TATIANE VITO CAMILOTI

SUPORTE SOCIAL EM BEZERROS LEITEIROS APÓS A MOCHAÇÃO: DOR, INTERAÇÃO SOCIAL E CONSUMO DE ALIMENTOS SÓLIDOS

Londrina
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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutor.

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BANCA EXAMINADORA

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Orientador: Prof. Dr. José Antônio Fregonesi -
Universidade Estadual de Londrina - UEL

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Profa. Dra. Ana Maria Bridi
Universidade Estadual de Londrina - UEL

_____

Profa. Dra. Andressa Amorim Cestari
Universidade Estadual de Londrina - UEL

_____

Profa. Dra. Andreia De Paula Vieira
Universidade Positivo - UP

_____

Prof. Dr. Agostinho Ludovico
Universidade do Norte do Paraná - UNOPAR

Londrina, 29 de Fevereiro de 2016.

Dedico este trabalho aos meus pais Helenice e Laércio,
à minha irmã Patrícia, aos meus sobrinhos Nicholas e Noah,
e ao meu noivo Leandro.

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"A compaixão para com os animais é das mais nobres virtudes da natureza humana".

Charles Darwin

CAMILOTI, Tatiane Vito. **Suporte social em bezerros leiteiros após a mochação**: dor, interação social e consumo de alimentos sólidos. 2016. 92f. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2016.

RESUMO

O suporte social pode ajudar o animal a enfrentar melhor às adversidades encontradas durante a vida. Neste contexto, o objetivo deste trabalho foi avaliar os efeitos do ambiente social no comportamento de bezerros leiteiros após a mochação com ferro quente. No primeiro experimento, 14 bezerros machos da raça Holandesa foram alojados em baias individuais ou em grupo dinâmico com outros bezerros e presença de suas mães no período noturno e foram mochados aos 37 ± 3 dias de idade. Comportamentos como movimentos rápidos de orelhas e cabeça, coçar a cabeça com os membros traseiros ou contra paredes e objetos e auto lamber-se foram observados diretamente por 20 minutos em cada uma das seguintes horas após a mochação 1, 2, 3, 4, 5, 7, 9, 12 e 24. Nas primeiras horas observadas (1, 2, 3 e 4), bezerros criados individualmente apresentaram mais movimentos de coçar a cabeça do que animais em grupo (P=0,01) e também mais comportamentos de lamber-se (P=0.04). No período equivalente as horas 5, 7 e 9, bezerros criados individualmente também apresentaram mais comportamento de coçar a cabeça (P=0,02) que bezerros criados em grupo. Houve interação entre observaçao e ambiente social no período equivalente as horas 12 e 24 para o comportamento de lamber-se e bezerros individuais expressaram mais desse comportamento que os animais criados em grupo social complexo após a mochação. No segundo experimento, 24 bezerros machos da raça Holandesa foram alocados em baias individuais ou em pares, e a idade e procedimento de mochação foram os mesmos utilizados no primeiro experimento, porém dessa vez metade dos animais passou pelo procedimento de mochação sem aplicação do ferro quente primeiro e a outra metade sofreu a mochação propriamente dita primeiro. Todos os animais em pares foram expostos duas vezes ao ambiente experimental, sendo a primeira vez como atores (mochados) e a segunda vez como observadores (parceiro mochado). Comportamentos relacionados a dor como coçar a cabeça, movimentos rápidos de cabeça e interações sociais entre os pares foram observados diretamente por 20 minutos nas horas 1, 2, 3, 4, 5, 7, 9, 12, 24 e 36 após o procedimento de mochação sem aplicação do ferro quente e a mochação propriamente dita, e o consumo de alimentos sólidos foi mensurado toda manhã. Não houve nenhum efeito do ambiente social nos comportamentos de dor e no consumo de ração total misturada. O consumo de grãos foi maior para os bezerros criados em pares que bezerros criados individualmente (P=0,003). Os bezerros expressaram mais interações sociais quando o segundo bezerro passou pelos procedimentos nos pares do que quando o primeiro bezerro passou pelos procedimentos nas horas 1, 2, 3 e 4 após a mochação (P=0,03). Nesse mesmo período de tempo houve uma diminuição de movimentos rápidos de cabeça quando o segundo bezerro passou pelos procedimentos comparando com o primeiro. Nas horas 12 e 24 após a mochação, as interações sociais entre os animais foram maiores quando o segundo bezerro foi mochado. Conclui-se que a mochação a ferro quente é dolorosa em bezerros dessa idade e somente um parceiro não foi suficiente para diminuir a expressão de comportamentos de dor . Interações sociais podem ajudar na recuperação da dor. Outros estudos devem ser realizados para melhor compreender os efeitos de grupos sociais na superação da dor e outros estados afetivos de bezerras leiteiras.

Palavras-chave: Bem estar animal. Bovino de leite. Comportamento. Dor. Estado afetivo.

CAMILOTI, Tatiane Vito. **Social support in dairy calves after disbudding**: pain, social interaction and solid feed intake. 2016. 92p. Thesis (Doctor´s Degree in Animal Science) – Universidade Estadual de Londrina, Londrina, 2016.

ABSTRACT

Social support can help the subject to cope with and and adapt better to the adversities during life. In this context, the aim of this study was to evaluate the effects of social environment on the behavior of dairy calves after hot iron disbudding. In the first experiment, 14 male Holstein calves were housed in individual pens or in a dynamic group with other calves and the presence of their dams during the night and were disbudded at 37 ± 3 days old. Behaviors such as ear flicks, head shakes, head rubbs using hind foot or against objects and self grooms were direct observed for 20 minutes in each of the following hours after disbudding 1, 2, 3, 4, 5, 7, 9, 12 and 24. In the first hours after disbudding (1, 2, 3 and 4), calves raised individually showed more head rubs than calves housed in group (P=0.01) and also showed more self grooms (P=0.04) behavior. In the period of hours 5, 7 and 9 individual calves also expressed more head rubs behavior (P=0.02) than calves housed in group. There was interaction between observation and social housing on the period of hours 12 and 24 for self grooming bahevior and individual calves expressed more of this behavior than calves reased in the complex social group after disbudding. In the sencond experiment, 24 male Holstein calves were allocated in individual pens or in pairs. The age and disbudding procedure were the same used in the first experiment, but this time half of the animals were sham disbudded first and the other half were disbudded first. All calves housed in pairs were exposed to the experimental environment twice over two rounds, once as the actor (disbudded) and once as the observer (the partner being disbudded). Pain related behaviors such as head rubs and head shakes, and social interactions between pair housed calves were direct observed for 20 minutes in the hours following sham disbudding and actual disbudding 1, 2, 3, 4, 5, 7, 9, 12, 24 and 36, and feed consumption was measured every morning. No effects of social environment on pain related behaviors and total mixed ration intake was found. However, grain intake was higher for paired calves than single calves (P=0.003). Calves expressed more social interactions when the second calf of the pairs was sham or disbudded than when the first calf was sham disbudded or disbudded in the period of hours 1, 2, 3 and 4 after the procedure (P=0.03). In the same period of time head shakes behavior decreased when the second calf of the pairs was sham or actual disbudded than when the first calf suffered the procedure. In the hours 12 and 24 after disbudding, social interactions were higher when the second calf of the pair was disbudded. In conclusion, hot iron disbudding is very painful to calves in this age and only one partner was not enough to decrease the expression of pain related behaviors. Future studies should be performed to better understanding of social group effects on pain recovery and other affective state of dairy calves.

Keywords: Animal welfare. Dairy cattle. Behavior. Pain. Affective state.

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1 INTRODUÇÃO

A ciência do bem-estar animal é uma ciência "mandatória", isto é, derivou-se de demandas éticas da sociedade sobre a qualidade de vida dos animais (FRASER et al., 1997). Com o desenvolvimento da ciência do bem-estar animal, três principais preocupações surgiram em relação à forma que os animais são tratados: saúde, vida natural e estado afetivo.

Do nascimento ao desmame, a bezerra leiteira passa por desafios de grande estresse e desconforto que podem comprometer seu bem-estar. Dentre eles, podemos citar a separação de sua mãe logo após o nascimento, o isolamento de outros animais com a criação individual, a mochação e o desmame. Bezerras são freqüentemente criadas individualmente em baias ou abrigos móveis com o objetivo de diminuir a contaminação de doenças no rebanho, porém tem aumentado na literatura trabalhos mostrando os benefícios da criação de bezerras em duplas ou grupos pequenos, como por exemplo, a socialização e brincadeiras locomotoras, maior consumo de alimentos e diminuição do estresse causado pelo desmame (DE PAULA VIEIRA et al., 2010; DE PAULA VIEIRA et al., 2012b). O contato social parece ser importante para as bezerras, visto que elas demonstraram estarem dispostas a trabalhar pressionando um painel para ter acesso a uma companhia (HOLM et al., 2002). Além disso, bezerras criadas em grupo enfrentam melhor o estresse causado pela troca de alimentos sólidos (COSTA et al., 2014).

A mochação é uma prática comum em propriedades leiteiras e é realizada com o objetivo de prevenir possíveis lesões causadas pelos chifres, nos próprios animais e também nas pessoas que trabalham diariamente com eles. Dentre os métodos de mochação, os mais utilizados são ferro quente, pasta cáustica e a descorna cirúrgica. Porém, é considerada uma prática dolorosa e sedativos, anestesias locais e analgésicos podem ser usados para amenizar a dor durante e após o procedimento (STAFFORD; MELLOR, 2005).

Nesse contexto, este trabalho encontra-se dividido em duas partes: a primeira parte é uma breve revisão de literatura sobre os principais pontos críticos de bem-estar na criação de bezerras leiteiras, a segunda parte consiste de dois artigos que relatam os efeitos de diferentes ambientes sociais nas respostas dos bezerros à dor assim como interações sociais e consumo de alimentos sólidos após a mochação.

2 REVISÃO DE LITERATURA

2.1 BEM-ESTAR ANIMAL

A ciência do bem-estar animal surgiu como uma resposta às preocupações éticas sobre o tratamento que damos aos animais e nossas responsabilidades e ações sobre a qualidade de vida a ser disponibilizada a eles. Desta forma, bem-estar animal é uma ciência "mandatória", pois surgiu deste interesse ético da sociedade sobre o bem-estar dos animais (FRASER, 2012).

No século XX, aproximadamente durante o período que corresponde às duas Guerras Mundiais e à Grande Depressão, a sociedade ocidental estava mais preocupada com a sobrevivência humana e com as necessidades básicas da vida. Os problemas relacionados aos animais eram, em grande parte, considerados de baixa prioridade. Nesse período, nas décadas de 1950 e 1960, os cidadãos dos países industrializados passaram preocuparem-se com outros assuntos além de segurança pessoal e necessidades básicas da vida. A publicação do livro Máquinas Animais, da escritora britânica Ruth Harrison em 1964, foi um fato marcante nessa transição de preocupações com o bem-estar animal, chocando seus leitores ao descrever as condições de vida das galinhas, porcos e bezerros nos sistemas de produção em confinamento que cada vez mais se tornavam comuns (FRASER, 2012).

Em resposta às manifestações públicas de preocupação com o bem-estar de animais criados em fazendas industriais, o governo britânico criou o comitê Brambell, encarregado de "investigar o bem-estar de animais mantidos sob sistemas intensivos de produção". As opiniões expressas, as preocupações e questões específicas de bem-estar animal examinadas por esse Comitê tiveram uma grande influência sobre os processos de investigação de bem-estar animal (RUSHEN et al., 2008).

Assim iniciou-se a Ciência do Bem-Estar Animal. O primeiro desafio para os cientistas foi definir bem-estar animal. Tais definições de bem-estar animal consideravam fatores culturais, científicos, religiosos, pessoais e até mesmo políticos (SWANSON, 1995). Ao menos três preocupações éticas são comumente expressas em relação à qualidade de vida dos animais: 1) viver uma vida natural, através do desenvolvimento natural e superação de estados negativos bem como da exibição de capacidades naturais, (2) livres de estado prolongados e intensos de medo e dor, bem como de outros estados afetivos negativos, e (3) funcionamento satisfatório de saúde, crescimento normal e normalidade dos sistemas fisiológicos, reprodutivos e comportamentais (FRASER et al., 1997).

Claramente estes diferentes tipos de preocupações sobre o bem-estar animal se sobrepõem (FRASER et al., 1997), ou seja, as três questões sobre o que é considerada melhor qualidade de vida para os animais estão relacionadas. Dessa forma, o bem-estar do animal é proporcionado quando estado afetivo, funcionamento biológico e vida natural se entrelaçam.

2.2 RELAÇÃO MATERNO - FILIAL E SEPARAÇÃO VACA - BEZERRA

Um dos pontos cruciais que determinam o sucesso de um sistema de produção de leite é a forma com que as bezerras são criadas. Bezerras bem criadas significam animais desmamados em boas condições para continuarem seu crescimento até reproduzirem e produzirem leite (CAMPOS; LIZIEIRE, 2005).

Muitos fatores podem influenciar o bem-estar de bezerras em sistemas produtores de leite, destacando-se as instalações, manejo nutricional e sanitário, manejo dos animais e interações com o tratador e práticas como o transporte (STULL; REYNOLDS, 2008).

Independente do sistema de criação adotado é fundamental que os manejos nutricional, sanitário e reprodutivo sejam realizados de forma adequada e que os animais recebam conforto e cuidado.

Bezerras de vacas zebuínas, normalmente encontradas em sistemas extensivos de produção, permanecem com as vacas durante a ordenha para facilitar a descida do leite. Neste sistema, as bezerras geralmente ficam com as mães em piquetes desde o nascimento até o desmame, ou até o final da lactação da vaca. Rebanhos de raças especializadas, normalmente encontradas em sistemas intensivos de produção, não exigem a presença da bezerra na hora da ordenha, assim vaca e bezerra são separadas logo após o nascimento e a bezerra é aleitada com o uso de mamadeira ou balde, sendo criada em baias individuais em galpões, abrigos móveis, ou piquetes.

Em tais sistemas, é possível garantir o fornecimento de colostro adequado para assegurar a transferência de imunidade passiva. O colostro a ser fornecido deve ser de boa qualidade e deve ser fornecido com até seis horas após o nascimento (STOTT et al., 1979a; STOTT et al., 1979b; WEAVER et al., 2000). De acordo com Stott et al. (1979c), bezerros que ingeriram o colostro diretamente da mãe apresentaram maiores taxas de absorção de imunoglobulinas que bezerros ingerindo o colostro através de mamadeira. Besser et al. (1991) concluíram que a falha na transferência de imunidade passiva para o bezerro

depende da quantidade e qualidade do colostro ingerido e não apenas do método escolhido, ou seja, é independente de ingestão direta da mãe ou de métodos artificiais como mamadeira e tubo esofágico.

O bem-estar da bezerra e da vaca pode ser prejudicado com a prática de separação logo após o nascimento, pois na natureza a bezerra necessita da presença da mãe que facilita o processo de desmame natural e gradualmente quando a bezerra possui aproximadamente 8-11 meses de idade (REINHARDT; REINHARDT, 1981).

Contudo, há estudos que comprovam que quanto maior o tempo que a vaca e sua cria ficam juntas após o nascimento, maior é o estresse dos animais quando a separação é realizada (LIDFORS, 1996; WEARY; CHUA, 2000; FLOWER; WEARY, 2001). A possível explicação para esta resposta à separação é o forte vínculo entre mãe e cria estabelecido logo nas primeiras horas do nascimento (EDWARDS; BROOM, 1982). Este vínculo é caracterizado por comportamentos como lamber um ao outro, fornecimento de alimento, calor e proteção, deitar-se em contato, atividades sincronizadas, e proximidade entre mãe e filha (JOHNSEN et al., 2015). Hudson e Mullord (1977) observaram que o contato de apenas 5 minutos da vaca com a bezerra após o nascimento foi suficiente para a formação de um forte e específico vínculo maternal.

Em um estudo recente, Johnsen et al., (2015) demonstraram que o vínculo entre a vaca e sua cria é criado independentemente se o bezerro obtém o leite para consumo somente de sua mãe. Esses pesquisadores testaram os efeitos de diferentes métodos de fornecimento do leite para o bezerro na criação do vínculo entre mãe e filho. Os métodos estudados foram bezerros alimentados somente por meio de um alimentador automático, somente mamando em suas mães durante a noite, ou combinado, podendo mamar no alimentador automático durante o dia e na mãe durante a noite e concluíram que independente da mãe ser a principal fonte de leite, quando os bezerros eram colocados juntos de suas mães durante a noite, o tempo passado perto de suas mães foram os mesmos nos três tratamentos, assim como o tempo passado lambendo uns aos outros (mãe e cria).

Weary e Chua (2000) estudando os efeitos de diferentes tempos de separação vaca-bezerra, de seis horas, um dia ou quatro dias após o nascimento, verificaram um aumento de respostas ao estresse causado pela separação tardia como o aumento da movimentação na baia, passaram mais tempo com a cabeça para fora da baia e também ficaram mais tempo em pé do que aqueles separados logo após o nascimento. As vacas separadas de seus bezerros após quatro dias apresentaram aproximadamente quatro vezes

mais vocalizações do que aquelas separadas após seis horas e um dia. Não houveram diferenças no ganho de peso dos bezerros e na produção de leite das vacas nesse estudo.

Em um outro estudo, Flower e Weary (2001) demonstraram que vacas e bezerros se movimentaram mais e colocaram a cabeça para fora da baia mais vezes quando separados após 15 dias de nascimento se comparados aos bezerros separados com um dia de vida. As vacas neste mesmo grupo apresentaram maiores taxas de vocalização. Entretanto, em termos de desempenho, animais mantidos com as mães por um período prolongado de tempo antes da separação ocorrer, apresentaram maiores taxas de ganho de peso que bezerros separados logo após o nascimento. A produção de leite de ambos os grupos se igualou quando foi considerada a produção entre 15 e 150 dias.

Metz (1987) também encontrou maior ganho de peso em bezerros mantidos 10 dias com as mães quando comparados com bezerros separados logo após o nascimento. Valníčková et al. (2015) demonstraram que os bezerros que permaneceram com suas mães por quatro dias após o parto cresceram muito mais até o final da segunda semana quando comparados com bezerros criados individualmente ou em pares. Esperou-se um maior ganho de peso dos bezerros mantidos com as mães, pois os mesmos ingeriram leite *ad libitum* (METZ, 1987; FLOWER; WEARY, 2001; VALNÍČKOVÁ et al., 2015). No estudo de Khron et al (1999), bezerros mantidos com as mães por quatro dias também obtiveram maiores taxas de ganho de peso que aqueles separados logo após o nascimento, porém isto aconteceu também com os bezerros mantidos com as mães, mas que não podiam mamar nas mesmas. Isto demonstra que a influência da presença da vaca no ganho de peso dos bezerros vai além da obtenção do alimento, no caso, do livre acesso ao leite.

A separação vaca-bezerra pode causar danos cognitivos aos bezerros mantidos em um grupo social complexo com outros bezerros e acesso as suas mães e outras vacas durante a noite (DAROS et al., 2014). Em um teste cognitivo realizado em bezerros após a separação das mães, Daros et al. (2014) encontraram uma resposta pessimista dos animais quando testados na identificação de telas de computador com diferentes cores. Meaguer et al., (2014) comparando animais criados individualmente com aqueles mantidos em um grupo social complexo, verificaram que animais criados sozinhos apresentam maiores dificuldades de aprendizado quando a tarefa a ser realizada é invertida, no caso, utilizaram também telas de diferentes cores para dar a recompensa com leite, ou punir sem o leite.

Os benefícios de manter vacas e bezerras juntas por tempo prolongado também refletem da facilitação de socialização das bezerras (KHRON et al., 1999; FLOWER; WEARY, 2001). Flower e Weary (2001) verificaram que bezerros que foram separados das

mães após duas semanas de nascimento, quando foram introduzidos a um bezerro não familiar as seis semanas de vida, demonstraram comportamentos sociais mais intensos (lamber, dar cabeçadas e esfregar a cabeça contra o bezerro desconhecido) do que aqueles que foram separados das mães um dia após o nascimento.

2.3 Criação Individual Versus Criação em Grupo

É muito comum em propriedades produtoras de leite a adoção da criação individual. Bezerras em aleitamento são muito suscetíveis a patógenos e a criação individual pode minimizar a propagação de doenças. O sistema de instalação individual tem como base o princípio de reduzir a transmissão de patógenos através do contato mínimo entre os animais (STULL; REYNOLDS, 2008). Abrigos e baias individuais permitem a observação da saúde e do consumo de alimentos de cada bezerra pelo tratador e também facilitam o acesso ao bezerra, caso procedimentos médicos ou de manejo sejam necessários (STULL; REYNOLDS, 2008). A agressão entre as bezerras e a competição por alimentos é reduzida com a criação individual (RUSHEN et al., 2008).

Porém, nesse sistema de criação individual há desvantagens, sendo que a falta de interação entre os animais pode resultar em falhas no desenvolvimento de comportamentos sociais adequados (BROOM; LEAVER, 1978; DE PAULA VIEIRA et al., 2012a). Baias individuais não permitem o contato social e os movimentos naturais dos animais são restritos devido ao espaço físico limitado que é normalmente utilizado (RUSHEN et al., 2008).

Por outro lado, a criação de bezerras em grupo é baseada no princípio que bovinos leiteiros são animais sociáveis (gregários) e o alojamento em grupo permite o desenvolvimento dos comportamentos e interações sociais em rebanho (STULL; REYNOLDS, 2008). Chua et al. (2002) demonstraram que bezerras leiteiras alojadas em pares passam até 2% do seu dia em contato social.

A criação em grupo também permite a oportunidade de exercícios e brincadeiras entre as bezerras do mesmo grupo (STULL; REYNOLDS, 2008). O comportamento de brincar pode ser um bom indicador de bem-estar de animais jovens (HELD; ŠPINKA, 2011). Jensen et al. (1999) demonstraram durante um teste social que bezerras criadas em grupo apresentaram maiores interações sociais com outras bezerras do que aquelas criadas individualmente. Duve et al., (2012) verificaram que bezerras alojados

individualmente e alimentados com menor quantidade de leite (5 L comparados com 9 L) passaram menos tempo brincando que aqueles alojados em pares ou com as mães.

Duve e Jensen (2012) demonstraram que bezerros previamente criados em pares a partir da terceira semana de vida possuíam maior cognição social quando criados em grupos que bezerros criados individualmente com contato social limitado. Valníčková et al. (2015) observaram comportamentos de brincar em bezerros criados individualmente, com a mãe até quatro dias de idade ou em grupos e concluíram que bezerros criados em instalações individuais são privados de níveis naturais de brincadeiras, demonstrados por níveis baixos de comportamentos de brincar espontâneos em sua baia de origem e alto efeito de compensação quando colocados em ambientes maiores com companhias. De Paula Vieira et al. (2012a) também observaram que bezerras criadas individualmente reagem mais à novidade social e ambiental quando comparados com bezerras alojados em pares, ou seja, demonstram maior cognição social.

O contato social parece ser muito importante para bezerros leiteiros. Bezerros estão dispostos a executarem tarefas que necessitam alto gasto de energia e uso de força para terem contato com uma companhia familiar quando treinados por condicionamento operante. Por exemplo, eles pressionam um painel com suas cabeças para abrir um portão que lhes permitem entrar em outra baia onde está o outro bezerro (HOLM et al., 2002), possuem alta motivação para interagirem socialmente. Os mesmo autores demonstraram que essa motivação para o contato com o parceiro é maior para o contato total do corpo do que somente o contato com a cabeça do outro animal.

Bezerros demonstram um vínculo maior com bezerros familiares a eles do que com aqueles que eles nunca tiveram contato (FÆREVIK et al., 2006; DUVE; JENSEN, 2011). Por meio de um teste de preferência social, Færevik et al. (2006) observaram que bezerros leiteiros escolheram passar mais tempo na área onde havia um bezerro familiar a eles que na área com o bezerro não familiar. Duve e Jensen (2011) avaliando o comportamento de bezerros também em testes de preferência social demonstraram que bezerros criados com uma companhia desde o nascimento ou a partir da terceira semana de idade estabelecem um vínculo maior com sua companhia que os bezerros criados com contato social limitado.

É possível se obter sucesso na criação em grupos em termos de saúde e desempenho dos animais. Hänninen et al. (2003) comparando bezerros criados individualmente ou em grupo de quatro animais não encontraram diferença na taxa diária de crescimento dos animais e a incidência de diarréia foi menor nos animais criados em grupo que nos animais alojados individualmente. Valníčková et al. (2015) concluíram que bezerros

criados em grupos cresceram mais até a décima semana de idade comparados com bezerros criados individualmente ou com a mãe até quatro dias de idade. Chua et al., (2002) demonstraram que a criação de bezerras em dupla também traz benefícios aos animais em termos de interações sociais e aumento do espaço para movimentação sem desvantagens na saúde e ganho de peso dos animais. De Paula Vieira et al., (2010) também demonstraram maior ganho de peso de bezerras criadas em dupla e maior cognição quando usaram um alimentador automático pela primeira vez.

2.4 SUPORTE SOCIAL

Animais sociais vivem em grupos e se comunicam para cooperar e manter o grupo de forma que todos os membros sejam beneficiados. Além disso, a interação social e a comunicação também se tornam essenciais para a proteção contra as ameaças ambientais (KIKUSUI et al., 2006).

Suporte social pode ser descrito em termos dos benefícios trazidos pelos parceiros sociais que facilita o indivíduo a enfrentar e se adaptar melhor às mudanças (COBB, 1976). Quando animais de uma mesma espécie estão juntos, eles apresentam melhor recuperação de experiências aversivas e estressantes (KIKUSUI et al., 2006).

O termo amortecimento ou neutralização social, do inglês *social buffering*, refere-se à habilidade do parceiro social em reduzir amplamente o impacto do estressor (KANITZ et al., 2014), alterando respostas dos sistemas cardiovascular, imune, endócrino e também comportamental (HENNESSY et al., 2009; RAULT, 2012). Os parceiros sociais podem tanto aliviar, piorar ou não influenciar a resposta do animal ao estresse. Tais respostas podem variar de acordo com o tipo de estressor, familiaridade com o parceiro, tamanho do grupo, espécie, estado emocional do parceiro, gênero, entre outros fatores (RAULT, 2012).

O estresse do parceiro social pode afetar nos efeitos de *social buffering.* Kiyokawa et al. (2004) estudaram a influência do estresse do parceiro no efeito de *social buffering* em ratos e descobriram que os parceiros que não haviam recebido previamente choque elétrico foram mais efetivos que os parceiros que receberam o choque na diminuição do estresse quando alojados com um rato que também havia sido condicionado a sentir medo com a aplicação de choques nas patas. Os autores concluíram que a presença de uma companhia social alivia as respostas comportamentais de um sujeito e os parceiros que não levaram choque foram mais efetivos para diminuírem estas respostas.

Estudos em diversas espécies demonstram que a separação social resulta em respostas negativas ao estresse como, por exemplo, aumentos na vocalização, taxa de batimento cardíaco e concentração de cortisol plasmático em novilhas (BOISSY; LE NEINDRE, 1997), aumento da atividade locomotora e aumento das taxas de vocalização em ovinos e caprinos (LYONS et al., 1993) e aumento dos níveis de cortisol em macacos quando separados do grupo pela primeira vez (GORDON et al., 1992).

Bezerras leiteiras também respondem à separação social e à familiaridade da companhia. Færevik et al. (2006) estudando o comportamento de bezerros machos e fêmeas as seis semanas de idade, separaram os animais de seus grupos de origem com a presença de um bezerro familiar, em outro teste com um bezerro não familiar e por último o bezerro foi separado e colocado na baia experimental sozinho. Os resultados mostraram que os bezerros vocalizaram significativamente mais quando deixados sozinhos ou junto com um bezerro não familiar do que quando foram colocados com um bezerro familiar (sem vocalização). Também apresentaram maior imobilidade e menor exploração da baia quando deixados sozinhos.

Jensen et al. (1997) demonstraram que bezerras leiteiras com três meses de idade criadas individualmente, apresentam mais comportamentos relacionados ao medo como relutância para entrar no ambiente e altas taxas de batimento cardíaco quando introduzidas a um novo ambiente social com uma bezerra não familiar ou quando isoladas em um novo ambiente.

Bezerras alojadas individualmente são mais reativas à novidade ambiental e social quando comparadas a bezerras criadas em pares. De Paula Vieira et al. (2012a) demonstraram que bezerras leiteiras criadas individualmente passaram mais tempo em pé e correndo e defecaram com maior freqüência que os animais criados em pares. Também quando testadas com uma companhia passaram menos tempo correndo, apresentaram uma latência maior para interagir socialmente e passaram mais tempo envolvidas em interação social com uma bezerra não familiar que animais criados em pares.

2.4.1 Dor e Suporte Social

A dor pode ser definida como uma experiência sensorial e emocional desagradável associada a uma lesão real ou potencial dos tecidos (INTERNATIONAL ASSOCIATION FOR THE STUDY OF PAIN, 1994). A dor altera a fisiologia e o

comportamento do animal para reduzir ou evitar danos, reduzir a possibilidade de reincidência e para promover a recuperação (MOLONY; KENT, 1997).

A dor no animal pode ser mensurada por meio de avaliação dos comportamentos, como por exemplo, isolamento do grupo, comportamento e posturas de repouso, vocalização, comportamentos estereotipados, entre outros. A dor também pode ser mensurada por meio de medidas fisiológicas como, por exemplo, avaliação dos níveis de cortisol sanguíneo e taxa de batimento cardíaco, entre outros (ANIL et al. 2002).

Langford et al. (2006) estudando a modulação social da dor como evidência de empatia em ratos verificaram que as respostas comportamentais à dor são moduladas pela presença de uma companhia de mesma espécie. Os autores observaram um aumento de respostas comportamentais à dor causadas pela injeção de ácido acético, nesse caso contorções, em ratos alojados em pares e familiares uns aos outros (irmãos ou companheiros de gaiola) quando ambos receberam a injeção, comparados com ratos testados individualmente. A resposta encontrada não foi a mesma quando os pares foram testados com os animais desconhecidos para eles.

Alguns anos após os resultados obtidos por Langford et al. (2006), um estudo também com ratos foi realizado por Gioiasa et al. (2009) e obtiveram a resposta contrária. Observando a resposta à dor de injeção de formalina em ratos testados individualmente ou em duplas (somente um dos ratos recebendo a injeção ou ambos) concluíram que a resposta comportamental à dor não foi diferente quando os animais foram testados individualmente ou quando somente um rato recebeu a injeção na dupla. Porém, quando os dois animais da dupla receberam a injeção de formalina, a resposta comportamental à dor diminuiu (comportamento de lamber-se). Os autores sugeriram que essa discrepância dos resultados pode ter sido influenciada pelo tipo de injeção utilizada, no primeiro estudo o ácido acético e no segundo, a formalina, assim como a relação dos ratos utilizados no estudo de Gioiasa et al. (2009) não eram irmãos e nem estranhos uns aos outros, pois foram alojados juntos por uma semana antes do teste ser realizado e já tinham uma forte relação de dominância e submissão estabelecida.

O grau de parestesco também parece estar associado com o grau de *social buffering* transmitido em ovinos (GUESGEN et al., 2014). Guesgen et al. (2014) estudaram os efeitos do contexto social no comportamento de dor causado pelo corte de cauda em cordeiros alojados com cordeiros familiares e relacionados uns aos outros (gêmeos), familiares porém não relacionados e não familiares e demonstraram que todos os animais apresentaram um aumento nos comportamentos de dor depois do procedimento, porém animais do grupo

familiares e relacionados (gêmeos) apresentaram um menor aumento dos comportamentos de rolar e dar coices que os outros grupos indicando um efeito do *social buffering* nesses cordeiros.

Colditz et al. (2012) não encontraram evidências comportamentais de *social buffering* na dor causada pela castração em cordeiros com o uso de diferentes métodos (faca, anel ou simulação de castração). Os animais foram alojados em grupos de três cordeiros castrados em cada método ou grupo de três cordeiros sendo um castrado em cada método e todos os grupos alojados com suas mães. O fato dos animais terem a presença das mães pode ter influenciado na resposta à dor.

2.4.2 Alimentação e Suporte Social

O ambiente social parece influenciar o consumo de alimentos sólidos de bezerras leiteiras por meio da facilitação social. De Paula Vieira et al. (2010), comparando bezerras criadas individualmente ou em pares, verificaram que o consumo de concentrado foi maior durante o período de aleitamento para aquelas bezerras criadas com uma companhia que aquelas criadas sozinhas na baia e também apresentaram benefícios após o desmame com consumo de concentrado e ganhos de peso maiores comparados com bezerras criadas individualmente. Costa et al. (2015) também demonstraram que bezerras alojadas em dupla apresentam maior consumo de concentrado que aquelas alojadas individualmente e esse consumo também é maior quando comparado com bezerras que foram colocadas em dupla com aproximadamente 40 dias de idade demonstrando que a idade que a bezerra é colocada com uma companheira pode influenciar na facilitação social.

Alojar as bezerras com animais mais velhos também contribui para o comportamento de consumo de alimentos sólidos. De Paula Vieira (2012b) observaram o comportamento de consumo de bezerras alojadas em grupo de três animais jovens ou em grupo com dois animais jovens e uma bezerra mais velha e concluíram que a presença de um animal mais velho não influenciou no consumo de concentrado, porém o número de visitas e tempo gasto no alimentador foi maior nas bezerras alojadas com um animal mais velho e já desmamado. Já no período após o desmame, bezerras alojadas com uma companhia mais velha passaram mais tempo no alimentador e apresentaram maior consumo de concentrado. Os autores concluíram que a companhia mais velha estimula o comportamento de consumo e crescimento antes e depois do desmame, bem como serve de referência social para as bezerras mais jovens.

A criação de bezerras leiteiras em um ambiente social complexo, representado por um grupo de bezerras de diferentes idades e acesso à mãe e outras vacas durante a noite, reduz a neofobia alimentar (COSTA et al. 2014). Esses autores compararam bezerras criadas individualmente com bezerras criadas num ambiente social complexo e verificaram que o consumo de alimentos sólidos novos como feno e cenoura foram maiores nas bezerras criadas num ambiente social e também apresentaram menor latência para se aproximar e comer os alimentos novos.

2.5 MOCHAÇÃO OU DESCORNA

A prevenção do crescimento dos chifres (mochação) ou sua remoção cirúrgica (descorna) são práticas comuns em fazendas leiteiras (STAFFORD; MELLOR, 2005), realizadas com o objetivo de reduzir o risco de lesões em tratadores e outros animais do rebanho e para tornar o manejo de animais confinados menos perigoso (GRAF; SENN, 1999). Para prevenir o seu crescimento, os chifres e o tecido circundante são removidos utilizando-se uma variedade de métodos incluindo cauterização por calor (ferro quente) ou química (pasta cáustica) e a remoção cirúrgica (VICKERS et al., 2005). A escolha do método depende em grande parte da preferência e experiência do produtor bem como da idade do animal, mas a cauterização por calor ou química são as utilizadas com mais freqüência para mochar bezerros nas primeiras semanas de vida (VICKERS et al., 2005).

A cauterização pelo calor é feita em bezerros nas primeiras quatro a seis semanas de idade. O botão do chifre e seu tecido generativo são destruídos por uma barra aquecida, geralmente com uma ponta côncava que queima o botão e o tecido circundante por alguns segundos (WEAVER, 1986). Já na cauterização química uma pasta de hidróxido de sódio ou hidróxido de cálcio é aplicada para destruir o botão do cornual (WEAVER, 1986). Esses produtos químicos cauterizam os tecidos continuamente durante todo o período em que a substância está em contato com os tecidos do animal (STAFFORD; MELLOR, 2011).

Todos os métodos de mochação envolvem a destruição do tecido e é evidente que os bezerros sentem estresse e dor (GRAF; SENN, 1999). Diversos autores já demonstraram cientificamente que tanto um método quanto o outro são procedimentos muito dolorosos para os bezerros (GRAF; SENN, 1999; GRØNDAHL-NIELSEN et al., 1999; VICKERS et al., 2005). No entanto, Vickers et al. (2005), comparando os dois métodos, concluíram que a mochação com ferro quente provoca mais dor nos animais mesmo quando

esse procedimento é associado ao uso de sedativo e anestesia local enquanto que a mochação com pasta cáustica foi realizada somente com o uso do sedativo.

A dor causada por diferentes métodos de mochação tem sido avaliada utilizando-se respostas fisiológicas, comportamentais e de produção, antes, durante e depois do procedimento com ou sem anestesia ou analgesia sistêmica (STAFFORD; MELLOR, 2005). Medidas comportamentais como chacoalhar a cabeça, esfregar a cabeça em paredes e objetos e movimentos rápidos das orelhas podem ser usadas como indicativos de dor após o procedimento de mochação (GRAF; SENN, 1999; GRØNDAHL-NIELSEN et al., 1999; VICKERS et al., 2005; MORISSE et al., 1995; FAULKNER; WEARY, 2000; HEINRICH et al., 2010). Os comportamentos relacionados à dor podem ser utilizados como bons indicadores da duração e das diferentes fases de uma experiência dolorosa (STAFFORD; MELLOR, 2005).

Como forma de amenizar a dor durante o procedimento, o uso de anestesias locais como a lidocaína, tem sido estudado com resultados satisfatórios (MORISSE et al., 1995; GRAF; SENN, 1999; GRØNDAHL-NIELSEN et al., 1999). Porém, um estudo realizado por Vickers et al. (2005), não encontrou efeitos do uso de lidocaína na redução de comportamentos de dor em bezerros mochados com pasta cáustica, o que pode ser explicado pelo modo de ação da pasta cáustica. Para atravessar a membrana celular lipídica hidrofóbica, o sal deve se dissociar, mas o anestésico deve se reassociar uma vez dentro da célula nervosa para formar o cátion que bloqueia o afluxo de íons de sódio e impede a condução nervosa. Os ingredientes ativos da pasta cáustica são hidróxido de sódio e hidróxido de cálcio, ambas as bases muito fortes. Como as bases destroem o tecido, o aumento do pH pode afetar o equilíbrio da solução anestésica, resultando em menos cátions disponíveis para bloquear os receptores de sódio e assim, a interrupção da função do anestésico (VICKERS et al., 2005).

Bezerros mochados com o uso da anestesia local ainda precisam de contenção, e devem também ser contidos enquanto o anestésico local é administrado. O uso de sedativo (como a xilazina) pode essencialmente eliminar as respostas dos bezerros na aplicação da anestesia local e a necessidade de contenção física durante a administração da anestesia local e da mochação (VON KEYSERLINGK et al., 2009).

O uso de medicamentos antiinflamatórios após a mochação torna-se necessário, pois a lidocaína, o anestésico local mais popular, é efetiva somente nas primeiras duas a três horas após sua aplicação (FAULKNER; WEARY, 2000). Heinrich et al. (2010) testaram o efeito do uso do antiinflamatório meloxicam no comportamento de bezerros após a mochação com ferro quente e demonstraram que os animais tratados com esse medicamento

tiveram menor movimento de orelha durante 44 h após a mochação comparando com os animais recebendo placebo. Os mesmos animais também apresentaram menos movimentos de cabeça durante as primeiras nove horas depois da mochação. Esses resultados sugerem que o uso de meloxicam foi efetivo em reduzir a dor pós-cirúrgica.

O estresse causado por esse procedimento extremamente doloroso também pode ser visto através das alterações fisiológicas dos animais. O método mais comum de se avaliar o estresse fisiologicamente é a concentração de cortisol em amostras de sangue. A mochação causa um rápido pico inicial no cortisol plasmático, que corresponde à dor aguda de dano nos tecidos e ao manejo dos animais, que diminui por aproximadamente sete a nove horas antes de voltar aos níveis iniciais, indicando a presença de uma resposta inflamatória (STAFFORD; MELLOR, 2005).

Estudos têm mostrado o aumento do cortisol após a mochação (LADEN et al., 1985; GRAF; SENN, 1999; GRØNDAHL-NIELSEN et al., 1999; HEINRICH et al., 2009). O uso do antiinflamatório meloxicam após a mochação, além de ter sido demonstrado satisfatório na redução de comportamentos de dor (HEINRICH et al., 2010), também foi eficiente na melhora dos índices fisiológicos como cortisol e nas taxas de frequência respiratória e cardíaca de bezerros mochados com ferro quente (HEINRICH et al., 2009). Grøndahl-Nielsen et al. (1999) demonstraram também aumento da freqüência cardíaca nos animais mochados com ferro quente sem uso de sedativo ou analgésico comparados aos animais que tiveram a mochação simulada com o uso de anestesia local.

Em relação ao consumo e ganho de peso, Laden et al. (1985) não encontraram diferença estatística em bezerras mochadas com 8 semanas de idade comparadas com o bezerras do grupo controle. Grøndahl-Nielsen et al. (1999) também não obtiveram diferença estatística no consumo de alimentos e ganho de peso de bezerros mochados a ferro quente com ou sem o uso de sedativo, anestésico local e analgesia.

A dor causada pela mochação, além de alterações visíveis de comportamento e mudanças fisiológicas nos bezerros, pode também influenciar no estado emocional dos animais. Neave et al., (2013) testaram os efeitos da dor após a mochação com ferro quente no processo cognitivo de bezerros leiteiros. Os animais foram treinados para responder de forma diferente a telas de vídeo brancas e vermelhas e, em seguida, foram testados com cores ambíguas, com tonalidades entre o branco e o vermelho antes e depois da descorna. Depois da mochação, os bezerros foram mais propensos a julgar cores ambíguas como negativo. Os autores concluíram que esse comportamento “pessimista” indica que a dor pós mochação com ferro quente resulta numa mudança negativa no estado emocional dos animais.

A mochação tem sido mostrada como um procedimento de intensa dor e estresse para os animais. Assim, a combinação do sedativo, anestesia local e o uso de antiinflamatório reduzem a resposta à dor durante a mochação e nas horas seguintes ao procedimento (VON KEYSERLINGK et al., 2009).

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3 OBJETIVOS

3.1 Objetivo Geral

Avaliar os efeitos do ambiente social nos comportamentos relacionados à dor, interação social e consumo de alimentos sólidos após a mochação com ferro quente em bezerros da raça Holandesa.

3.2 Objetivos Específicos

a) Investigar os efeitos do ambiente social complexo, ou seja, um grupo dinâmico de bezerros, mães e outras vacas, na expressão de comportamentos relacionados à dor nas horas seguintes à mochação com ferro quente.

b) Investigar os efeitos da criação de bezerros em pares na expressão de comportamentos relacionados à dor nas horas seguintes à mochação com ferro quente.

c) Avaliar a modulação social da dor entre dois bezerros do mesmo par quando a mochação é realizada primeiro em um animal e depois no segundo animal.

d) Avaliar o efeito do ambiente social no consumo de alimentos sólidos, como concentrado inicial e ração totalmente misturada, no dia anterior à mochação e no dia da mochação.

4 ARTIGO 1[1]

Interpretive Summary: Effects of Complex Social Environment on Pain Behavior of Dairy Calves after Disbudding. ***Camiloti et al. Page 000-000.*** Disbudding in dairy calves is a very painful procedure intended to facilitate management and reduce the risk of injuries to humans and other animals. Social buffer is known to alleviate environmental stress in other species. In this study, calves were housed individually or housed in a complex social group pen with access to its mother at night and hot iron disbudded. Calves expressed less head shaking and self grooming behavior when they had the companion of other calves in the group with access to their mothers and other cows.

EFFECTS OF SOCIAL ENVIRONMENT ON PAIN BEHAVIOR AFTER DISBUDDING

Effects of Complex Social Environment on Pain Behavior after Disbudding in Dairy Calves

T.V. Camiloti*† J. A. Fregonesi*†, M. A. G. von Keyserlingk* and D. M. Weary*

Behavior of dairy calves before and after disbudding: effect of the social environment

* Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, V6T1Z4, Canada

† Universidade Estadual de Londrina, Rod. Celso Garcia Cid PR 445 Km 380, Campus Universitário, Londrina, 86051-990, Brazil

Corresponding author's email: tatianevito@hotmail.com

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ABSTRACT

Dairy calves are commonly separated from their mothers soon after birth and they are raised individually up to weaning. Before weaning dairy calves are disbudded to facilitate management and to reduce the risk of injuries to human and other animals. However disbudding is an invasive and painful procedure and social contact is known to minimize the effects of stressful experiences. In this study, we evaluated how the social environment with the presence of other calves and the mother can affect calf responses to pain after disbudding. Fourteen Holstein male calves were housed in either individual pens or in a dynamic group pen with other calves and could have access to their mothers and other cows at night. They were disbudded with hot iron at 37 ± 3 days old, direct observed for the following hours after the procedure (1, 2, 3, 4, 5, 7, 9, 12 and 24 hours) for pain related behaviors as ear flicks, head rubs, head shakes and self grooms for 20 minutes. For ear flicks, head rubs and head shakes observed, calves expressed more pain behaviors in the gap of the peak of the pain (hour 5, 7 and 9) after disbudding for both housing systems. Individual housed calves expressed more head rubbing behavior in Period 1 (hours 1, 2, 3 and 4) and Period 2 (hours 5, 7 and 9) than complex social housed calves. During Period 3 (hours 12 and 24) calves showed more self grooming behavior when they were housed alone comparing with those housed in the group having full contact with their mothers and other cows at night after disbudding. The results show that hot iron disbudding is a very painful procedure when performed in one month old calves and a complex social environment can alleviate pain related behavior as head rubbing and self grooming.

KEY WORDS: animal welfare, behavior, individual housing, social complexity, social buffering.

INTRODUCTION

The horn itself consists of dense keratin that is produced at the corium, and has several behavioral and physiological functions to cattle (Knierim et al., 2015). In dairy calves, disbudding is a commonly performed procedure intended to facilitate management and reduce the risk of injuries to humans and other animals. Horn buds are typically removed with a caustic paste or a hot iron and regardless of the method of disbudding or dehorning this procedure is extremely painful to the animals (Stafford and Mellor, 2005). Behaviors as ear flicks, head rubs and head shakes are widely used to relate to pain in several disbudding studies (Grøndahl-Nielsen et al., 1999; Vickers et al., 2005; Faulkner and Weary, 2000) and those behaviors can last for several hours following the disbudding (Heinrich et al., 2010). Pain-related behaviors can be used to measure the duration and characterize the different phases of a painful experience (Stafford and Mellor, 2005). Stress and the acute pain caused by this procedure can be alleviated by a combination of local anesthetic and anti-inflammatory treatment, as well as sedation to facilitate handling during the process (Morisse et al., 1995; Vickers et al., 2005; Knierim et al., 2015).

Dairy farms commonly adopt individual housing systems to raise the calves and the individual facility system is based on the principle of reducing the transmission of pathogens through minimal contact between animals (Stull and Reynolds, 2008). Cattle are gregarious animals, i.e. they are animals that live in groups in their natural habitat. Social contact is very important for growing calves. De Paula Vieira et al. (2012) showed that calves housed individually are more reactive to environmental and social novelty when compared with calves housed in pairs. Calves reared in pairs also show reduced responses to weaning (De Paula Vieira et al., 2010). Play behavior is a natural behavior and can be used to indicate the presence of good welfare in calves (Jensen et al., 1998). Social contact with a companion increases the frequency of play behavior and also the competitive success of the calves (Duve

et al., 2012). Holm et al. (2002) showed the motivation of calves to have the companion of other calf and found that calves are willing to work hard to gain access to a peer when trained in an operant conditioning task, such as pressing a panel with their heads to open a gate that allows them to enter another pen where there was another calf.

Most of the dairy farms separate the calves from their dams soon after birth and further social contacts with the mother are usually prevented (Roth et al., 2009). The dam is an important social partner for young calf. Hudson and Mullord (1977) found that 5 min contact with a calf immediately post partum was sufficient for the formation of a strong, specific maternal attachment with that calf. Recently, Johnsen et al (2015) showed that the bond between cow and calf goes beyond the milk source for the calf.

Socially housed animals typically recover more quickly from stressful experiences (Kikusui et al., 2006). Social separation induced struggling and large increases in vocalization, heart rate and plasma cortisol concentration in heifers (Boissy and Le Neindre, 1997) and locomotor activity and vocal rates in sheep and goats (Lyons et al., 1993). Kiyokawa et al. (2004) studied the influence of a partner's stress response in rats and found that nonshocked partners were more effective than shocked partners at stress attenuation when housed with a rat that also was fear conditioned by the application of footshocks. The presence of the partner rat attenuated behavioral responses of the subject and nonshocked partners were more effective at attenuating these responses.

The benefits of housing calves with a social partner have been explored in relation to some stressors experienced by calves, but the painful experience of disbudding has not yet been explored. Therefore, the aim of the following study is to evaluate the effect of social support (i.e. the presence of a group of calves, the dam and other cows) on pain responses of dairy calves during the hours following hot iron disbudding. We predicted that calves housed

in the complex social group would express less pain related behavior than individual housed calves.

MATERIAL AND METHODS

This experiment was conducted at the University of British Columbia's Dairy Education and Research Center, located in Agassiz, British Columbia, Canada, between March and April of 2013. All procedures carried out in the current study were approved by the University of British Columbia Animal Ethics Committee.

Animals and Housing

Fourteen Holstein dairy bull calves were randomly assigned either to individual (n = 7) or to complex social group housing (n = 7) systems. Individual housed calves weighed an average of 46.2 ±7.2 kg (mean ± SD) and group housed calves weighed an average of 42.7 ± 5.1 kg at birth. Individual housed calves were separated from the dam within 6h of birth, housed in sawdust-bedded single pen (1.2 x 2m) and allowed auditory but not visual contact with other calves. Calves housed in a complex social group were kept with their dams in the calving pen (4 x 4m) for 3 d after parturition and moved together to the experimental pen. The experimental pen included a pen of 12 sand-bedded free stalls and a sawdust bedded creep area to the calves. It consisted in a dynamic group of cows and calves varying in size from 4 and 8 cow-calf pairs over the course of the study. All cows were fitted with udder nets (Large Mesh Udder Support, Franksville Specialty Company, Phillips, WI) right after parturition to prevent calves suckling from the dam. During the day, cows and calves were housed adjacent with fence-line contact that allowed physically interaction during the day (7AM to 7PM) and calves had access to the dams during the night (7 PM to 7 AM) through two gates connecting calves and cows' pens.

Calves of both housing systems were fed 4 L of colostrum by bottle within 6 h of birth. Only colostrum over 50 mg Ig/mL estimated by the use of a colostrometer was used to feed the calves. Blood samples were collected from the jugular vein 24 hours after the first feeding of colostrum and serum was analyzed using a refractometer. Only calves that presented values higher than 5.5 g/dL of serum proteins were included in the trial.

All calves were fed 8 L/day of whole pasteurized milk by bottle divided in two meals, at 7 AM and 4:30 PM. Concentrate starter, TMR (total mixed ration) and water were provided *ad libitum*.

Once a week a general health check of the calves was done including rectal temperature, navel healing, breathing and heart rate, runny nose and eyes, general skin appearance and skin hydration following the standard operating procedures of the farm and when calves were diagnosed as ill, they were treated by the herd veterinarian of the farm. Also weekly, all the pens were cleaned and the dirty sawdust was changed for fresh bedding.

Disbudding procedure

Calves were disbudded at 37 ± 3 (mean ± SD) days of age, weighing 67.4 ± 6.5 kg (mean ± SD) at approximately 9 AM. For this procedure, fifteen minutes before disbudding, calves were sedated with an intramuscular injection of xylazine (Rompun, 2%, Bayer Inc., Ontario; 0.25 mg/kg body weight). Ten minutes after administration of xylazine, a local anaesthetic (5 mL per side of 2% Lidocaine; Ayerst Veterinary Labs, Ontario) was injected subcutaneously into the corneal nerve of each horn bud, located along the occipital groove midway between the horn bud and eye (Faulkner and Weary, 2000). Five minutes after the administration of lidocaine, an electrically heated hot-iron dehorner (Rhinehart X-30) was applied to each horn bud for approximately 15s. Every calf of the complex social group was

isolated from the group to be disbudded using a plywood panel and then put back to the pen after the procedure was done.

Behavior Measurements

Behaviors previously related to pain after disbudding (Table 1) were monitored by direct observations of the calves in 9 periods of 20 min each following the hours 1, 2, 3, 4, 5, 7, 9, 12 and 24 after disbudding (Faulkner and Weary, 2000). Direct observation was performed by the same observer during all period of the study.

To control variation in pain responses of the procedure, baseline data were collected the day before the actual disbudding, without the administration of any drug. For baseline data, the same behaviors were observed in the day prior to the disbudding day, and the methodology used to collect behavior data was exactly the same as the one used after disbudding.

Statistical Analyses

All analyses were performed with SAS (version 9.3, SAS Institute Inc., Cary, NC). Data were checked for normality using the UNIVARIATE procedure, and when necessary, data was transformed. Significance was declared at $P < 0.05$.

A Factorial 2 x 2 experimental design was tested. The effects of housing (Individual versus Complex Social Group) and observation (before versus after disbudding) on pain related behavior variables as ear flicks, head rubs, head shakes and self grooms and housing system x observation interaction were tested with the calf as the experimental unit using the MIXED procedure in SAS. Behavioral responses had a peak after hour 5 following the disbudding, so data was analyzed separately in 3 periods: Period 1 (hours 1, 2, 3 and 4 after disbudding); Period 2 (hours 5, 7 and 9); and Period 3 (hours 12 and 24 after disbudding).

RESULTS

In Period 1, no interaction was found between observation (before and after disbudding) and housing system (Individual and Complex social group) but a tendency for self grooming behavior was found (P = 0.05). In this period, ear flicking (EF) and self grooming (SG) were statistically significant when we compared before and after disbudding, showing an increase of EF (P = 0.03) and a decrease of SG (P = 0.002) after disbudding. Head rubbing (HR) and SG were statistically significant when housing system treatments were compared (P = 0.01; and P = 0.04, respectively).

In Period 2, no observation x housing system interaction was found in all variables observed. In this period, where in this study calves expressed more pain behaviors (peak of pain) after disbudding, the responses of EF, HR and head shaking (HS) when observation (before and after disbudding) were compared were P = 0.007, P = 0.03, and P = 0.01, respectively. For HR, it also showed significant value within Individual x Complex social group housing (P = 0.02). In this period, individual calves expressed more HR than complex social group housing calves (mean ± SD; 10.2 ± 11.2 for individual calves and 2.7 ± 3.3 for group calves).

In Period 3, we found interaction between observation X housing system treatment only for SG behavior (P = 0.04). Calves raised individually showed more self grooming (14.3 ± 13.0) than complex social group housed calves (2.6 ± 2.6). In the other behaviors observed no interaction was found. EF and HS showed difference between before x after disbudding observations (P = 0.02; and P = 0.003, respectively) with these behaviors increasing after the procedure.

DISCUSSION

There are evidences in the scientific literature that a companion or a group housing provides many benefits to dairy calves, for example, it increases feed intake and weight gain (De Paula Vieira et al., 2010; De Paula Vieira et al., 2012; Costa et al., 2015), also it helps animals to cope better with environment and social novelty (Jensen et al., 1997; Jensen et al., 1999; Duve and Jensen, 2011) and stressful challenges to welfare of dairy calves as separation from other calves, (Færevik et al., 2006), weaning (De Paula Vieira et al., 2010) and complex social housing reduces food neophobia (Costa et al., 2014). In this study, we showed that complex social group housing with the presence of other calves, the dam and other cows during the night had some benefits to calves in terms of pain relief or decrease the expression of this pain after hot iron disbudding. To our knowledge, this study was the first to evaluate the effect of social buffering on pain behavior in dairy calves after disbudding procedure.

Pain related behavior observed in this study as ear flicking, head shaking and head rubbing were previously showed by several authors a relation with pain after hot iron disbudding in calves (Faulkner and Weary, 2000; Vickers et al., 2005; Heinrich et al., 2010). In this study, when baseline data (before disbudding) was compared to after disbudding behaviors data, it was clear that all pain related behaviors observed increased after this procedure. These results are consistent with Morisse et al. (1995), when comparing 4 h before dehorning with 4 h after dehorning and found an increase of HS and scratching the lesion with the hind foot after heat cauterization and caustic paste dehorning with or without anaesthesia. Heinrich et al. (2010) also showed that all calves increased pain related behaviors after dehorning comparing to sham dehorning baseline levels.

In this study, the peak of pain based in all pain related behaviors observed, occurred between hours 5 and 9 after disbudding. These results are consistent with those found by Faulkner and Weary (2000), that showed peak of HS and ear EF 6 h after dehorning and

Heinrich et al. (2010) that also showed the peak after approximately 6 h following dehorning, but our results are in contrast with Morisse et al. (1995), these authors found that all behavior indicative of pain or discomfort disappeared with 4 h and for Vickers et al. (2005), more HS were present in the first 4 h following dehorning.

We direct observed behaviors until 24 h following disbudding and we found differences in HS comparing baseline versus after disbudding which means that calves were still feeling some discomfort after this period. Faulkner and Weary (2000) also found pain related behaviors until 12 h for HS and 24 h for EF after dehorning and Heinrich et al. (2010) showed that increases in behavior previously associated with dehorning pain relative to the sham dehorning period were present to varying degrees during the 44 h study period.

It was found interaction between observation and housing system treatments only for self grooming (SG) on Period 3. In this period, before disbudding, calves raised individually showed less SG than calves in the complex group and after disbudding the opposite happened, with individual calves expressing more SG than calves in the group, showing more discomfort probably caused by the pain.

Individual calves showed more head rubbing against pen wall and using the hind foot to scratch the head in Period 1 and Period 2 and a tendency to show more of this behavior also in Period 3 than did group housing calves with the presence of the mother and other cows. Individual calves also showed more self grooming behavior in Period 1 comparing to group housed calves. Social support that can be described as the benefits brought by social partners that could help an individual to cope with challenges (Rault, 2012) is a possible argument to explain these findings. In dairy calves, Duve et al. (2012) showed that social contact with other calves and the dam decreased struggle responses to restraint and increased play behavior and competitive success, but to our knowledge there is nothing related to pain after disbudding procedure in literature. The presence of the dam could explain our findings related

to decrease of head rubbing behavior in complex social group housing. Hild et al. (2010), measuring the effect of the pain caused by thermal stimulation in lambs, found that lambs staying close to the ewes and resting synchronously were both associated with lower sensitivity to thermal stimulation and Blass et al. (1995) found that maternal contact and suckling reduced pain responsivity in rat pups.

The other calves allocated in the group also can have had influence in head rubbing behavior. Social buffering effects caused by partners related to pain are documented in rats (Kiyokawa et al., 2004). In contrast, Colditz et al. (2012) found no effect of social contact in pain behaviors related to different methods of castration in suckling lambs that could have had influenced of the mother, which has showed by Hild et al. (2010) that the presence of the dam can have some effects in lambs pain tolerance. In another study with sheep, social buffering did not happened when tail docking lambs had the companion of a familiar unrelated or unfamiliar unrelated lamb, however when tested with a familiar related partner (twin) showed a smaller increase in rolling and kicking during the procedure than the other groups (Guesgen et al., 2014).

Our results provide some evidences of calf to calf or mother to calf transmission or a possible buffering effect of behavioral responses or the effect of the complex social environment that was provided to these animals comparing with calves housed alone. Further work is required to investigate the effects of the companion on pain recovery after disbudding in dairy calves.

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Table captions

Table 1. Ethogram used for pain related behavior for behavioral analysis (adapted of Heinrich et al., 2010).

Table 2. Behavioral responses (mean ± SD; events) of calves housed individually (n = 7) compared with calves housed in a complex social housing with other calves, dam and other cows (n = 7) before and after hot iron disbudding. Period 1 = hours 1, 2, 3 and 4; Period 2 = hours 5, 7 and 9; and Period 3 = hours 12 and 24 after disbudding. Behaviors: EF = ear flicking; HR = head rubbing; HS = head shaking; and SG = self grooming.

Table 3. Mean ± SD (Observation * Housing interactive effect) of self grooming (SG) behavior responses observed in Period 3 (hours 12 and 24 after disbudding).

Camiloti et al. Table 1.

Behavior	Description
Ear flicking	Calf rapidly moves one or both ears to the front and back independent of a head shake. Each time movement constitutes an ear flick.
Head shaking	Calf rapidly shakes head from one side to the other. Recorded as one behavior each time movement after a resting position.
Head rubbing	Calf lifts hind leg to scratch top of head with foot or rubs head against sides of the pen, floor, gate, food and water buckets and other calves for the group treatment.
Self grooming	Calf licks parts of its body. Recorded as one behavior each time movement after a resting position.

Camiloti et al. Table 2.

	Observation (OBS)		Housing (HO)		P – value[1]		
	Before Disbudding	After Disbudding	Individual	Complex social	OBS	HO	OBS X HO
Period 1							
EF	5.1 ± 4.1b	11.8 ± 11.1a	11.5 ± 10.7	5.4 ± 5.4	0.03	0.08	0.15
HR	3.3 ± 2.9	1.8 ± 2.3	3.8 ± 3.1a	1.3 ± 1.3b	0.09	0.01	0.09
HS	1.7 ± 1.9	2.0 ± 2.9	2.6 ± 3.1	1.1 ± 1.0	0.81	0.14	0.54
SG	15.1 ± 9.1a	4.9 ± 5.7b	12.8 ± 11.0a	7.1 ± 5.8b	0.002	0.04	0.05
Period 2							
EF	3.3 ± 2.9b	78.3± 95.6a	62.8 ± 97.7	18.9 ± 40.0	0.007	0.09	0.10
HR	3.1 ± 3.0b	9.9 ± 11.5a	10.2 ± 11.2a	2.7 ± 3.3b	0.03	0.02	0.12
HS	2.4 ± 2.3b	34.6 ± 40.6a	25.4 ± 41.8	11.6 ± 19.1	0.009	0.23	0.21
SG	13.6 ± 10.3	11.2 ± 8.4	15.8 ± 9.8	9.1 ± 7.7	0.43	0.09	0.59
Period 3							
EF	2.2 ± 2.5b	27.0 ± 37.2a	23.9 ± 38.6	5.3 ± 6.7	0.02	0.06	0.08
HR	1.2 ± 2.1	2.8 ± 3.1	3.1 ± 3.1	0.9 ± 1.7	0.06	0.05	0.85
HS	0.9 ± 1.0b	7.8 ± 7.5a	5.6 ± 7.9	3.1 ± 4.2	0.003	0.26	0.20
SG	3.9 ± 3.9	8.4 ± 10.7	9.1 ± 10.4	3.3 ± 3.9	0.06	0.10	0.04

[1] P – value are shown for OBS (Observation Before X After Disbudding), HO (housing system Individual X Complex Social Housing), and the interaction between OBS X HO (observation X housing system).

a,b Within observation (Before Disbudding X After Disbudding) and housing treatment (Individual X Complex Social housing) in the same line indicate significant ($P<0.05$) differences.

Camiloti et al. Table 3.

Observation	Housing	
	Individual	Complex social
Before disbudding	3.9 ± 3.0b	4.0 ± 5.0b
After disbudding	14.3 ± 13.0a	2.6 ± 2.6b
P – value[1]		
P – value observation	0.104	
P – value housing	0.062	
Interaction	0.038	

[1] P – value are shown for observation (Before X After Disbudding), housing system (Individual X Complex Social Housing), and the interaction between Observation X Housing.
a,b Mean ± SD indicate significant ($P<0.05$) differences.

5 ARTIGO 2[2]

Interpretive Summary: Pain Behavior and Social Interactions of Dairy Calves after Disbudding. ***Camiloti et al. Page 000-000.*** Social contact is known to provide many benefits for animals, although dairy calves are commonly raised in single pens. Disbudding is an invasive procedure performed in these animals to prevent injuries. This study evaluated how a partner influences pain related behaviors, feed intake and social interactions after disbudding. In conclusion, one calf as companion was not enough to buffer pain socially, but pair housing had a higher solid feed intake at the disbudding day than individual calves. Increasing social interactions between calves of the same pair may have some influence decreasing head shakes behavior.

PAIN BEHAVIOR AND SOCIAL INTERACTIONS OF DAIRY CALVES

Pain Behavior and Social Interactions of Dairy Calves after Disbudding

T.V. Camiloti*†, J. A. Fregonesi*†, M. A. G. von Keyserlingk* and D. M. Weary*

* Animal Welfare Program, Faculty of Land and Food Systems, University of British Columbia, 2357 Main Mall, Vancouver, V6T1Z4, Canada

† Universidade Estadual de Londrina, Rod. Celso Garcia Cid PR 445 Km 380, Campus Universitário, Londrina, 86051-990, Brazil

Corresponding author's email: tatianevito@hotmail.com

[2] O artigo foi redigido de acordo com as normas do *Journal of Dairy Science* e será submetido para publicação nesta revista. Anexo 1.

ABSTRACT

Social contact has been described as beneficial to animals, however dairy calves are commonly raised in single pens. Disbudding is an invasive and painful procedure performed in dairy calves to prevent injuries. To test the effect of a social companion on buffering pain related behaviors, social interactions and feed intake after hot iron disbudding, dairy calves (n = 24) were randomly housed individually or in pairs. When calves were approximately 36 days old, half of the animals were sham disbudded and then disbudded three days later and the other half of the animals the order of the procedures was inversed. All calves in pairs were exposed to the experimental environment twice over two rounds, once as the actor (disbudded) and once as the observer (the partner being disbudded). Pain related behaviors as head rubbing and head shaking, and social interactions were direct observed for 20 minutes in the hours following dehorning (1, 2, 3, 4, 5, 7, 9, 12, 24 and 36h), and solid feed consumption was measured every morning. As predicted, independent of the procedure sequences, pain behaviors increased after disbudding. Housing system treatments (Individual x Paired calves) had no effects on pain behaviors and total mixed ration intake. However, grain intake was higher for paired calves than single calves (P=0.003). Calves expressed more social interactions in round 2 than in round 1 in the period of hours 1, 2, 3 and 4 after the procedure (P=0.03). In the same period of time, head shakes behavior decreased when the second calf of the pairs was sham or actual disbudded than when the first calf suffered the procedure. In the hours 12 and 24 after disbudding, social interactions were higher when the second calf of the pair was disbudded. In summary, the presence of only one partner may not be enough to provide social modulation of pain behaviors excepted for head shakes after hot iron disbudding, even when social interactions increased, but social contact with a partner increases grain intake.

KEY WORDS: dairy cattle, calf social behavior, housing, pain, feed intake.

INTRODUCTION

In dairy production systems young animals go through many stressing managements from calving to weaning. For example, calf-dam separation as soon as birth, disbudding and weaning. Disbudding is an invasive and very painful procedure performed in dairy calves to decrease the risk of injuries in humans and other animals, which the horns are cauterized commonly using an iron heated or caustic paste. Pain can be described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (International Association for the Study of Pain), and to reduce the pain caused by disbudding some farms adopt the use of sedative, local anaesthetics and anti-inflammatory drugs (Stafford and Mellor, 2011). Disbudding can affect behaviours as ear flicks, head shakes and head rubs (Faulkner and Weary, 2000; Vickers et al., 2005), physiological parameters as cortisol, heart and respiratory rates (Morisse et al., 1995; Graf and Senn, 1999; Heinrich et al., 2009) and also affects the emotional state of calves (Neave et al., 2013).

A companion is known to facilitate socialization and social development, minimize the stress after weaning (De Paula Vieira et al., 2010), an older companion during separation (De Paula Vieira et al., 2012) and reduce food neophobia (Costa et al., 2014). Social contact may be important to calves and they are able to work pressing a panel to have full contact with a partner (Holm et al., 2002). Social contact is also important in terms of feed intake and weight gain (De Paula Vieira et al., 2010; Costa et al., 2015), and social complexity may buffer stress during separation from a social reference from the group (De Paula Vieira et al., 2012). In other species, social contact is shown to have influence in pain caused by electrical chocks in rats (Kiyokawa et al., 2004), and tail docking in lambs (Guesgen et al., 2014).

Social interactions and play behavior are indicators of animal welfare (Boissy et al., 2007; Held and Špinka, 2011). Calves housed in pairs spend approximately 2% of the day in social contact (Chua et al., 2002). Play behaviour may be reduced during negative experiences

as pain caused by disbudding, although the body contact may be important when social buffering is considered (Mintline et al., 2013).

Studies showing the effect of disbudding on behaviour, physiological and emotional state of calves have been done, but few are trying to explain the effect of social contact on this procedure. The aim of this study was to evaluate the relevance of social contact on pain related behaviours in the hours following disbudding, as well as solid feed intake and social interactions between partners, testing the social modulation of pain hypothesis in dairy calves after this procedure.

MATERIAL AND METHODS

The experiment was carried out from April to November of 2013 at the University of British Columbia's Dairy Education and Research Center, located in Agassiz, British Columbia, Canada and it was approved by the University of British Columbia Animal Ethics Committee.

Animals and Housing

Twenty-four Holstein male calves were separated from the dam right after birth, and fed with at least 4 L of colostrums (with >50 g/L of IgG) by bottle. Only calves with serum protein > 5.5 g/dL were kept in this study. Calves were randomly allocated to individual (n = 8) or pair housing (n = 8 pairs) weighing at birth 42.2 ± 6.2 kg (mean ± SD) individual calves and 43.4 ± 4.5 kg paired calves. Experimental pens were sawdust bedded and measured 1.2 x 2.0 m for individual calves and 2.4 x 2.0 m for paired calves. Pens were cleaned once a week as well the check of health of all calves was done including body weight, rectal temperature, diarrhea scoring and respiratory system. When calves were diagnosed as ill, they were treated by the herd veterinarian of the farm. One calf from the individual housing and one calf from

the pair housing system were treated for respiratory disease, and also one calf from the individual housing and one calf from the pair housing system were treated for diarrhea.

Experimental Design and Disbudding Procedure

Sham disbudding was used as control treatment. All calves were sham disbudded and disbudded and the order of the procedures was switched in half of the animals in each housing system treatment. Four experimental unit of each housing system treatment started the experiment being sham disbudded and the other half disbudded first. This way, it was possible to evaluate the effects of the drugs on pain related behaviors after each procedure. The interval between one procedure and the other was three days.

Calves were disbudded approximately at 9AM averaging 35.8 ± 2 days old and weighing 64.8 ± 3.8 kg (mean ± SD) for individual calves and 67.3 ± 7 kg for paired calves. The procedure consisted in an intramuscular injection of the sedative xylazine of 0.2 mg/kg body weight (Rompun, 2%, Bayer Inc.) and ten minutes after administration of the xylazine, a local anaesthetic (5 mL per side of 2% Lidocaine; Ayerst Veterinary Labs, Ontario) was injected subcutaneously into the corneal nerve of each horn bud, located along the occipital groove midway between the horn bud and eye (Faulkner and Weary, 2000). Five minutes after the administration of lidocaine, an electrically heated hot-iron dehorner (Rhinehart X-30) was applied to each horn bud for approximately 15s. During the sham procedure, calves received injections of sedative and local anesthetic, exactly the same methodology and doses performed during the disbudding, however using the electrical dehorner cold this time.

Each calf in the pair housing system treatment was exposed to the test environment twice, once as the receptor of the action, i. e. disbudded, and once as the observer, not disbudded. Therefore, the first calf was sham or disbudded (round 1) and the same procedure was performed in the second calf of the pair (round 2) five days later. To perform both

procedures in paired-housed calves, a plastic divisor panel was used to separate the calves in different pens and they were put back together as soon as the procedure was concluded.

Behavior measurements

The behavior of each calf was direct observed during 36 hours following sham disbudding and disbudding in 10 periods of 20 min each, at 1, 2, 3, 4, 5, 7, 9, 12, 24 and 36 hours after the procedures (Faulkner and Weary, 2000). Direct observation was done by the same observer during all the experiment period.

The following pain behaviors previously related to disbudding were observed: head shaking (rapid movement of the head from one side to the other); head rubbing (scratch the head with the hind foot or against pen walls, buckets, floor or other animal of the pair); and social interactions (observed on paired-housed calves interactions as licking each other, body contact, sniffing each other, play behavior).

Milk and Solid Feeding

During all the experimental period, all calves were fed 8 L of pasteurized whole milk by bottle divided in two meals/day. Concentrate starter [Hi-Pro Medicated Calf Starter, Chilliwack, BC, Canada, Dry Matter (DM) of 89.5%; chemical composition shown as percent of DM, 90% DM; Crude Protein (CP) 21%, Neutral Detergent Fiber (NDF) 19%, Acid Detergent Fiber (ADF) 11%; medicated with coccidiostat (50 mg/kg of lasalocid sodium)] , TMR (shown as percent of DM; 26.1% of corn silage, 14.8% of grass silage, 10% of alfafa hay, 49% of concentrated mix; average of 49.1% DM; chemical composition CP 17%, NDF 32%, ADF 20%) and water were available for consumption *ad libitum.*

Solid feed intake was measured by consumption every morning approximately at 8:30AM and fresh feed and water were provided. Samples of feed were collected, kept frozen and sent to A&L Canada Laboratories Inc. (London, ON, Canada) to chemical analyses.

Statistical Analyses

All analysis was conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC) and data were checked for normality using the UNIVARIATE procedure and probability distribution plots in SAS. When necessary, data was transformed. Significance was declared at $P < 0.05$.

A Factorial 2 x 2 experimental design was tested to evaluate the effects of housing system (Individual versus Pair) and observation (sham disbudding versus actual disbudding) on pain related behavior variables

Pain behavior. The effect of housing (Individual x Pair) and observation (Sham x Actual Disbudding) on head shakes and head rubs (all measured in number of events) was tested by obtaining the mean value of the pen (i.e., calf for individual treatment and the mean of the 2 calves per pen in the pair treatment) and was analyzed using the MIXED procedure in SAS. The model included the effect of housing, observation, order (the order that sham and disbudding were performed) and tested housing x observation interaction. Behavioral responses had a peak after hour 5 following the disbudding procedure, so data was analyzed separately in 4 periods: Period 1 (hours 1, 2, 3 and 4 after sham and disbudding); Period 2 (hours 5, 7 and 9); Period 3 (hours 12 and 24); and Period 4 (hour 36 after sham and disbudding).

Round. This analysis was performed only on the paired calves comparing round 1 (i. e., the first calf of the pair being disbudded) and round 2 (i. e., second calf of the pair being disbudded) on variables head rubs, head shakes and social interactions between the calves.

The effect of round (Round 1 x Round 2) and observation (Sham x Actual Disbudding) was analyzed using the MIXED procedure in SAS and it was also tested separately for each period: Period 1 (hours 1, 2, 3 and 4 after sham and disbudding); Period 2 (hours 5, 7 and 9); and Period 3 (hours 12 and 24 after sham and disbudding). The model included the effect of observation, hour, round and tested round x observation interaction and calf was considered as random effect.

Feed Intake. This analysis was performed comparing individual calves with paired calves only on round 1 to obtain data of one calf being disbudded alone x one calf being disbudded with a companion, using the pen (i. e., calf or pair) as the experimental unit. The effect of housing system (Individual x Pair) and the day (-1: day prior to disbudding; and day 0: day of disbudding) on TMR and calf starter intake was analyzed using the MIXED procedure in SAS. The model included the effect of housing treatment, day and tested housing x day interaction.

RESULTS

Pain behavior. For pain related behaviors observed, no differences were found when housing systems were compared and interactions tested in any of the 4 periods observed. In Period 1, head rubbing showed an increase after disbudding when observations (sham x actual disbudding) were compared ($P = 0.002$). In Period 2, increases in head rubbing and head shaking could be observed after disbudding when observations were compared ($P = 0.004$; $P < 0.0001$, respectively). We did not find any effect in Periods 3 and 4 (Table 1). The order that the procedures were performed (sham or disbudding first) had no influence on pain behaviors observed excepted for head rubbing on Period 2 ($P = 0.008$).

Round. In Period 1, interaction between observation and round was found for head shaking ($P = 0.02$). Comparing observations (Sham x Actual Disbudding) in Period 1, when

the effect of round 1 and 2 was tested on pain related behaviors, we found differences on head rubbing ($P = 0.006$) and head shaking ($P = 0.04$) increasing these behaviours after disbudding (Table 2). When rounds were compared on the same period of time, social interactions increased in round 2 ($P = 0.03$). Head shaking was higher in round 1 (10.6 ± 9.4 events) than in round 2 (2.9 ± 4.7 events) after actual disbudding procedure (Table 3).

In Period 2, no observation x round interaction was found (Table 2). We only found results when observations were compared, and again head rubs and head shakes increased after disbudding ($P = 0.003$; and $P < 0.0001$, respectively).

In Period 3, we found interaction between observation (Sham x Actual Disbudding) and social interaction ($P = 0.03$). In this period, social interactions increased in round 2 when the second calf of the pair was disbudded (Table 3).

No significant results were found in Period 4 (Table 2).

Feed Intake. Intake of TMR (total mixed ration) was similar ($P = 0.15$) comparing disbudding (Day -1, day before disbudding x Day 0 when actual disbudding was performed) and the same was found when we compared individual versus pair housing systems treatments ($P = 0.38$). Nevertheless, there was a tendency for disbudding x housing system interaction for grain intake on the dehorning period ($P = 0.05$). Intake of grain also did not differ when disbudding (Day -1 x Day 0) was compared ($P = 0.31$), but was higher for paired calves ($P = 0.003$) than for individual calves when housing system was compared (Table 4).

DISCUSSION

Disbudding is a very painful procedure and many authors has shown the effects that this practice has on behavioral (Faulkner and Weary, 2000; Vickers et al., 2005; Heinrich et al., 2010), physiological (Morisse et al., 1995; Gran and Senn, 1999, Heinrich et al, 2009), and also on emotional state of calves (Neave et al., 2013). In our knowledge, no study so far

reported the effects of pain caused by disbudding on social interactions and intake of solid feed of those animals and also how social contact affect the expression of pain related behaviors after disbudding. The most common behaviors related to the pain caused by disbudding observed in calves were ear flicking, head shaking and head rubbing and some studies shown that those behaviors can be noticed in hot iron disbudded calves (Grøndahl-Nielsen et al., 1999; Faulkner and Weary, 2000) as well as in caustic paste disbudded calves (Morisse et al., 1995; Vickers et al., 2005).

In this study, when sham disbudding and disbudding were compared, we observed increase in head shakes and head rubbing in the first 9 hours after disbudding procedure. These results are consistent with Grøndahl-Nielsen et al. (1999), Faulkner and Weary (2000) and Heinrich et al. (2010) that also verified an increase of these behaviors after the procedure. One of our objectives was to evaluate the effect of a social companion on pain behaviors after disbudding and for this topic no differences between calves raised individually or in pairs were found. These results are consistent to those presented by Guesgen et al. (2014), studying pain in tail docked lambs also found no social buffering effects between the lambs when they were tested with familiar unrelated and unfamiliar unrelated lambs, however, when tested with familiar related (twins), lambs showed a smaller increase in rolling and kicking than the other groups. Colditz et al. (2012), studying the social buffering effect on pain caused by different methods of castration in lambs did not find evidences of transmission of buffering on behavioral responses.

Our findings are in contrast with our previous study, which was found that animals raised in a dynamic group of other calves having visual and partial body contact with the dam and other cows of the group, expressed less head rubbing. The result in the current study can be explained because calves had as companion only one calf, and maybe the number of partners has some influences on this situation and is not enough to provide the social

reference effect. Another point to be considered is that this study was developed during spring/summer months, with temperature increasing and naturally the increase of flies in the environment, and both could have had affect on those behaviors.

We tested the effect of round on pain related behaviors and social interactions in calves housed in pairs, which once the calf was suffering the disbudding and later the same calf was observing its partner being disbudded. The objective was to evaluate the effect of the previous experience with the situation in the expression of pain and on the social support given to the partner. Effect of round was found on pain related behaviors in Period 1 comparing round 1 and round 2 with the second calf that was disbudded expressing less head shaking than the first calf of the pair expressed when it was disbudded and this result can be explained by the fact that in this period social interactions increased when the second calf was disbudded and this could have decreased the expression of this behavior. However for all other periods and for head rubbing behavior no significant result was found, showing that independently of the calf watching its partner suffering with pain, the pain caused by hot iron disbudding procedure cannot be minimized by previous experience in the hours where the peak of pain occurs (Period 2) or when pain related behaviors started to decrease (Period 3). Most of our findings are in contrast with Guesgen et al. (2014), which found that round affected the expression of pain in tail docked lambs, animals docked in the second exposure to the test environment displayed less pain behaviors such as kicks and head shakes, excepted for head shaking in Period 1, decreasing this behavior in round 2.

As we discussed above, social modulation of pain only happened in Period 1 with round x observation for head shaking behavior, but did not happen when we compared individual versus pair housed calves, where only one calf was not enough to provide such benefit in terms of expression of pain related behaviors. However, when we tested the effect of round on social interaction, we found that social behaviors as licking, sniffing each other,

playing behaviors and body contact increased when the second disbudding happened in the pair housed calves (round 2) in Period 1 (first 4 hours following the procedure), while the calf was still under some effect of the sedation caused by xylazine injection. There was interaction between observation (Sham x Actual Disbudding) and round (round 1 x round 2) for social interaction behavior in Period 3 (hours 12 and 24 following the procedure), which means that the procedure had effects on rounds, however this social interaction was not enough to buffer the effects on pain related behaviors in this period. In a study with adult male rats, Kiyokawa et al. (2004) showed that the presence of a partner rat attenuated fear behavioral responses on fear-conditioned shocked rats, although nonshocked partners, rats that did not have the painful experience previously, were more effective than shocked partners to attenuate fear responses as freezing and resting behaviors. In our study the opposite happened, with social interactions increased in round 2, when one calf of the pair had the painful experience early.

Social housing is also known to improve social facilitation in terms of solid feed intake. In our study, paired-housed calves showed a higher ingestion of grain than did individually housed calves and this finding is consistent with De Paula Vieira et al. (2010); Costa et al. (2015); and Jensen et al. (2015). In our knowledge, this is the first study evaluating the effects of disbudding on feed intake on 5 weeks old dairy calves. TMR consumption was equal for both housing systems. Our results are consistent with Costa et al. (2015), which found that calves housed in pairs in the first 3 days of life eat more concentrate starter than calves housed individually or housed in pairs at 6 weeks old, but they also did not found the same effect between treatments for TMR intake. Our results contrasts with that of Phillips (2004), which comparing solid feed intake of calves raised in group versus individually found that group animals ate more grass than individual calves, but the intake of starter was the same for both treatments. The level of milk provided is likely to have effect on solid feed intake (Khan et al., 2011). In our study, calves received 8L of milk/day, in contrast

with Phillips (2004), that fed the calves just 4L of milk/day likely increasing the motivation of the calves to eat concentrate. Jensen et al. (2015) found that paired-housed animals had a greater concentrate intake than individually housed calves for the animals that were fed high level of milk, but no effect of housing was found in calves receiving low level of milk.

In conclusion, the presence of one companion was not enough minimized the effects of the pain caused by hot iron disbudding on behaviors even with the increase of social interactions between pair housed calves when the second calf suffered the procedure excepted for head shaking behavior. Social facilitation effect was notable when concentrate started was compared, demonstrating that social housing is also important for feed intake on the disbudding day.

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Table captions

Table 1. Behavioral responses (mean ± SD; events) of calves housed individually (n = 8) compared with calves housed in pairs (n = 8) during sham disbudding and actual hot iron disbudding. Period 1 = hours 1, 2, 3 and 4; Period 2 = hours 5, 7 and 9; Period 3 = hours 12 and 24; and Period 4 = hour 36 after the procedures. Behaviors: HR = head rubbing; and HS = head shaking.

Table 2. Behavioral responses (mean ± SD; events) of calves housed in pairs (n = 8) during sham disbudding and actual hot iron disbudding. Calf 1 = first calf disbudded of the pair; and Calf 2 = second calf disbudded of the pair. Period 1 = hours 1, 2, 3 and 4; Period 2 = hours 5, 7 and 9; Period 3 = hours 12 and 24; and Period 4 = hour 36 after the procedure. Behaviors: HR = head rubbing; HS = head shaking; and SI = social interaction.

Table 3. Mean ± SD (Observation * Round interactive effect) for head shaking (HS) behavior responses observed in Period 1 (hours 1, 2, 3 and 4 after sham or actual disbudding procedure). and for social interaction (SI) behavior responses observed in Period 3 (hours 12 and 24 hours after sham or actual disbudding procedure) of calves housed in pairs (n = 8).

Table 4. Mean ± SD of grain and TMR (total mixed ration) intake (Kg) of calves housed individually (n = 8) compared with calves housed in pairs (n = 8) during Day -1 (day prior to disbudding day) and Day 0 (actual disbudding day).

Camiloti et al. Table 1.

	Observation (OBS)		Housing (HO)		P – value[1]			
	Sham	Actual	Individual	Pair	Order	OBS	HO	OBS X HO
Period 1								
HR	2.0 ± 2.6b	5.4 ± 6.1a	3.0 ± 4.2	4.1 ± 5.3	0.45	0.002	0.67	0.67
HS	1.8 ± 2.1	4.6 ± 6.9	1.3 ± 1.3	4.1 ± 6.2	0.74	0.10	0.08	0.26
Period 2								
HR	3.1 ± 1.9b	7.5 ± 7.2[a]	4.6 ± 3.4	5.7 ± 6.8	0.008	0.004	0.54	0.23
HS	5.1 ± 9.3b	20.7 ± 31.7a	8.4 ± 12.2	15.2 ± 28.6	0.55	<.0001	0.38	0.06
Period 3								
HR	2.3 ± 2.7	2.7 ± 3.3	3.1 ± 3.1	2.3 ± 3.0	0.35	0.61	0.53	0.07
HS	2.4 ± 3.8	6.6 ± 13.7	2.4 ± 3.2	5.5 ± 12.2	0.22	0.07	0.39	0.17
Period 4								
HR	2.2 ± 3.2	3.0 ± 5.1	3.9 ± 6.2	1.9 ± 2.7	0.38	0.29	0.39	0.52
HS	0.5 ± 1.3	2.2 ± 5.3	1.3 ± 2.5	1.4 ± 4.5	0.80	0.17	0.82	0.72

[1] P – value are shown for Order (order procedures were performed – sham first X actual disbudding first), OBS (observation before X after dehorning), HO (Housing system – Individual X Pair housed calves) and OBS X HO (interaction between observation and housing system treatment).

a,b Within observation (Sham Disbudding X Actual Disbudding) and housing treatment (Individual X Pair) in the same line indicate significant ($P<0.05$) differences.

Camiloti et al. Table 2.

	Observation		Round		P – value[1]		
	Sham	Actual	Round 1 Calf 1	Round 2 Calf 2	Obs	Round	ObsX Round
Period 1							
HR	1.9 ± 2.6b	5.9 ± 6.7a	4.3 ± 6.2	3.5 ± 4.7	0.006	0.68	0.58
HS	3.1 ± 4.2b	6.7 ± 8.2a	6.8 ± 7.8	3.0 ± 4.9	0.04	0.17	0.02
SI	3.9 ± 2.7	5.1 ± 7.0	2.4 ± 1.5b	6.6 ± 6.7a	0.31	0.03	0.37
Period 2							
HR	2.9 ± 3.4b	8.5 ± 8.2a	5.0 ± 7.5	6.4 ± 6.2	0.003	0.55	0.43
HS	5.1 ± 9.6b	25.3 ± 37.1a	12.8 ± 19.0	17.6 ± 36.3	< .0001	0.68	0.78
SI	5.6 ± 3.5	4.8 ± 2.8	5.2 ± 3.2	5.2 ± 3.2	0.45	0.96	0.96
Period 3							
HR	1.6 ± 1.9	2.9 ± 3.7	2.6 ± 3.0	2.0 ± 3.1	0.09	0.64	0.21
HS	2.2 ± 3.9	8.7 ± 16.5	3.7 ± 4.4	7.2 ± 16.8	0.05	0.49	0.36
SI	3.1 ± 3.2	3.4 ± 3.0	3.5 ± 3.1	3.0 ± 3.1	0.73	0.65	0.03
Period 4							
HR	1.7 ± 2.1	2.1 ± 3.2	1.9 ± 2.1	1.9 ± 3.2	0.66	0.96	0.92
HS	0.5 ± 1.5	2.2 ± 6.1	0.4 ± 0.7	2.3 ± 6.2	0.28	0.25	0.48
SI	1.8 ± 2.4	2.9 ± 2.3	2.4 ± 2.4	2.3 ± 2.4	0.18	0.85	0.38

[1] P – value are shown for Obs (Observation sham disbudding X actual disbudding), Round (first calf disbudded X second calf disbudded), Obs X Round (interaction between observation and round).

a,b Within Observation (Sham Disbudding X Actual Disbudding) and Round (first calf disbudded X second calf disbudded) in the same line indicate significant ($P<0.05$) differences.

Camiloti et al. Table 3.

Observation / Behaviors	Period 1 Head Shakes	Period 3 Social Interactions
	Round 1 (Calf 1)	
Sham Disbudding	3.0 ± 3.8b	4.5 ± 3.5a
Actual Disbudding	10.6 ± 9.4a	2.5 ± 2.6b
	Round 2 (Calf 2)	
Sham Disbudding	3.2 ± 5.4b	1.6 ± 2.3b
Actual Disbudding	2.9 ± 4.7b	4.4 ± 3.3a
P – value[1]		
P – value Observation	0.04	0.73
P – value Round	0.18	0.65
P – value Obs X Round	0.02	0.03

[1] P – value are shown for observation (Sham X Actual Disbudding), Round (first calf disbudded X second calf disbudded), and the interaction between Observation X Housing.
a,b Mean ± SD in the same column indicate significant (P<0.05) differences.

Camiloti et al. Table 4.

	Disdudding (DISB)		Housing (HO)		P – value[1]		
	Day -1	Day 0	Individual	Pair	DISB	HO	DISB X HO
Grain	0.06 ± 0.06	0.06 ± 0.06	0.02 ± 0.04b	0.10 ± 0.06a	0.31	0.003	0.05
TMR	0.20 ± 0.20	0.16 ± 0.15	0.13 ± 0.18	0.23 ± 0.16	0.38	0.15	0.56

[1] P – value are shown for DISB (Day -1: day before disbudding X Day 0: disbudding day), HO (Individual X Pair housing systems) and DISB X HO (interaction between Disbudding day and Housing system treatment).

a,b Within Housing (Individual X Pair housing systems) in the same line indicate significant ($P<0.05$) differences.

6 CONCLUSÃO

Com a crescente demanda da sociedade para questões de bem-estar animal e melhora na qualidade de vida desses animais, tornam-se necessários estudos com o objetivo de responder essas questões e aplicar de forma prática manejos que diminuam ou até mesmo elimine qualquer risco ao bem-estar de animais de produção.

A separação de bezerro de sua mãe logo após o nascimento, a criação individual e procedimentos dolorosos como a descorna podem ter impacto negativo na criação de bezerras leiteiras.

O presente trabalho mostrou que a criação de bezerros em um ambiente social complexo pode trazer benefícios em termos de expressão de comportamentos de dor após a descorna com ferro quente. Foi possível também verificar que a presença de um único companheiro no momento da descorna não foi suficiente para promover o suporte social necessário na diminuição dessa dor, porém mostrou que as interações sociais aumentam quando um animal que já sofreu o procedimento vê o companheiro passar pela mesma dor indicando dessa forma empatia entre os animais. O contato social com um parceiro antes e após a descorna demonstrou também ajudar na facilitação social através do consumo de concentrado inicial desses animais.

Futuros estudos devem ser desenvolvidos com o objetivo de descrever mais profundamente essa relação entre os animais após procedimentos dolorosos e caracterizar os possíveis fatores que potencializam o suporte social em bezerras leiteiras.

ANEXOS

ANEXO 1

Journal of Dairy Science (Instructions to Authors)

J. Dairy Sci. 98:Instructions 1–17

Journal of Dairy Science® Instructions to Authors[1]

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These instructions detail the form and style required by the *Journal of Dairy Science* (JDS) for papers submitted for publication. Papers that do not follow the form and style of the journal may be rejected without review. We recommend that authors refer to these instructions when preparing manuscripts, when incorporating requested changes into revisions after review, and when checking author proofs.

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All research animals should be acquired, retained, and used in compliance with federal, state, and local laws and regulations. The authors should state explicitly that IACUC (or equivalent) approval was obtained before commencement of the study. Authors should make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments should be conducted in accordance with the principles and specific guidelines presented in *Guidelines for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd ed. (available from the Federation of Animal Science Societies, 1800 S. Oak St., Suite 100, Champaign, IL 61820; http://www.fass.org/). Methods of killing experimental animals must be described in the text. When describing surgical procedures, the type and dosage of the anesthetic agent must be specified.

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1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
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Types of Articles

Full-Length Research Papers. The majority of papers published in JDS are full-length research articles. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts and methods, and sufficient background information

[1]Revised March 2015.

that would be required for thorough understanding by scientists in other disciplines. The results of experiments published in the journal must be replicated, either by replicating treatments within experiments or by repeating experiments.

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Hot Topics. Papers submitted for this section must report on a completed experiment testing a timely, original hypothesis of importance to an area of dairy science. The work may be preliminary in nature, but with sufficient data so that the hypothesis is clearly tested. Results may point to avenues for fruitful, in-depth analyses. Reports must contain an explicitly stated hypothesis and objectives, with sufficient detail in methodology for repetition of the work, as well as a results section, a brief discussion, and references. Total page limits for text, tables, figures, and references must be no more than 4 journal pages (approximately 10 typewritten pages minus space for tables and figures). The manuscript should contain a title and short abstract but not separate sections. The total number of tables and figures should be no more than 3; references should be minimal. The first page must have HOT TOPICS in capital letters on the header line.

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*Department of Animal Science, and
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List only pertinent references. No more than 3 references should be needed to support a specific concept.

Table 1. Effect of garlic oil, diallyl disulfide, allyl mercaptan, monensin, and lovastatin on a 17-h in vitro batch culture rumen microbial fermentation trial

	Treatment[1]						
Item	Control	GAR300	DAD300	ALM300	MON	LOV	SEM
pH	6.6	6.7	6.7	6.6	6.6	6.6	0.01
Apparent disappearance of DM, %	61.0^{a}	50.7^{b}	51.2^{b}	60.4^{a}	53.9^{b}	62.4^{a}	1.11
Fiber digestibility							
NDF, %	56.8^{a}	44.3^{b}	41.4^{b}	55.9^{a}	39.3^{b}	60.0^{a}	1.73
ADF, %	53.7^{a}	36.8^{b}	34.9^{b}	52.5^{a}	30.7^{b}	57.0^{a}	2.03
Gas, μmol	4,674.8^{a}	3,756.9^{cd}	3,359.7^{d}	4,388.2^{ab}	4,009.6^{bc}	4,673.1^{a}	123.34
CH_4, μmol	417.3^{a}	110.1^{d}	131.3^{d}	335.9^{b}	241.7^{c}	396.3^{a}	21.56
Total VFA, m*M*	49.3^{a}	39.7^{c}	38.8^{c}	45.4^{b}	45.7^{ab}	48.4^{ab}	1.17
Individual, mol/100 mol							
Acetate	61.2^{a}	54.3^{d}	53.9^{d}	58.3^{b}	56.4^{c}	61.1^{a}	0.53
Propionate	22.6^{d}	25.8^{c}	28.3^{b}	22.8^{d}	34.2^{a}	22.8^{d}	0.78
Butyrate	12.5^{c}	16.5^{a}	14.0^{bc}	15.0^{ab}	6.6^{d}	12.4^{c}	0.60
Branched-chain VFA	2.0^{a}	1.7^{b}	1.7^{b}	2.0^{a}	1.4^{c}	2.0^{a}	0.10
C2:C3	2.7^{a}	2.1^{b}	1.9^{c}	2.5^{a}	1.6^{d}	2.7^{a}	0.07
CH_4 (μmol):VFA (μmol)	0.20^{a}	0.05^{d}	0.07^{cd}	0.15^{ab}	0.10^{bcd}	0.17^{ab}	0.00
N-NH_3, mg/100 mL	16.7^{ab}	16.6^{bc}	19.0^{a}	17.2^{ab}	14.4^{c}	16.4^{bc}	1.10

$^{a-d}$Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: GAR300 = 300 mg/L *Allium sativa* (garlic oil); DAD300 = 300 mg/L diallyl disulfide; ALM300 = 300 mg/L allyl mercaptan; MON = 12.5 mg/L monensin; LOV = 5 mg/L lovastatin.

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Journals

Buch, L. H., A. C. Sorensen, J. Lassen, P. Berg, J.-A. Eriksson, J. H. Jakobsen, and M. K. Sorensen. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. J. Dairy Sci. 94:1540–1551. http://dx.doi.org/10.3168/jds.2010-3137.

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Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.

The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen or the paddock is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.

Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should

include a blocking factor, or the initial measurement should be included as a covariate.

A parameter [mean (μ), variance (σ^2)], which defines or describes a population, is estimated by a statistic ($\bar{x}$, s^2). The term *parameter* is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

Standard designs are adequately described by name and size (e.g., "a randomized complete block design with 6 treatments in 5 blocks"). For a factorial set of treatments, an adequate description might be as follows: "Tryptophan at 0.05 or 0.10% of the diet and niacin at 5, 10, or 20 mg/kg of diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks, each block consisting of littermates." Note that a factorial arrangement is not a design; the term "design" refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not "statistically significant" is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by "±" to a number implies that the second value is its standard error (not its standard deviation) unless otherwise specified. Adequate reporting may require only (1) the number of observations, (2) arithmetic treatment means, and (3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is appropriate to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple comparison tests should be used only to compare means of treatments that are unstructured or not related. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. Means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms *significant* and *highly significant* traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P-value is preferred to the use of these terms. For example, use ". . . we observed a difference ($P < 0.05$) between control and treated samples" rather than ". . . we observed a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.03$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (alpha) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 2 or 3 places after the decimal (2 significant digits are usually sufficient). Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of Type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a beta error, not an alpha error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant

information contained in the data is sacrificed. In most cases, 2 or 3 significant digits (not decimal places) are sufficient.

Sensory Data

Sensory data should comply with "*Invited Review:* Sensory Analysis of Dairy Foods," *Journal of Dairy Science* 90:4925–4937. http://dx.doi.org/10.3168/jds.2007-0332.

Nomenclature

Genes and Proteins. The journal recommends using internationally accepted symbols for genes and proteins; such symbols may be used without definition. Symbols for specific genes and proteins can be obtained by querying the gene database of PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Nomenclature rules for humans, nonhuman primates, and livestock are available at http://www.genenames.org, and rules for mice and rats are at http://www.informatics.jax.org/mgihome//nomen/strains.shtml. Gene symbols should be shown in italics (e.g., *SERPINA14*) and proteins in roman text (e.g., SERPINA14). Gene symbols are generally shown in all uppercase letters (e.g., *LHB*), except in mice and rats, where only the first letter is capitalized (e.g., *Lhb*)

Single Nucleotide Polymorphisms. The increasing number of SNP association studies and the improvements in bovine genome annotation require a standardized SNP nomenclature for unequivocal and correct SNP identification. Additionally, information regarding the SNP investigated should be easily accessible in a publicly available database. Therefore, all relevant SNP included in a study should be listed with their unique RefSNP (rs) or submitted SNP (ss) number (if rs number is not yet available) as indicated in the public domain NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/snp). If the SNP investigated do not yet have an entry in the NCBI dbSNP database, the authors of the manuscript are responsible for submitting all the required information to NCBI (see http://www.ncbi.nlm.nih.gov/projects/SNP/) for depositing the SNP into the database and obtaining a unique ss number for the SNP. In the text of the manuscript, use the rs/ss number of the SNP or an alternative standardized nomenclature.

Microorganisms. All microorganisms must be named by genus and species. The name of the genus must appear in full the first time that the microorganism is cited in the abstract, in the body of the paper, and in each table and figure legend. Thereafter, the genus can be abbreviated by its first initial unless it will be confused with other microorganisms cited in the paper, in which case each genus should be abbreviated to use enough letters to avoid confusion (e.g., *Strep.* vs. *Staph.*). The formal, binomial names of all microorganisms should be in italics. Specific strain designations and numbers should be used when appropriate. Authorities are not required.

For microorganisms that are genetic variants of a parent strain, the genotypic and phenotypic properties should be cited according to the procedures described by Demerec et al. (1966) in *Genetics* 54:61–76. Phenotypes should be identified by 3 letters; the first is capitalized. Genotypes should be identified by 3 lowercase italic letters. Superscript plus (+) signs are used to refer to a wild-type. The serial isolation number is placed after the locus symbol for mutations. The delta symbol is used to indicate deletions. Nomenclature for bacterial plasmids should be cited according to Novick et al. (1976) in *Bacteriological Reviews* 40:168–189.

Enzymes. Mention of an enzyme should include the EC number.

In Vitro Antimicrobial Susceptibility Tests

Please refer to the JDS policy in Appendix 4 of this document.

Miscellaneous Usage Notes

Abbreviations. Abbreviations should not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by their abbreviation (e.g., IgG, CD). Abbreviations may be used in heads within the paper if they have been first defined within the text. The inside front cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal (or the updated list at http://www.journalofdairyscience.org) for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used fewer than 3 times after first use must be spelled out in full rather than abbreviated. Do not use capitalized whole words (e.g., CORN) as treatment abbreviations, or single-letter abbreviations that could be confused with chemical elements (e.g., P, C, S). All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first

time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter.

The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural forms of abbreviations do not require "s." Chemical symbols and 1-letter and 3-letter abbreviations for amino acids do not need definition. Bacterial genus names are abbreviated according to the guidelines recommended in *Scientific Style and Format* (8th ed.), and the abbreviated form should not be shown in bold at first use. Units of measure, except those in the standard JDS abbreviation list, should be abbreviated according to standard SI usage and do not need to be defined. See Appendix 2 for a list of commonly used terms.

International Words and Phrases. Non-English words in common usage (i.e., given in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, ad libitum, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

Capitalization. Breed and variety names are to be capitalized (e.g., Holstein, Danish Red). Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used. Proper nouns should be capitalized.

Numbers and Units. The *Journal of Dairy Science* uses the Council of Science Editors' number style given in the eighth edition of *Scientific Style and Format.*

Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits; a comma separator must be used in numbers greater than 999 (e.g., 2,478). Measures must be in the metric (SI) system; however, US equivalents may be given in parentheses. Units of measure not associated with a numeric value must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use.

General Usage. Note that "and/or" is not permitted; choose the more appropriate meaning or use "x or y or both."

Use the slant line only when it means "per" with numbered units of measure or "divided by" in equations. Use only one slant line in a given expression: e.g., g/cow per day. The slant line may not be used to indicate ratios or mixtures.

Use "to" instead of a hyphen to indicate a range of values in text; an en-dash may be used to indicate a range in parenthetical text or tables.

Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <) when these signs occur between 2 items.

Items in a series should be separated by commas: a, b, and c.

Restrict the use of "while" and "since" to meanings related to time. Appropriate substitutes include "and," "but," or "whereas" for "while" and "because" or "although" for "since."

Commercial Products. The use of names of commercial products should be minimized. When a commercial product is being tested as part of the experiment, the manufacturer and location should be given parenthetically at first mention in text, tables, and figures, but, when possible, the generic name should be used thereafter. Only generic names should be used in article titles. Trademark symbols and registration marks should not be used and will be removed.

Avoid describing a method as "per manufacturer's instructions." If the product goes out of production, the method will be lost to readers. Many products come with literature references; try to use references that can be found by other researchers to describe a method being used.

Supplemental Information

The following information is available online and updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles and words used in citations is available in Appendix 3.

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/cuu/Units/index.html.

Figure and Table Preparation Guidelines. Current information on figure and table preparation can be found at http://www.journalofdairyscience.org/.

Manuscript Central Instructions. Manuscripts are submitted at http://mc.manuscriptcentral.com/jds. Full user instructions for using the Manuscript Central system are available at http://mc.manuscriptcentral.com/jds/index.html?mode=instruction.

Appendix 1
ABBREVIATIONS

Revised December 2013

The following abbreviations may be used without definition in the *Journal of Dairy Science*. In addition, abbreviations of all chemical elements, common combinations of chemical elements, SI units of measure used with a value, and common amino acids (3-letter and 1-letter abbreviations) should be used without definition. Abbreviations are generally not permitted in the title, running head, and key words. Plural abbreviations do not require "s".

Unrestricted Use

AA = amino acid
ACTH = adrenocorticotropin
AMP, ADP, ATP = adenosine mono-, di-, or triphosphate
ANOVA = analysis of variance
ATPase = adenosine triphosphatase
BLUP = best linear unbiased predictor
BSA = bovine serum albumin
cDNA = complementary deoxyribonucleic acid
DEAE = diethyl amino ethyl
DNA = deoxyribonucleic acid
DNase = deoxyribonuclease
EDTA = ethylenediaminotetraacetate
ELISA = enzyme-linked immunosorbent assay
FSH = follicle-stimulating hormone
GAPDH = glyceraldehyde 3-phosphate dehydrogenase
GnRH = gonadotropin-releasing hormone
HEPES = *N*-2-hydroxyethyl piperazine-N′-ethanesulfonic acid
HPLC = high performance (pressure) liquid chromatography
IFN = interferon
Ig = immunoglobulin
IL = interleukin
LH = luteinizing hormone
mAb = monoclonal antibody
mRNA = messenger ribonucleic acid
NAD = nicotinamide adenine dinucleotide
NADP = nicotinamide adenine dinucleotide phosphate
$NADPH_2$ = reduced nicotinamide adenine dinucleotide phosphate
PAGE = polyacrylamide gel electrophoresis
PCR = polymerase chain reaction
$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$
REML = restricted maximum likelihood
RFLP = restriction fragment length polymorphism
RIA = radioimmunoassay
RNA = ribonucleic acid
RNase = ribonuclease
rRNA = ribosomal ribonucleic acid
Tris = tris(hydroxymethyl)aminomethane
UHT = ultra-high temperature
USDA = United States Department of Agriculture
UV = ultraviolet

Define in Abstract; Unrestricted Use Elsewhere

ADF = acid detergent fiber
ADG = average daily gain
ADL = acid detergent lignin
ADIN = acid detergent insoluble nitrogen
AI = artificial insemination
BCS = body condition score
BHBA = β-hydroxybutyrate
bST = bovine somatotropin
BTA = *Bos taurus* autosome
BUN = blood urea nitrogen
BW = body weight
CI = confidence interval*
CLA = conjugated linoleic acid
CN = casein
CNS = coagulase-negative staphylococci
CoA = coenzyme A
CP = crude protein
CV = coefficient(s) of variation*
DCAD = dietary cation-anion difference
df = degrees of freedom*
DHI(A) = Dairy Herd Improvement (Association)
DIM = days in milk
DM = dry matter
DMI = dry matter intake
EAA = essential amino acid
EBV = estimated breeding value
ECM = energy-corrected milk
ETA = estimated transmitting ability
FA = fatty acid
FAME = fatty acid methyl esters
FCM = fat-corrected milk
FFA = free fatty acids
GC-MS = gas chromatography-mass spectrometry
GLC = gas-liquid chromatography
h^2 = heritability*
HTST = high temperature, short time
IGF = insulin-like growth factor
IMI = intramammary infection
LA = lactalbumin
LG = lactoglobulin
LPS = lipopolysaccharide
LSD = least significant difference*
LSM = least squares means*
ME = metabolizable energy
MIC = minimum inhibitory concentration
MP = metabolizable protein
MUFA = monounsaturated fatty acids
MUN = milk urea nitrogen
n = number of samples*
NAN = nonammonia nitrogen
NDF = neutral detergent fiber
NDIN = neutral detergent insoluble N
NDM = nonfat dry milk
NEAA = nonessential amino acid
NEFA = nonesterified fatty acids
NE_G = net energy for gain
NE_L = net energy for lactation
NE_M = net energy for maintenance
NFC = nonfiber carbohydrates
NPN = nonprotein nitrogen
NRC = National Research Council
NS = nonsignificant*
NSC = nonstructural carbohydrates
OM = organic matter
PBS = phosphate-buffered saline
PMNL = polymorphonuclear neutrophilic leukocyte
PTA = predicted transmitting ability
PUFA = polyunsaturated fatty acids
r = correlation coefficient*
R^2 = coefficient of determination*
QTL = quantitative trait loci
RDP = rumen-degradable protein
RUP = rumen-undegradable protein
SARA = subacute ruminal acidosis
SCC = somatic cell count
SCM = solids-corrected milk
SCS = somatic cell score
SD = standard deviation*
SDS = sodium dodecyl sulfate
SE = standard error*
SEM = standard error of the mean*
SFA = saturated fatty acids
SNF = solids-not-fat
SNP = single nucleotide polymorphism
SPC = standard plate count
TCA = trichloroacetic acid
TDN = total digestible nutrients
TMR = total mixed ration
TS = total solids
UF = ultrafiltration, ultrafiltered
VFA = volatile fatty acids

*Use generally restricted to tables and parenthetical expressions.

Appendix 2

Selected Units and Terms

The following abbreviations and terms can be used without definition in the *Journal of Dairy Science*.

afternoon	p.m.
atomic mass unit	amu
atmosphere	atm
base pair	bp
calorie (gram)	cal
celsius (with number)	°C
centimeter	cm
centimeter, square	cm^2
circa	ca.
centimorgan	cM
centipoise	cP
central processing unit	CPU
colony-forming unit	cfu
counts per minute	cpm
counts per second	cps
crossed with, times	×
cubic	cu
cubic centimeter	cc, cm^3
cubic millimeter	mm^3
curie	Ci
cycles per second (hertz)	Hz
day(s)	d
dalton	Da
deci	d (prefix)
deciliter	dL
equivalents	Eq
foot-candle	use lx
gram	g
gravity	*g*
hectare	ha
hour(s)	h
inside diameter	i.d.
international unit	IU
intramuscularly	i.m.
intraperitoneally	i.p.
intravenously	i.v.
joule	J
kilo	k (prefix)
kilobase	kb
kilobyte	KB
kilocalorie	kcal
kilogram	kg
Klett units	KU
kiloelectron volts	keV
kilopascal	kPa
liter	L
logarithm (natural)	ln
logarithm (base 10)	$\log_{10}$
lux	lx
mega	M (prefix)
meter	m
metric tonne	tonne/t
micro	µ (prefix)
microcurie	µCi
microeinstein	µE
microfarads	µF
microgram	µg
microliter	µL
milli	m (prefix)
milliliter	mL
millimeters of mercury	mm Hg
millimolar (concentration)	m*M*
millimole (mass)	mmol
minute(s)	min
molar (concentration)	*M*
molar (mass)	mol
mole (number, mass)	mol
month(s)	mo
morning	a.m.
nano	n (prefix)
newton	N
normal (concentration)	*N*
nanogram	ng
osmolality	use mmol/kg
outside diameter	o.d.
parts per billion	µg/kg
parts per million	mg/kg
pascal	Pa
pico	p (prefix)
picogram	pg
plaque-forming unit	pfu
probability	*P*
rennet activity unit	RU
revolutions per minute	rpm
second(s)	s
siemens	S
species	spp.
subcutaneous	s.c.
subspecies	ssp.
unit	U
volt	V
volume	vol
volume/volume	vol/vol (use parenthetically)
watt	W
week(s)	wk
weight/volume	wt/vol (use parenthetically)
year(s)	yr

Amino Acids

alanine	Ala
arginine	Arg
asparagine	Asn
aspartic acid	Asp
citrulline	Cit
cysteine	Cys
glutamic acid	Glu
glutamine	Gln
glycine	Gly
histidine	His
isoleucine	Ile
leucine	Leu
lysine	Lys
methionine	Met
ornithine	Orn
phenylalanine	Phe
proline	Pro
serine	Ser
threonine	Thr
tryptophan	Trp
tyrosine	Tyr
valine	Val

Appendix 3

Abbreviations of Frequently Cited Journals

Acta Agric. Scand. A Anim. Sci.
Acta Endocrinol.
Acta Theriol.
Adv. Carbohydr. Chem. Biochem. (since 1968)
Adv. Exp. Med. Biol.
Adv. Genet.
Adv. Protein Chem.
Adv. Vet. Sci. Comp. Med. (since 1969)
Agric. Biol. Chem.
Am. J. Anat.
Am. J. Clin. Nutr.
Am. J. Clin. Pathol.
Am. J. Obstet. Gynecol.
Am. J. Opthalmol.
Am. J. Pathol.
Am. J. Physiol.
Am. J. Vet. Res.
Anal. Biochem.
Anal. Chem.
Anat. Rec.
Anim. Behav.
Anim. Breed. Abstr.
Anim. Feed Sci. Technol.
Anim. Prod.
Anim. Reprod. Sci.
Ann. Biol.
Anim. Biochim. Biophys.
Ann. New York Acad. Sci.
Ann. Rech. Vet.
Ann. Zootech. (Paris)
Annu. Rev. Biochem.
Annu. Rev. Pharmacol. Toxicol. Antibiot. Chemother.
Appl. Anim. Ethol.
Appl. Environ. Microbiol. (since 1976)
Arch. Biochem. Biophys.
Arch. Gefluegelkd.
Arch. Tierernahr.
Arch. Tierz.
Asian-australas. J. Anim. Sci.
Aust. J. Agric. Res.
Aust. J. Biol. Sci.
Aust. J. Dairy Technol.
Aust. J. Exp. Biol. Med. Sci.
Aust. Vet. J.
Bacteriol. Rev.
Behav. Processes
Biochemistry
Biochem. J.
Biochem. Biophys. Res. Commun.
Biochimie
Biochim. Biophys. Acta
Biol. Reprod.
Biol. Technol.
Biometrics
Bioscience
Bio/Technology (New York)
Biotechnol. Bioeng. Biotechnol. Lett.
Br. J. Nutr.
Br. Vet. J.
Cancer Res.
Can. Inst. Food Sci. Technol. J.
Can. J. Anim. Sci.
Can. J. Comp. Med.
Can. J. Genet. Cytol.
Can. J. Physiol. Pharmacol.
Can. J. Zool.
Can. Med. Assoc. J.
Carbohydr. Res.
Cell. Tissue Res.
Cheese Rep.
Chem. Ind. (Lond.)
Clin. Chem.
Clin. Chim. Acta
Clin. Endocrinol.
Clin. Toxicol. Comp. Biochem. Physiol. (now in series: (A Comp. Physiol., B Comp. Biochem., C Comp. Pharmacol., or C Comp. Pharmacol. Toxicol.)
Compend. Contin. Educ. Proc. Vet.
Cornell Vet.
CRC Crit. Rev. Biochem.
Cult. Dairy Prod. J.
Curr. Opin. Biotechnol. Dairy Field
Dairy Ind. Int.
Dairy Sci. Abstr.
Dev. Biol.
DNA Cell Biol. (since 1989)
DNA (New York); changed in 1989 to DNA Cell Biol.
Domest. Anim. Endocrinol.
Dtsch. Tierarztl. Wochenschr.; continued in 1972 by DTW Dtsch. Tierarztl. Wochenschr.
Electrophoresis
Endocrinology
Eur. J. Biochem.
FASEB J.
FEBS Lett.
Fed. Proc. (now FASEB J.)
FEMS Microbiol. Immunol.
Fertil. Steril.
Food Eng. (New York)
Food Res.
Food Technol.
Gastroenterology
Gen. Comp. Endocrinol.
Gene (Amst.)
Genet. Sel. Evol.
Genetics
Horm. Behav.
Immunol. Today

Indian J. Dairy Sci.
Infect. Immun.
Int. Dairy J.
Int. J. Food Microbiol.
Jpn. Agric. Res. Q.
J. Agric. Food Chem.
J. Agric. Sci. [(Camb.) if published in England (before 1991)]
J. Am. Oil Chem. Soc.
J. Am. Vet. Med. Assoc.
J. Anim. Sci.
J. Appl. Physiol.
J. AOAC; continued in 1992 by J. AOAC Int.
J. Bacteriol.
J. Biol. Chem.
J. Br. Grassl. Soc.
J. Cell Biol.
J. Cell Physiol.
J. Chromatogr.; continued in 1994 by J. Chromatogr. A and B
J. Clin. Endocrinol. Metab.
J. Clin. Invest.
J. Clin. Pathol. (Lond.)
J. Comp. Pathol.
J. Cult. Dairy Prod.
J. Dairy Res.
J. Dairy Sci.
J. Endocrinol.
J. Environ. Pathol. Toxicol. Oncol.
J. Exp. Anal. Behav.
J. Exp. Biol.
J. Exp. Med.
J. Food Sci.
J. Food Prot.
J. Gen. Microbiol.
J. Gen. Physiol.
J. Hered.
J. Immunol.
J. Immunol. Methods
J. Infect. Dis.
J. Lab. Clin. Med.
J. Leukocyte Biol.
J. Lipid Res.
J. Mol. Biol.
J. Morphol.
J. Nutr.
J. Pharmacol. Exp. Ther.
J. Physiol. (Lond.) [or (Paris)]
J. Range Manage.
J. Reprod. Fertil.
J. Sci. Food Agric.
J. Soc. Dairy Technol.
J. Texture Stud.
J. Toxicol. Environ. Health
J. Ultrastruct. Res.
J. Ultrastruct. Mol. Struct. Res.
J. Vet. Med. Ser. A or B
J. Vet. Res. J. Zool. (Lond.)
Jpn. J. Zootech. Sci.
Lab. Anim.
Lait
Lebenson. Wiss. Technol.
Lipids
Livest. Prod. Sci.; continued in 2006 by Livest. Sci.
Milchwissenschaft
Mol. Cell. Endocrinol.
Mol. Gen. Genet. Nature (Lond.)
Neuroendocrinology
New Engl. J. Med.
Neth. J. Agric. Sci.
Neth. Milk Dairy J.
Nutr. Res.
Rev. N.Z. J. Dairy Sci. Technol.
Obstet. Gynecol.
Onderstepoort J. Vet. Res.
Pharmacol. Rev.
Physiol. Rev.
Physiol. Zool.
Poult. Sci.
Proc. Natl. Acad. Sci. USA
Proc. Soc. Exp. Biol. Med.
Process Biochem.
Protein Expr. Purif.
Recent Prog. Lipid Res.
Reprod. Fertil. Dev.
Res. Vet. Sci.
Science
Sci. Tecn. Latt. Cas.
Theor. Appl. Genet.
Theriogenology
Toxicol. Appl. Pharmacol.
Transgenic Res.
Vet. Clin. North Am. Food Anim. Pract.
Vet. Immunol. Immunopathol.
Vet. Rec.
Vet. Res. Commun.
Z. Tierz. Zuchtungsbiol.; continued in 1985 by J. Anim. Breed. Genet.
Zentralbl. Veterinarmed. A, B, or C; continued in 2000 by J. Vet. Med. Ser. A or B
Z. Lebensm. Unters. Forsch.

Appendix 4

Journal of Dairy Science Policy on In Vitro Antimicrobial Susceptibility Tests

Authors should avoid the use of the term "antibiotic" when referring to a specific agent unless that agent is naturally occurring and unmodified (e.g., penicillin). The broader term "antimicrobial agent" is preferred because it includes naturally produced agents, semisynthetic agents, and totally synthetic agents. The term "susceptibility" should be used instead of "sensitivity." Authors unfamiliar with antimicrobial susceptibility testing should obtain CLSI (formerly NCCLS) document M31 (Clinical Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, PA 19087-1898) for specific information regarding antimicrobial susceptibility testing of veterinary pathogens. CLSI or NCCLS equivalent methods for antimicrobial susceptibility testing available outside the US are also acceptable. A list of these methods is available at http://www.oie.int/eng/normes/mmanual/a_00021.htm.

Two methods are generally used to generate antimicrobial susceptibility data: the agar disk diffusion (ADD) method and the minimum inhibitory concentration (MIC) method. The use of the term "Kirby-Bauer" to refer to the ADD method is incorrect and should be avoided. The correct citation for this method is the "disk diffusion method of Bauer et al." The ADD method is a qualitative method and results should be reported as susceptible, intermediate, or resistant (SIR). If zone of inhibition diameters are reported, these should be reported in millimeters.

The MIC method is quantitative and results should be reported in micrograms per milliliter (μg/mL). The minimum summary statistics for reporting MIC results from multiple strains of an organism are the MIC_{50}, the MIC_{90}, and the range. The MIC_{50} and MIC_{90} represent the concentrations required to inhibit 50 and 90% of the strains, respectively. The MIC_{50} and MIC_{90} reported should be the actual concentrations tested, not values calculated from the actual data obtained. When <10 isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested. If more than a single drug is studied, insert a column labeled "test agent" between the columns listing the organisms and the columns containing the numerical data, and record data for each agent in the same isolate order. In addition, the percentage of strains categorized as susceptible, intermediate, or resistant may be reported. If only one of these categories is to be reported, the percent susceptible value is preferred. If the percentage of resistant isolates is to be reported for an agent, it should include isolates categorized as intermediate.

The percentage of strains susceptible or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by CLSI. Given the paucity of approved breakpoints for mastitis pathogens, authors may use breakpoints from other species (e.g., human breakpoints for ampicillin or canine breakpoints for enrofloxacin). However, authors must clearly state that the breakpoints are not approved for mastitis pathogens. Moreover, authors cannot assign breakpoints or use breakpoints from related antibiotics (except for class testing purposes) or breakpoints developed for other methods.

Authors must indicate that the appropriate quality control tests were performed. Information regarding the frequency of testing and the specific strains tested should be provided. The frequency of quality control testing and organisms tested should conform to the recommendations in the CLSI standard (document M31) or equivalent. A single statement in the manuscript indicating that the results obtained for the quality control documents were within published ranges is acceptable. However, authors may be requested to provide the quality control information during the manuscript review cycle.