by Nilson (1963) who described it as “a diffuse, acute, subacute or chronic aseptic pododermatitis usually involving the tissues of several hooves and leading to local and mostly systemic clinical signs.” Ossent et al. (1997) characterized laminitis as the state presenting a rapid onset of claw pain and lameness. Greenough et al. (2007) has broadened the definition to “a systemic disease with local manifestations in the claw”. Various forms of laminitis have been described including acute, subacute, chronic and subclinical (Bradley et al., 1989; Bergsten, 1994); all forms have in common the supposed relation with nutrition and feeding practices such as high levels of non-fibrous carbohydrates (NFC), protein and poor NDF.

The classic theory consisted in the association between lowered rumen pH (or subacute ruminal acidosis (SARA) with laminitis. In summary the hypothesis started with initial local rumen disturbance (rumen acidosis) leading to a systemic metabolic insult causing vascular dysfunction in the hoof, with further degeneration at the dermal-epidermal junction in the lamellar region of the hoof and loss of structural integrity. Consequently the distal phalanx undergoes a movement that provoke compression of the sole corium, leading to the development of lesions in the horn capsule as well as discolouration in the sole and white line (Ossent and Lischer 1995, 1998; Randall et al.,2018).

However the relationship between nutrition/feeding practices and subclinical laminitis is still not completely understood. It is known that others factors are also important in laminitis onset, such as housing conditions, management practices, calving, floor type, feeding frequency management, mixed lots of animals with different ages, evidencing the multifactorial nature of this health condition (Nocek,1997; Lean et al.,2013). This systematic review aims to study the causal relationship between rumen acidosis caused by ingestion of highly fermentable carbohydrates and the occurrence of laminitis in cattle.

Materials and methods

Research question

The search strategy aimed to find peer-reviewed scientific literature that studied the relationship between rumen acidosis or SARA and laminitis in cattle.

Searches were systematically performed using the same strategy words the following databases: (1) Web of Science; (2) Pubmed; (3) Science Direct and (4) Scopus. The literature search was based on key concepts in terms of PICO (P= population, I= intervention, C= comparator and O=outcome). The literature search terms used were “(ruminant* OR bovine OR cattle_OR steer* OR cow* OR heifer*) AND (Acidosis OR SARA OR rumenitis OR “rumen pH”) AND (“Gait Disorders” OR lame* OR CHDL OR laminitis OR "sole ulcer" OR "white line disease" OR claudication OR “claw horn disruption”)”.

Eligibility criteria for studies

Before the screening, a first filter was used when selecting the abstracts. First removal of abstracts was based in information concerning the title and journal of publication, removing those studies performed in humans (346), studies involving other animal’s species such as horses, sheep or fishes (15), book chapters (105) and studies in plants (14).

The first step of assessment of screening questions consisted in the application test on 30 randomly selected abstracts. The screening questions were applied in all abstracts by two independent reviewers considering information in the title, abstract and key words. The questions were: 1) Does this abstract investigate primary research?; 2) Does this abstract investigate rumen acidosis in dairy or beef cattle?; and 3) Is this rumen acidosis associated with locomotion problems in these animals? All screening outputs were tabulated using Microsoft Excell.

When both reviewers responded ‘Yes’ for all questions, the abstract was considered eligible (fulfilling the criteria) and passed to the next step; and when both responded ‘No’ to at least one of the questions, the citation was excluded. Data conflicts were resolved by consensus, and the full manuscript was accessed when agreement was not attained.

Data collection process and items extracted

Extract form was piloted tested in 2 papers, and only one reviewer was responsible for this step. A form to evaluate the quality of the assessment and extraction of the informations for primary research studies was made using Microsoft Word. Data extracted from the studies included population, intervention, outcome measurements, results, and manuscript information (journal name, author(s) name(s), year of publication, and original language). An Excel sheet was built with the extracted data with the information collected.

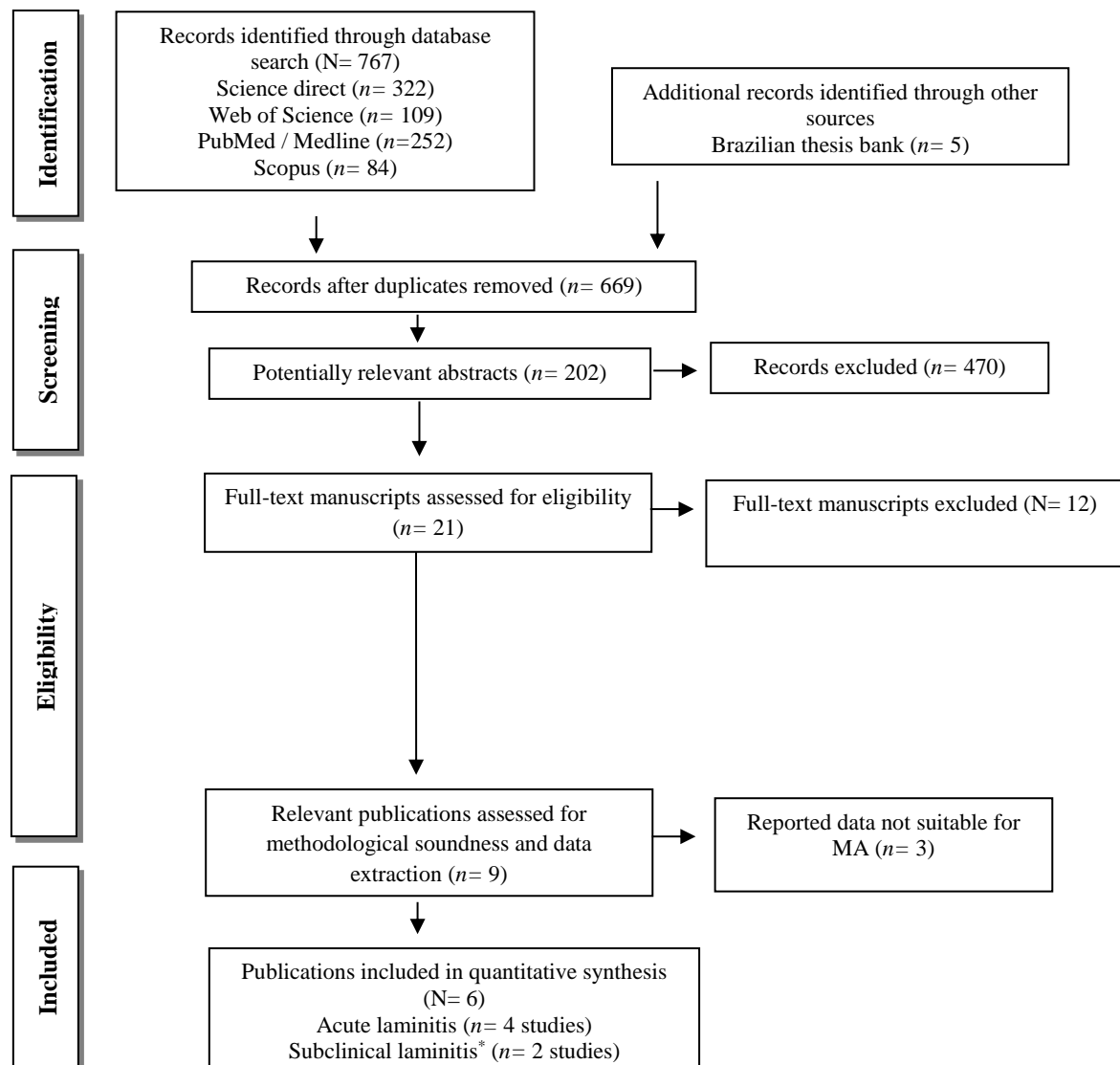


Fig.1: Flow diagram outlining the screening process for the systematic review of relationship between rumen acidosis and laminitis. Adapted from PRISMA guidelines (Moher et al. 2009).

Results

A total of 767 citations were obtained: 109 in Web of Science (1); 252 in Pubmed (2); 322 in Science Direct (3); 84 in Scopus (4) and 5 Brazilian thesis bank (5) (Fig 1). Electronic citations obtained were imported into the reference manager Mendeley and the duplicates were manually removed.

After the first screening of the candidate abstracts, 21 results were selected to be read as full paper, and only 4 results were selected to pass to next step. In addition, two thesis were selected when the grey literature was checked. A total of 6 citations were considered in the data collection process. The database was composed by 4 papers and 2 thesis which were considered in this systematic review: 2 papers and 2 thesis about induced rumen acidosis with the objective of observing occurrence of acute laminitis, 2 papers with intention of study occurrence of subclinical laminitis. Rumen acidosis was induced by two ways: dietary supply of NFC and oral administration of oligofructose. Researches that studied the acute laminitis, induced acute acidosis based in the methodology described by Thoenfer et al. (2004) that consisted in divided the dose of oligofructose in 3 subsequent days. Characteristics of the relevant experiments can be seen in table 1.

Table 1: General characteristics of the relevant experiments

Author(s)	Language	Study design	Country	Animals	Breeds	Intervention	Laminitis occurrence
Momcilovic et al., 2000	English	Controlled trial	EUA	16 steers	Holstein	Induced by feeding NFC?	Subclinical
Donovan et al., 2004	English	Controlled trial	EUA	98 cows	Holstein	Induced by feeding NFC?	Subclinical
					3 Jersey; 6 Holstein - friesian; 2 Ayrshire ; 1 Guernsey	Induced by oligofructose	Acute
Thoenfer et al., 2004	English	Controlled trial	Not reported	12 heifers		Induced by oligofructose	Acute
Danscher et al., 2009	English	Quasi-experiment	Denmark	8 heifers	Holstein	Induced by oligofructose	Acute
Noronha, 2017	Portuguese	Controlled trial	Brazil	6 steers	Zebuine Cross-breed	Induced by oligofructose	Acute
Sousa, 2017	Portuguese	Controlled trial	Brazil	29 cows	Nelore	Induced by oligofructose	Acute

Induction of ruminal acidosis

Experimental studies that induced acute acidosis with oligofructose ministered via intraruminal used the minimum dose 13g/kg and maximum 21g/kg (table 2), the induction period lasted three days and the animals were evaluated for the clinical status, blood and rumen pH. Parameters were taken at least 3 days prior to induction and continued for a maximum of 9 days. Randomized studies induced acidosis with high energy level using 1.64 to 1.78 Mcal NE/kg DM in dairy cows or 81.2% of TDN in dairy calves. Detailed information about animals groups and type of induction are described in table 2.

Table 2: Characteristics of induction and experimental groups

Author(s)	Type of Induction	Experimental groups
Momcilovic et al., 2000	High energy (TDN 81.3%;Low energy (71.3); High protein (20%); Low protein (15%)	LE+ LP; LE+HP; HE+LP; HE+HP
Donovan et al., 2004	Low NEL Pre partum (1.52 Mcal/kg);Low NEL Pos (1.71 Mcal/kg); High NEL Pre partum (1.64 Mcal/kg);High NEL Pos partum (1.78 Mcal/kg)	H/L ; H/H; L/L; L/H (Pre/Pos partum)
Thoefner et al., 2004	Oligofructose ministered orally 13, 17 and 21g/Kg	Oligofructose animals; Control animals
Danscher et al., 2009	Oligofructose orally 17g/Kg	Before; After
Noronha, 2017	Oligofructose orally 13 and 17g/Kg	Pilote group; experimental group
Sousa, 2017	Oligofructose orally 15,3g/Kg	Medicated group; Non-medicated group

In order to minimize errors in diagnosing rumen acidosis, the studies included in this review also used more than one indicator to characterize acidosis (*see details in table 3*). In the present systematic review, the studies measured rumen pH and other rumen characteristics and verified clinical symptoms to ascertain whether animals were acidotic.

Table 3. Indicators of rumen acidosis

Author(s)	Indicators of acidosis	Time of rumen pH collection	Collection method of rumen fluid to determine pH
Momcilovic et al., 2000	rumen pH; Total VFA	0 to 48h	via an esophageal tube
Donovan et al., 2004	rumen pH	14 d prepartum and 8, 22, 70 d postpartum	rumenocentesis
Thoefner et al., 2004	rumen pH; feces pH; rumen contractions	72 to 48 h	not reported
Danscher et al., 2009	general demeanor; feces consistency; rumen pH	72 to 216 h	via ruminal intubation
Noronha, 2017	rumen pH; ruminal motility; ruminal clinical examination	0 to 28 h	via ruminal cannula
Sousa, 2017	rumen pH; osmolarity of rumen fluid	0 to 72 h	rumenocentesis

Ruminal acidosis was found in 5 studies, and rumen pH was considered the main indicator. The trials measured rumen pH in time intervals ranging from 0-24h (Sousa, 2017), 0 to 72h ; and until 216h (more details in table 3). The lowest rumen pH values were 4,4; 4,3; 4,3; 4,6 and 4,8 was observed at 9 h, 10h, 18h, 9h and 24h after acidosis induction by Thoefner et al. (2004), Danscher et al. (2009), Sousa (2017) and Noronha (2017), respectively.

Subacute rumen acidosis was provoked by Donovan et al. (2004) feeding different levels of energy and fiber calculated for avoid serious digestive and metabolic diseases. Subacute acidosis was settled when rumen pH fell bellow 5.5 as a conservative cutoff. These authors did not verify significant relation between dietary treatment, parity group and rumen pH. However, they reported that 41% of cows fed high levels of energy during postpartum were classified as having rumen acidosis based in rumen pH bellow 5.0.

Clinical signs of acidosis

The proportion of the animals that developed any signs of clinical acidosis were 75% in Momcilovic et al. (2000), as they observed that six out eight calves fed with high energy and protein levels were recumbent in the first 24 hours, presented signs of anorexia, depression, discomfort, lethargy, stiffness, muscular tremor and diarrhea. The authors reported high intake of water during the first 12h after rumen acidosis induction (Thoefner et al, 2004), probably due to increased osmolarity in rumen, the hypovolemic state probably was caused by high levels of acid lactic, depending on the occurrence of

diarrhea and high heart rate All heifers used in the studies of Thoenfer et al. (2004) and Danscher et al. (2009) submitted to acute rumen acidosis induction using oligofructose developed signs of systemic metabolic acidosis, as both studies reported anorexia and depression in the first day of acidosis induction. Thoenfer et al. (2004) reported profuse and watery diarrhea that began at 9 h and continued until 33 h after induction. Five out six animals (experimental group) showed transient fever episode, concomitantly with diarrhea. In the study conducted by Danscher et al. (2009), all heifers developed watery diarrhea starting between 6 and 24 h after rumen acidosis induction, although some differences in the intensity signs of systemic disease were found, e.g. the heaviest heifers tended to show more severe symptoms of clinical acidosis symptoms such faster decline of rumen contractions and high heart rate than lighter heifers. Sousa (2017) reported 100% of success in inducing rumen acute acidosis in Zebu cattle and the authors described that induced heifers showed depression, ataxia and fasciculations. Noronha (2017) did not report severe clinic signs of acidosis, except high respiratory rates in both groups. The author reported that some animals presented slight discomfort while walking, and it was related to depression that is common in rumen acidosis cases.

Diagnosis of Laminitis

The methods used to diagnose acute and subclinical laminitis found in this systematic review are listed in table 4.

Table 4: Diagnostic methods for detecting laminitis

Author(s)	Diagnosis of laminitis
Momcilovic et al., 2000	Radiographed hooves; surface temperature and hooves inspection
Donovan et al., 2004	Hoof score; locomotion score
Thoenfer et al., 2004	Hoof testing; lameness examination and digital pulse strength, post-mortem biopsy
Danscher et al., 2009	Hoof testing; evaluation of tarso-crural joints; weight-shifting and locomotion score
Noronha, 2017	Hoof testing; locomotion score and biopsy of the claw
Sousa, 2017	Hoof testing; locomotion score, thermography and forces plates

Indicators of Acute Laminitis

Studies that induced acute laminitis used hoof test to demonstrate the local signs of the disease, thus positive reactions with frequency of 83.3 %, 43% and 93% in relation to the total number of animals induced were reported by Thoenfer et al. (2004), Danscher et al. (2009) and Sousa, (2017) respectively. Strongest positive results in hoof testing was observed 30 to 33h after acidosis induction (Thoenfer et al, 2004; Danscher et al., 2009), while Sousa (2017) reported after 24h. Other method used to evaluate the local signs of acute laminitis was foot palpation (Thoenfer et al.,2004), which consisted in palpation of digital arteries in an attempt to detect increased pulse amplitude. Palpation of tarso-crural

joints to detect some distension as well as weight-shifting behavior were considered as signs of laminitis (Danscher et al., 2009) (Danscher et al., 2009). Thermography was used to verify an expected temperature increase during the initial inflammatory phase (Momcilovic et al., 2000; Sousa, 2017) and considered an indicator of laminitis.

Although the distinct ways used by the authors to recognize local signals of acute laminitis, the most relevant method was hoof testing. Foot palpation used by Thoenner et al. (2004) resulted in highly variable results. Thermography used by Thoenner et al. (2004) generated data not shown in the paper. Sousa (2017) reported that infrared thermography had 96% of sensitivity and 60% of specificity to identify acute signals of acute laminitis. Clinical diagnosis for acute laminitis included the evaluation of lameness occurrence and it was usually assessed using locomotion score. In the studies, Thoenner et al. (2004) observed lameness in 66% of total induced animals after 39 h. Danscher et al. (2009) observed high locomotion scores (indicating severe lameness) between 60-120 h after rumen acidosis induction, reporting that 29% of cows received score 3 (scale from 1 to 5, 1 = normal and 5 = severely lame) and 32% of cows were scored with 2. Sousa (2017) reported that 85.1, 11.1% and 3.7% of induced cows were scored 2, 3 and 4 respectively. Danscher et al. (2009) and Sousa (2017) used the same scale based in Sprecher et al. (1997), and based in the fact it is possible to presume whereas these two studies found moderate lameness.

Four studies in this systematic review induced acute laminitis using oligofructose, and three of these studies were able to demonstrate consistent clinical change compatible with acute laminitis (Thoenner et al., 2004, Danscher et al., 2009 and Sousa, 2017). However, one study (Noronha, 2017) did not find any differences in hooves sensitivity and in locomotion score 28 hours after induction. Although no differences were found for sensitivity and locomotion score, Noronha (2017) observed histological changes observed in the biopsy (*in vivo*) of the digit dermis such as edema, inflammatory infiltrate, morphological alterations of the basal epidermis and basement membrane alterations, which are compatible with the inflammatory process triggered by the carbohydrate-mediated rumen overload. Thoenner et al. (2004) also found clear differences between lamellar regions of induced and control animals in histopathological examination of claw biopsies (*post-mortem*), with dermal hemorrhage and edema after 48 h of induction in 10 cows and after 72 h in other 2 cows.

Indicators of Subclinical Laminitis

Two studies used in this review examined the hooves of animals during a more extended period with objective to detect anatomical changes compatible with the subclinical manifestation. Momcilovic et al. (2000) performed hooves inspection at 3 and 7 mo after the administration of the induction diet. On the first exam, they found the following frequency of 93.7% (15/16) cases of hardship groove, 31.2% (5/16) cases of hoof wall separation, 31.2% (5/16) cases of sole and heel erosion (5/16) and just one case of double

sole (1/16). On the second exam the frequency of hardship groove was 68.7 (11/16) and just one case (1/16) for hemorrhage of sole, sole erosion (1/16) and heel erosion (1/16) were found at 7 mo. It is worth to consider that no significant differences were detected in the occurrence of diseases between diet groups, and hemorrhage of sole and double sole were recorded in animals fed the low energy diet. Another way to check subclinical laminitis signs was done by Donovan et al. (2004) using hoof score method (Greenough and Vermunt,1991) that consists in classify the occurrence of hemorrhages and sole ulcers. The authors suggested association between dietary treatment and hoof scores in zones 3 and 4 (area abaxial wall-bulb junction and sole-bulb junction). This experimental study submitted cows during transition period (pre and pos partum) to different levels of energy in the diet. The authors also noticed that as the days in lactation increased, there was an increase in the hoof score for all treatment diets. Around lactation peak (65 DIM) animals fed low energy in pre-partum and high energy in pos-partum showed significant higher hoof scores than the others groups that received high energy –high energy; high energy-low energy and low energy-low energy respectively in pre-partum/pos-partum. The hoof score classification used by these authors applied a 5-point scale (0 = no hemorrhages or discoloration, 1 =slight discoloration or yellow staining, 2 = moderate ecchymotic hemorrhages, 3 = severe hemorrhages or secondary horn disintegration, and 4 = exposed corium /sole ulcer) for access evidence of subclinical laminitis. These changes are calls subclinical because they do not necessarily change the gait of the animals. Donovan et al. (2004) did not verify significant correlation between lameness scores and hoof scores.

Discussion

The diagnostic of laminitis should consider the different stages of disease development and also the different forms of acute, subclinical and chronic cases. Acute laminitis is considered as a systemic disease with local manifestations (Greenough et al, 2007). Some symptoms are only expected in the acute form such as claw pain, accessed by animal's reaction during the hoof testing. A positive reaction to hoof testers can be anything from a mild flinch to raring up and snatching the hoof away from the person testing.. Acute inflammation of the coronary border cause claw pain and also may change the color of skin (redness) (Ossent and Lischer,1998). Despite subjectivity hoof testing presupposes that a positive reaction corresponds to the stage of development of the disease that involves inflammation of lamellar region (Ossent and Lischer,1998). To minimize errors, the authors considered positive response in the hoof test when two consecutive positive pain reactions were detected in the same claw. One author (Noronha, 2017) did not verify any differences in hooves sensitivity and locomotion score 28 h after induction, maybe due to the sampling time, as other authors reported some effect on sensitivity 39 hours after induction (Thoefner et al.,2004; Danscher et al.,2009). Noronha (2017) highlighted the differences between *Bos taurus taurus* and *Bos taurus indicus* following the acidosis

induction and also the differences in the claw conformation. The color of claw tended to be darker in Zebu and this characteristic is associated with the hardness and strength of the material (Dietz and Prietz,1981; Petersen et al.,1982), decreasing the development of laminitis.

Claw was observed before the changes in the locomotion score (Thoefner et al, 2004; Danscher et al., 2009).The causal relationship between between the increased reaction in the hoof testing (claw pain) and locomotion score (lameness) is questioned by Danscher et al. (2009). According to these authors, joint pain contributed primarily to changes in the locomotion score, while hoof pain may be responsible for late-phase lameness. They also observed that claw pain persisted during the trial, whereas lameness decreased, indicating that claw pain may not be the sole source of acute lameness in this model. Increased hooves sensitivity after induction using oligofructose could indicate alterations of lamellar region. Thoefner et al. (2005) supported this hypothesis, when they observed histological evidence of lamellar pathology after induction with oligofructose.

Investigations on the lamella were done by Thoefner et al. (2004) and Noronha et al. (2017) in the present systematic review. Histological alterations characterize the prodromal phase of disease development, when the obvious clinical signs could not be observed (Andersson and Berman,1980). Momcilovic et al. (2000) used radiographed images and hoof temperatures taken 48 hours after feeding a diet with high energy and protein contents, to study the early signs of laminitis before the occurrence of clinical signs. The authors could not detect any significant difference between treated and control animals. The duration of the initial phase of the insult until the appearance of the first clinical signs is still unclear. Momcilovic et al. (2000) used radiographed the hooves in an attempt to detect changes in rotation of distal phalanx. This alteration is explained due weakness of the suspensory tissues of the distal phalanx that is thought to occur during the development of laminitis (Shearer and Van Amstel, 2017).

Laminitis is recognized by physiologic or pathologic changes in the laminar corium (suspensory apparatus of distal phalanx). In theory, the release of vasoactive substances during acidosis decreases the blood flow to the corium and cause significant degeneration and damage to the dermal–epidermal junction in a short term. Consequently the sinking and downward displacement of distal phalanx may occur, increasing the compression-related injury of the corium and of the digital cushion beneath. Hemorrhage, thrombosis, and variable amounts of necrosis may be related to the contusion-related trauma caused by the sinking of distal phalanx, and the development of sole ulcers and white line disease could occur over a period of 8 to 12 weeks or more after the initial events. Alterations in the hoof characteristics and deformations in the horn tissue are expected during subclinical and chronic processes of laminitis (Nocek,1997; Ossent and Lischer, 1998; Blowey and Weaver,2011)

Subclinical laminitis is usually considered as a consequence of subacute rumen acidosis (SARA), based on the hypothesis that the high intake of energy (NFC) can damage the rumen mucosa, releasing toxic substances which can enter into the bloodstream, attaining the microcirculation at the claws. These toxins activate the matrix metalloproteinases (MMPs), which are enzymes that act degrading the collagen fibers, which in turn are responsible for maintaining distal phalanx inside the claw horn capsule (Riley et al., 2002). However the calving period itself has been associated with hormonal and metabolic changes that affect the connective tissues of hoof wall, suspensory apparatus (Webster, 2001, 2002; Tarlton et al., 2002). Reinforcing this idea, Knott et al., (2007) demonstrated that calving affected on the suspensory apparatus causing the increase of laxity. These authors defend the point of view that calving may be a potentially primary factor involved in the pathogenesis of claw lesions, but this theory is impossible to be applied for male beef cattle. Danscher et al. (2010) did not verify the effect of acute rumen acidosis (in a protocol with acidosis induced by oligofructose) could on the physiological support of suspensory tissue at 24 and 72 h after induction.

Consistent with this result Momcilovic et al. (2000) they not find phalangeal rotation in the first 48 h after setting rumen acidosis. Although they related some alterations in hoof characteristics that could be interpreted as evidences of subclinical laminitis three months after the onset of rumen acidosis, but their results were not significantly different between treatments, and also some animals fed the low energy+protein diet developed alterations in claw horn conformation. Trying to understand what causes the lesions, some questions may be raised: Does phalangeal rotation would be paramount for the development of claw horn lesions? Does changes in the third phalanx occur after acidosis or subacute acidosis insult? What other factors can influence? The relationship between acidosis and subclinical laminitis is more complicated to study in field trials, due to the multifactorial nature of this process, which has been recognized in the literature (Nocek, 1997). The randomized intervention study done by Donovan et al. (2004) observed the interaction between days in milk (DIM) and the increase of hoof scores, and these authors did not find evidences about the relationship between low rumen pH, rumen acidosis and subclinical laminitis. In this study the cows were in transition period, when several factors could have influenced the outcome, like hormonal changes and decrease in body condition score after parturition. It is expected that during first weeks after calving a large mobilization of the body reserves takes place, leading to expressive loss of body fat. This effect might be observed in digital cushion, this structure is located below the distal phalanx, has the important damping function (Räber, 2004). When the digital cushion becomes thin the capacity force-dissipating is reduced, and it may contribute to development of claw lesions (Bicalho et al., 2009).

Physiological, metabolic and hormonal changes may have contributed to increase in hoof scores (Donovan et al., 2004), added to this the cows were housed in free-stall and sand bed. The floor surface has been recognized as important factor modulating the impact of biomechanical forces on the claw, as a direct effect of compressibility or indirect effects

related to abrasiveness (Telezhenko et al., 2009; Bergsten et al., 2015). When stalls and bedding are not optimal, cows will choose to spend more time standing in the stalls and walkways, that consequently may negatively affect the claw (Bicalho and Oikomonou, 2013).

Associations between rumen acidosis and acute laminitis were partially evidenced in studies with induction protocols, maybe because of the short duration of the disease and fewer factors to influence. Alterations after oligofructose overload such as claw pain and lameness could be interpreted as signs of acute laminitis, but it is necessary to investigate some histological evidence of the claw. In this systematic review only 2 papers investigated the lamellar pathology. Thoefner et al. (2004) realized *post-mortem* biopsies and Noronha (2017) biopsy fragments of the coronary region and the abaxial wall. Moreover, the mechanism underlying how acidosis could damage the lamellar tissue it is not fully understood. The theory that involves release of histamine and endotoxins needs to be more studied.

Conclusions

This systematic review sought to understand relation of cause and effect between laminitis and acidosis, however few studies were found with this theme, covering the whole process: feeding with high concentrate diet, setting of rumen acidosis and measuring alterations in the hoofs compatible with laminitis, and therefore it resulted in a very limited database. Wide divergences between feeding practices, acidosis and laminitis diagnose methods precludes performing meta-analysis with the present database. Studies about acute laminitis were partially comparable, and enabled us to partially demonstrate the existing relationship between acidosis and acute laminitis, however the mechanism of action is still unclear.

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CAPÍTULO III

CLAW MEASURES OF JERSEY COWS: AN ANATOMY STUDY

CLAW MEASURES OF JERSEY COWS: AN ANATOMY STUDY

PASSOS, L.T; FISCHER,V and SILVA-DEL-RÍO, N

Introduction

Lameness has been associated with pain and discomfort (Dyer et al., 2007; Tadich et al., 2013; Passos et al., 2017) and it is also associated with large economic losses (Archer et al., 2010; Reader et al., 2011). Trimming is performed to restore the distribution of forces on the digits (Shearer and van Amstel, 2001) and it is used in the healing process in some hoof diseases. In the recommendation's guide by Toussaint-Raven (1985) the "Dutch Method" recommends the cut the dorsal wall of the claw to 75 mm. On the other hand, some studies (Nuss and Paul.,2006; Tsuka et al.,2014; Archer et al.,2015) contradict this recommendation, claiming that 75 mm for dorsal wall length is not appropriated for all cases, and may lead to over trimming.

The main consequence consisted in withdraw excessively the sole horn in the toe, and might result in thinner soles. A minimum sole thickness threshold of 7 mm is recommended for maintaining protective function (Toussaint Raven,1989;Saunders et al.,2009). Anatomically, the junction of the hard claw wall and the softer sole horn is formed by the white line horn, which is considered a weak area in the horn capsule and may suffer trauma (Shearer and Van Amstel,2013) as initial insult. However this situation may evolved to more severe lesions as "toe-tip necrosis syndrome" with many possibilities such interphalangeal arthritis, osteomyelitis of the middle and proximal phalanges, flexor tendonitis, cellulitis, and the embolic spread of bacteria to the lungs, liver, and kidneys (Klofer, 2017).

Although it is recognized the importance claw conformation to maintain health of the digits, little is known about the optimal values to be use in the trimming of Jersey cows. The goals of the present study were to study the forelimb claws from Jersey and Jersey x Holstein crossbred cows: (1) to describe internal and external morphological characteristics, (2) to evaluate how external structures of claws correlates with internal structures (3) to identify from what value to DWL corresponding with adequate sole thickness.

MATERIALS AND METHODS

Cows and Herd Management

From February to May 2018, 1 dry-lot and 2 free-stall commercial dairies located in Tulare county (California, US) were visited on 20 occasions from 6 to 9 am before the arrival of the rendering pick-up truck. Feet from hind limbs of cadaver Jersey cows and Holstein × Jersey crossbreed cows were harvested above the claws using a cordless electrical saw (BDCR20B Black & Decker; Towson, Maryland,US). The feet were identified with the following information [side (right - left), date, name of dairy and cow ID] and placed in a 4-liter size ziplock bag (manufacture) for transportation to the lab of Veterinary Medicine Teaching Research Center. Once in the lab, the feet were stored in the freezer at - 20 °C until further examination and measurements.

Based in the cow's number ID we retrieved information about historical life as age, days in milk, parity, hoof trimming historical and hoof treatments such as foot bath. When available, death cause was registered.

Claw Observations and Measurements. Cadaver claws were thoroughly cleaned before examination using water and a scrubbing brush. A single observer (L.P.) assessed the integrity of all the claws as well as performed all the external and internal claw

measurements. Claw measurements were conducted with a 5.3 cm long mini digital protractor ($\pm 0.3^\circ$; 824 Digital Angle Detector, General Tools and Instruments LLC, New York, NY), a caliper (± 0.01 mm; model, manufacture, location) and a soft measuring tape (model, manufacture, location).

External Integrity of Claw (hoof inspection). The integrity of the horn capsule and adjacent structures was evaluated accessing the presence of following claw disorders based in ICAR Claw Health Atlas (Egger-Danner et al., 2015): vertical or horizontal fissures, penetrating foreign bodies, heel horn erosion, sole ulcers, and digital dermatitis and integrity of the soles.

External Morphological Measurements of Claw Conformation. The length of the dorsal wall (DWL) (Fig 1, Letter A) was measured from the border between the skin and the coronet (Nuss et al., 2011). The coronet was located using a 20 gauge-needle to identify the last soft tissue before the keratinized horn. The claw angle was obtained by placing a mini digital protractor in the middle of the dorsal border of the claw (Fig 1, Letter B). The claw height was measured from a side view by drawing a perpendicular line from the dorsal border of the coronet to the sole surface (Fig 1, Letter C). Heel height was measured by drawing a perpendicular line from the floor surface to the coronet at the heel (Fig 1, Letter D). The diagonal claw length was measured from the tip of the toe to the skin-horn junction at the heel (Fig 1, Letter E). The claw length was measured by drawing a line from the apex of the sole to the point on the floor surface where the bulb height ended (Fig 1, Letter F). On the plantar surface of the claw, the sole length was measured as the distance from the claw apex to the sole-bulb junction whereas the sole width was considered as the maximum distance, perpendicularly to the sole length, between the abaxial and axial borders of the claw in bulb area of the sole (Fig 1, Letter

G). The circumference of the coronary border was measured using a soft measuring tape that was wrapped around the coronet (Fig 1, Letter H). After external measurements each toe was divided sagittally using a band saw to measure the internal structures.

Internal Morphological Measurements of Claw Conformation. The internal dorsal wall length (Fig 2) was measured from the proximal limit of the wall horn, identified using a 20 G needle, to the distal tip of the internal dorsal wall border (Archer et al., 2015). Sole thickness (Fig 2) was measured at three different locations along the length of distal phalanx, apical margin (S1), concavity of ventral surface (S2) and in the final portion (flexor tuberosity) of distal phalanx (S3). The depth of the subcutaneous layer was measured at two different locations, below the apical margin of the distal phalanx (SUB1) and in the medial point of concavity of ventral face (SUB2). The thickness of the digital cushion (Fig 2) was measured below the final portion (flexor tuberosity) of distal phalanx. The distal phalanx length was measured directly between the distance apical margin to the final portion (flexor tuberosity) (Fig 2). Rotation of the distal phalanx was calculated based on congruent angles using SUB1, SUB2 and distal phalanx length measurements [anatomical references]. A small right triangle was drawn below the distal phalanx, the angle was calculated according to the trigonometric cosine relation. If the angle was above $|3.4|^\circ$ (either positive or negative) the distal phalanx was considered to be retroverted (negative) or anteverted (positive) relative to the sole surface (Adapted from Tsuka et al., 2014).

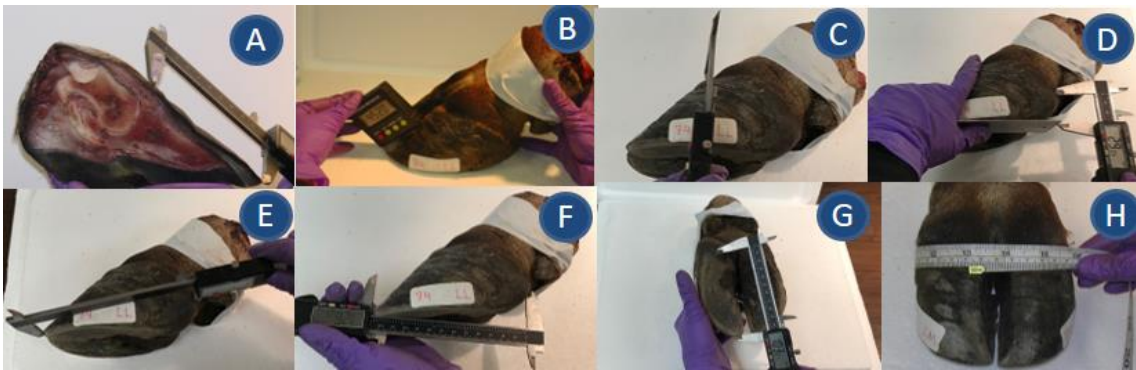


Fig 1: External Morphological Measurements of Jersey and Holstein x Jersey crossbred cows. A: length of dorsal wall; B: claw angle; C: claw height; D: heel height; E: diagonal claw; F: claw length; G: sole length; H: circumference of the coronary border.

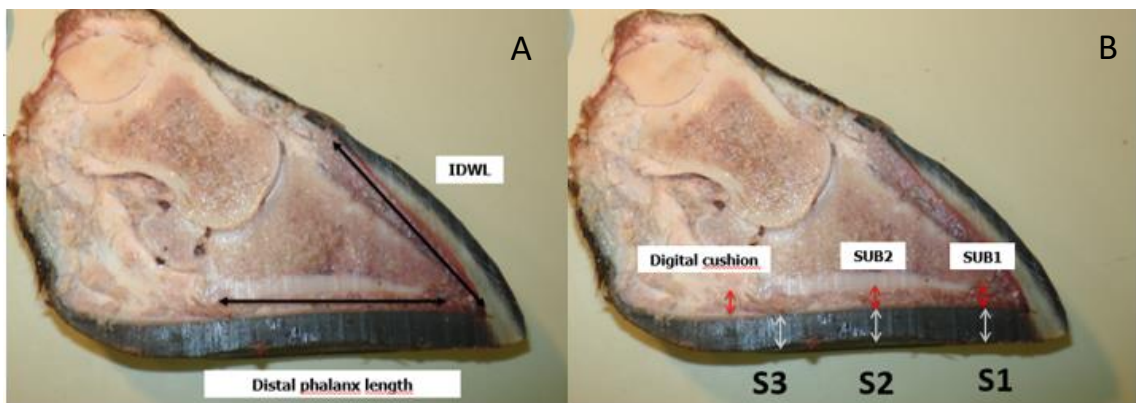


Fig 2: Internal Morphological Measurements of Jersey and Holstein x Jersey crossbred cows. A: IDWL; Internal dorsal wall length and Distal phalanx length. B: Digital cushion thickness; Sole thickness at S1, S2 and S3; SUB1 and SUB2: Subcutaneous layer at two different points.

Data analysis

Descriptive statistics were done using the following procedures of SAS[®]: MEANS, UNIVARIATE, FREQ and CORR. Proc FREQ was used to describe the general frequency of results and the distribution of results within the parameters considered ideal for the healthy conformation of the claw internally and externally. Pearson correlation coefficients were used to evaluate linear associations between internal claw measurements (S1, S2, S3, digital cushion and rotation of distal phalanx) and external claw conformation measurements (dorsal wall length, claw angle, heel height, coronary band width). Linear regression models were tested for sole thickness at S1, S2

and S3 and independent variables were chosen with forward stepwise option. Differences in claw measurements (S1, S2, S3, rotation of distal phalanx, DWL and heel height) were tested using ANOVA and considering the following categorical variables: breed (Jersey; Jersey crossbreed), system (dry lot; free-stall), side (lateral; medial), days in milk [first phase (1 to 100 d *post partum*), second phase (101 to 200 d *post partum*) and third phase (> 200 d *post partum*), age (<36 months; >36 months) and Parity (primiparous and multiparous). Interactions between categorical variables were not performed due to uneven and very low frequency of observations in some subclasses.

Results

Descriptive statistics

Mean, minimum and maximum values of external and internal morphological measurements are presented in table 1. A total of 296 claws were analyzed, 122 claws from dry lot system (41.2%) and 174 claws from free stall (58.7%), the distribution of data collected were 39.8% from herd 1, 41.2% from herd 2 and 18.9% from herd 3. The frequency of claws within genetic groups was 242 (81.7%) feet Jersey cows and 54 (18.2%) feet from Holstein × Jersey crossbreed cows. When cows were classified according lactation stage, 216 observations were in the first phase of lactation (72.9%); 28 in the second phase of lactation (9.4%) and 52 in the third phase (17.5%).

Distribution of the observations within parity revealed that 108 (43.9%) observations were from cows with one calving; 40 (16.2%) observations from cows with 2 calving and 98 (39.8%) observations from cows with 3 or more calving while observations from cows with age less than 36 months were 136 (45.9%) and more 36 months of age were 160 (54%).

The frequency of sole thickness values at S1 below the minimum value (7mm - thin) recommended (Toussaint Raven, 1989; Shearer and van Amstel, 2001) for this area was 29%. The frequency of thin soles across parity was 48, 19 and 33% for parity 1, 2 and ≥ 3 , respectively. The frequency of values for rotation of distal phalanx were 7% (21) negatives, 63% (187) positives and 30% of total values were considered within the limits associated with the normal position of the distal phalanx. Claws with positive values were more frequent in cows with $\geq 3^{\text{rd}}$ parity (96 cases), while those with negatives rotation

were distributed more evenly across parity: in cows with 1st parity (9 cases), \geq 3rd parity (8 cases) and 2nd (4 cases). Positive values for rotation of distal phalanx and sole thickness at S1 occurred simultaneously in 22% of total claws (296).

We found that only 51 claws showed values equal or above the recommended threshold for minimum sole thickness at S1 value ($=7$ mm) and values for rotation of distal phalanx within the range considered healthy ($0-3.4^\circ$). The external claw features considered as important at trimming had means values for DWL (mm) 75 and 78 (medial;lateral), heel height (mm) 23 and 26 (medial;lateral) and claw angle ($^\circ$) 48 and 42 (medial;lateral) in these 51 claws.

Correlations between external and internal measurements of Claw Conformation

The main characteristics observed externally at trimming (DWL, Heel height and claw angle) had few significant correlations with the internal measurements. The DWL and S1 were positively correlated ($P<.0001$) for lateral ($r=0.68$) and medial ($r=0.67$) claw. Sole thickness at S2 and S3 also correlated with DWL ($P<.0001$) in medial claws $r=0.49$; 0.54 , respectively and in lateral claws $r=0.58$; 0.63 . The heel height and S3 showed moderate correlation ($P<.0001$) considering lateral ($r=0.72$) and medial claws ($r=0.68$). The only external measurement that was correlated with rotation of the distal phalanx was heel height ($P<.0001$), presenting weak correlation in medial claws ($r=0.36$) and moderate correlation for lateral ($r=0.49$). The distal phalanx length was correlated with DWL ($P<.0001$) for lateral ($r=0.61$) and medial claws ($r=0.51$). Other external variables (circumference, diagonal claw, sole length and claw length) were positively correlated ($P<.0001$) with distal phalanx length in both lateral and medial claws with r values above 0.62 .

Table 1: Characteristics of external and internal morphological measurements (Mean, minimum and maximum values).

Variable	Mean	Minimum	Maximum	Coefficient of Variation (%)	Std Dev	Std Error
Age (months)	48.7	20	138	54.1	26.4	1.5
Parity	2.9	1	11	72.5	2.1	0.1
Dim (days)	91.4	1	428	123.7	113.1	6.6
Circumference (mm)	31.7	28	36.5	6	1.9	0.1
Dorsal wall length (mm)	74.5	59.3	103.9	10.5	7.8	0.4
Claw angle (°)	47.1	29.8	58.6	10.4	4.9	0.2
Claw height (mm)	62.2	47.5	83.5	9.5	5.9	0.3
Diagonal claw (mm)	120.1	89	153.7	10.1	12.2	0.7
Heel height (mm)	25.8	15	62.3	21.1	5.4	0.3
Sole length (mm)	94.1	69	121.4	10.8	10.2	0.5
Sole width (mm)	46.9	37	58.4	9.1	4.2	0.2
Claw length (mm)	118.1	89.6	152.2	9.2	10.9	0.6
Internal dorsal wall length (mm)	62.1	46.7	139.9	23.4	14.5	0.8
Sole thickness S1 (mm)	8.4	3.1	17.9	28.2	2.3	0.1
Sole thickness S2 (mm)	8.7	2.2	26	33.2	2.8	0.1
Sole thickness S3 (mm)	10.8	4	27.3	29.5	3.1	0.1
Subcutaneous layer 1 (mm)	2.3	0.8	4.3	25	0.5	0.0
Subcutaneous layer 2 (mm)	3.9	1.7	6.4	23	0.9	0.0
Digital cushion thickness (mm)	4.2	1.4	8	24.7	1	0.0
Distal phalanx length (mm)	54.9	38.1	82.6	11.9	6.5	0.3
Rotation of Distal phalanx (°)	4.4	-2.7	13.6	68.8	3	0.1

Regression model and variance analysis (Anova)

Regression models showed the linear effect of how the independent variables influenced the variation of S1, S2 and S3. The whole model explained 60% of the variation in S1, and the dorsal wall length was the most determinant variable, showing a partial $R^2 = 0.45$ (table 2). The whole model explained 41% of the variation in S2, dorsal wall length entered the template with 0.28 partial r-square (table 3). found that. The S3

regression model showed that the whole model explained 77% of the variation, and the heel height contributed with 0.53 (partial r-square) (table 4).

Table 2: Regression model of sole thickness at S1.

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	DWL	1	0.45	0.45	102.22	241.85	<.0001
2	Rotation of Distal phalanx	2	0.06	0.52	54.04	42.71	<.0001
3	Heel height	3	0.02	0.02	38.92	15.29	0.0001
4	Digital cushion thickness	4	0.01	0.56	29.88	10.16	0.0016
5	Sole width	5	0.01	0.57	21.29	10.05	0.0017
6	Diagonal claw	6	0.01	0.58	13.43	9.65	0.0021
7	Distal phalanx length	7	0.01	0.59	8.26	7.17	0.0078
8	Claw angle	8	0.00	0.60	7.75	2.52	0.1135
9	Circumference	9	0.00	0.60	7.13	2.64	0.1052

Table 3: Regression model of sole thickness at S2.

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	DWL	1	0.28	0.28	59.11	117.63	<.0001
2	Heel height	2	0.07	0.36	23.10	35.56	<.0001
3	Subcutaneous layer 1	3	0.01	0.37	17.36	7.40	0.0069
4	Sole width	4	0.01	0.39	12.65	6.54	0.0111
5	Sole length	5	0.01	0.41	6.13	8.52	0.0038
6	Distal phalanx length	6	0.00	0.41	5.04	3.11	0.0791

Table 4: Regression model of sole thickness at S3.

Step	Variable Entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	Heel height	1	0.53	0.53	303.69	338.74	<.0001
2	DWL	2	0.06	0.59	226.20	45.05	<.0001
3	Rotation of Distal phalanx	3	0.10	0.69	98.83	97.57	<.0001
4	Subcutaneous layer 1	4	0.02	0.72	65.56	29.18	<.0001
5	Digital cushion thickness	5	0.02	0.74	39.57	25.07	<.0001
6	Diagonal claw	6	0.00	0.75	31.89	8.91	0.0031
7	Sole width	7	0.01	0.77	13.31	20.21	<.0001
8	Claw angle	8	0.00	0.77	10.73	4.55	0.0338
9	Internal dorsal wall length	9	0.00	0.77	10.56	2.17	0.1419

Inspection of the scatter plot for values of dorsal wall length and sole thickness at S1 revealed that DWL above 77.0, 76.2 and 80.2 mm for 1st, 2nd, and \geq 3rd parity corresponded to a sole thickness > 7 mm at S1 (Fig.3).

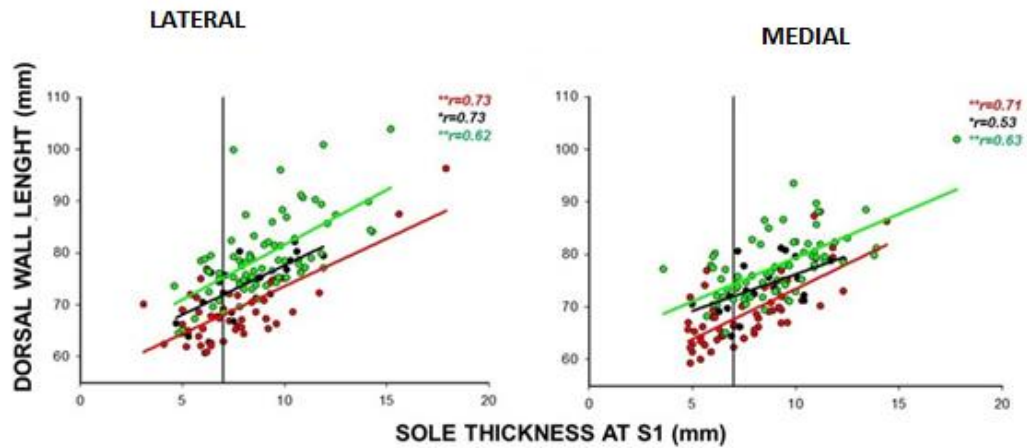


Fig 3: Scatter plot for dorsal wall length and sole thickness at S1 for cows (296 claws) on their 1st (red), 2nd (black) and \geq 3rd (green) parity.

Variance analysis (table 5) showed significant main effects for breed ($P < 0.05$) in sole thickness at S2, S3, rotation of distal phalanx and heel height. Higher values in S2, S3, rotation of distal phalanx and heel height were found for Holstein \times Jersey crossbreed compared with Jersey cows. We also found a tendency ($P < 0.10$) of higher values for DWL in crossbreed compared with Jersey cows. Type of system significantly affected ($P < 0.05$) sole thickness at S1, S2, S3 and DWL. Cows in free-stall system had high values for S1, S2, S3 and DWL than cows in dry lot system. The rotation of distal phalanx values had tendency ($P < 0.10$) to be lower in free-stall system compared with dry lot system. When we compared lateral and medial claws (side effect), we found significant lower values ($P < 0.05$) in medial claws for S3, rotation of distal phalanx and heel height compared with lateral claws. A tendency was found for S2 lower values in medial claws ($P < 0.10$) Age did not affect the claws traits.

Claws from cows classified in the first third of lactation had significant lower value for S1 when compared with cows at the third phase of lactation. Values for S2 were significantly higher in cows at the third phase of lactation compared with cows at the first and second lactation phases. Cows at second third of lactation showed tendency ($P < 0.10$) of lower S3 values when compared with cows in the first phase of lactation, while cows in the third phase of lactation were significant ($P < 0.05$) larger compared with cows at the first and second lactation phases. Cows at the third phase of lactation presented larger values of DWL ($P < 0.05$) compared with cows at the first and second lactation phases. Heel height values of cows at the second lactation phase were lower than those measured in cows at the first or the third phase of lactation ($P < 0.05$). Values for rotation of distal phalanx measured in cows at the second phase of lactation had a tendency ($P < 0.10$) of lower values compared with cows at the first and third phase of lactation.

Parity did not affected S1 and rotation of distal phalanx, while for S2 values cows classified as $\geq 3^{\text{rd}}$ parity showed significant larger values than cows at 1^{st} and 2^{nd} phases of lactation. While cows at the 1^{st} parity presented lower values for S3 than cows at 2^{nd} and $\geq 3^{\text{rd}}$ parity. Values for DWL and Heel height were significantly different for all categories of parity, higher values were found for claws classified as $\geq 3^{\text{rd}}$ parity.

Table 5: Variance analysis of claw conformation measurements in the breed, system, side, days in milk, age and parity.

Traits	Breed		System		Side		Days in milk			Age		Parity		
	JE	Cross JE	Dry lot	Free-stall	Lateral	Medial	1	2	3	<36 m	>36 m	1 st	2 nd	3 rd
Sole thickness at S1 (mm)	8.6a	9.0a	7.9a	9.6b	8.7	8.9	8.2b	8.7ab	9.4 ^a	8.2a	9.3a	8.3a	8.8a	9.2a
Sole thickness at S2 (mm)	8.6a	10b	8.7a	10b	9.6	9.0	8.7a	8.8a	10.4b	9.4a	9.3a	8.1a	9.1a	10.7b
Sole thickness at S3 (mm)	10.4a	11.7b	10.2a	11.9b	11.9a	10.2b	11.1bc	9.9c	12.2 ^a	10.6a	11.5a	9.9b	11.4a	11.9a
Rotation of P3 (°)	3.9a	5.5b	5.1	4.3	6.0b	3.4a	5.1a	3.9a	5.2 ^a	4.6a	4.8a	4.7a	4.7a	4.8a
Dorsal wall length (mm)	75.1a	77a	74.1a	77.9b	76.6	75.5	73.7a	75.4a	79b	75.6a	76.4a	70.5a	75.9b	81.6c
Heel height (mm)	24.8a	28b	25.9	27	28.7b	24.2a	26.9a	24.6b	27.8 ^a	27.1a	25.8a	23.8a	26.3b	29.1c

Letters show significant differences (P<0.05)

Discussion

The evaluation of the hooves' health condition by examining the external features of the bovine claw is a common practice at trimming and it aims to reestablish the appropriate weight-bearing within and between claws (Toussaint Raven, 1989; Shearer and van Amstel, 2001). Minimum length for dorsal wall (DWL), minimum heel height and adequate claw angle are the most common measurements used as indicators of the hoof health condition (Toussaint Raven, 1989; Shearer and van Amstel, 2001). However, there are doubts about association of these external characteristics with the correspondent internal traits of the claw, such as sole thickness (Tsuka et al., 2014; Archer et al., 2015). Our correlation results showed that only DWL and heel height were moderately correlated ($r > 60\%$) with the sole thickness at S1 and S2. The largest contribution of DWL and heel height explaining the variation in the linear regression model of S1 and S3 respectively (partial R^2) can be explained by the anatomical proximity of the structures. The horn tissues of the sole have the important function acting as unreactive barrier against trauma (Tomlinson et al., 2004). Previous studies (Toussaint Raven, 1989; Shearer and van Amstel, 2001) with Holstein cows indicated a minimal threshold value for sole thickness of 7 mm to avoid toe lesions such as thin sole, apical white line disease and toe ulcer (Kofler, 2017). Recently the ICAR Claw Health Atlas described thin sole as "sole horn yields, feels spongy, when finger pressure is applied". Cases of thin sole are frequently associated with over-trimming, cause suboptimal thickness of weight-bearing (Shakespeare, 2009). In the present study, we found a high frequency (48%) of S1 values below 7 mm for primiparous cows, and considering the existent literature may indicate

thin sole and such animals may present higher probability to develop toe lesions. We do not dispose trimming data on these animals to established an cause-effect between trimming and the low sole thickness registered. . Sanders et al. (2009) reported thin soles during initial phase of lactation, pointing out that the highest prevalence of thin sole–induced toe ulcers, toe ulcers, and white line lesions occurred within the first 15 DIM. Other study with Jersey heifers reported that sole thickness decreased between days 10 to 110 of lactation (Laven et al., 2012). The occurrence of thin sole at the beginning of lactation in the present study might be attributed to the abrupt diet changes frequently observed on the early lactation period.

In the present study we noticed that first phase of lactation had significant effect to lower values on S1, heel height and DWL, as during this period cows face metabolic and hormonal changes, and this can be reflected in the hoof healthy. Some authors (Webster, 2001; Tarlton et al., 2002) suggested that calving weakened the connective tissues of hoof wall suspending the pedal bone, consequently may cause the rotation of the distal phalanx. We noticed that 22% of the claws presents thin sole and positive values for rotation of distal phalanx and we inferred that these cows could have developed thin sole-induced toe ulcers (TSTU) (Greenough and Vermunt, 1991; Sanders et al., 2009).

The majority of the studies emphasized the importance of a minimum sole thickness at S1 to assure and adequate health status of the claws. But it seems to be not enough as in the present study we found some claws with S1 thickness > 14mm and DWL >100 mm (data no shown), were deformed, with visual aspect compatible to that described in the literature for laminitis (Vermunt and Greenough, 1995), when is typical overgrowth due the accelerated horn production.

The main way to prevent thin sole according to the literature is related with a minimum length for dorsal wall (75mm) proposed by Toussaint-Raven (1985). However some studies (Tsuka et al.,2014; Archer et al.,2015) demonstrated that this threshold is not always enough to avoid trauma Nuss and Paul (2006) suggested higher values of DWL to be used in functional trimming. It is important to emphasize that these studies were developed with Holstein cows, while for Jersey cows the adequate minimum threshold for DWL are not yet established. In Tsuka et al.(2014) study, sole thickness (S1) >7mm was correlate with DWL of 79.8 and 78.4 (at medial and lateral claws) in Holstein cows. Despite the limited number of claws, we found a similar result especially for multiparous Jersey cows.

Conclusions

We noted in this study some differences for description claws in Jersey cows and Holstein × Jersey crossbreed cows, larger values for S2, S3, rotation of distal phalanx and heel height were found in crossbreed cows. Dorsal wall length was the main attributed affecting sole thickness at S1 and S2. Only heel height as a external measurement that has positive correlated with rotation of distal phalanx. We found some evidences that dorsal wall length should be above 80 mm for DWL to assure adequate sole thickness (> 7 mm) at S1.

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CAPÍTULO IV

4.0. Considerações finais

Ao longo desses quatro anos de estudos, nossa intenção sempre foi a de buscar entender melhor a respeito dos fatores que desencadeiam claudicação não infecciosa em bovinos. Dedicamos nossa atenção em desenvolver dois estudos, primeiramente entender como a nutrição associa-se as desordens e se a relação causa-efeito poderia ser acessada através de revisão sistemática e meta-análise. Em um segundo momento nossa ideia foi entender como as estruturas anatômicas externas se relacionam com as internas e como isso impacta na saúde do dígito bovino.

Ao iniciarmos nosso primeiro estudo, nos deparamos com um grande desafio diante do baixo volume de publicações que envolvessem nutrição e laminite, chamou atenção que havia grande variabilidade na metodologia citada nos trabalhos, assim como os métodos de diagnósticos utilizados para avaliar a condição de laminite, este fato inviabilizou a meta-análise, concluímos com a revisão sistemática que existem indícios da relação entre casos agudos de laminite e nutrição, porém o mecanismo pelo qual ocorre permanece incerto. Não foi encontrado nenhum estudo com abordagem profunda de todas as fases da doença, que pudessem fazer a ligação entre o alto consumo de dieta energética, baixo pH ruminal, liberação de endotoxinas, chegada das toxinas na microcirculação do dígito e ocorrência de laminite.

Apesar da não direta ligação entre o estudo 1 e 2, durante a avaliação das patas no estudo pos-mortem foi constatado a presença de alterações tais como: rotação de terceira falange, deformações na parte córnea, linhas horizontais fundas na muralha (concavidade) unhas em formato de tesoura, chinelos, processos ulcerativos. Tais alterações são descritas na literatura como características de laminite subclínica de grande ocorrência no início da lactação, coincidentemente muitos dos nossos animais também estavam no primeiro terço da lactação. Baseado nesses fatos, nossa contribuição para a linha de pesquisa foi de que não há evidências que suportem a relação causa e efeito entre nutrição e laminite, tão pouco pode se afirmar sobre as ligações entre as fases da laminite, sobretudo em casos subclínicos, existe diversos fatores envolvidos que se confundem, entendê-los nos permitirá no futuro realizar um adequado manejo preventivo.

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