UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

FATORES ASSOCIADOS À QUALIDADE DO LEITE, HIGIENE ANIMAL E CONCENTRAÇÃO BACTERIANA NA CAMA DE VACAS LEITEIRAS CONFINADAS NO SISTEMA DE COMPOSTAGEM

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Botucatu – SP

Abril, 2015

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Dissertação apresentada junto ao Programa de Pós-Graduação em Medicina Veterinária para obtenção do título de Mestre.

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Data da defesa: 29 de abril de 2015

AGRADECIMENTOS

Dedico esse trabalho ao meu amigo e orientador Prof. Dr. José Carlos Figueiredo Pantoja, que sempre me ajudou, ensinou e incentivou a concluir essa tese.

Agradeço também ao veterinário Leonardo Dantas Da Silva pela motivação para que estudássemos o assunto e pela apresentação dos proprietários das fazendas onde realizamos o estudo.

Agradeço aos meus colegas de departamento que também me auxiliaram nas atividades de coletas nas fazendas e nas manipulações das amostras no laboratório.

Agradeço ao Professor Dr. Hélio Langoni por ceder o espaço no Laboratório NUPEMAS para beneficiar as amostras coletadas.

Agradeço aos proprietários que disponibilizaram suas fazendas para realizar o estudo, e aos seus funcionários que sempre foram prestativos em nos ajudar na coleta das amostras e dados.

Agradeço aos membros da banca de avaliação do exame de qualificação e defesa, professores José Luis Moraes Vasconcelos, Roberto Lyra Villas Boas, Hélio Langoni, e Paulo Fernando Machado pela contribuição para a melhoria desse trabalho.

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FÁVERO, S. Fatores associados à qualidade do leite, higiene animal e concentração bacteriana na cama de vacas leiteiras confinadas no sistema de compostagem. 2015. 107 p. Dissertação (Mestrado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, S.P.

Os objetivos foram descrever e identificar fatores da cama associados à concentração bacteriana no leite e na cama, índices epidemiológicos de mastite e higiene animal em rebanhos alojados no sistema de compostagem. Três fazendas foram visitadas mensalmente durante um ano. Amostras foram colhidas do leite do tanque e de casos de mastite para exame microbiológico. Foram avaliados escores de limpeza do flanco, perna, teta e úbere. Amostras da cama foram coletadas para estimar concentrações bacterianas e características físico-químicas. Matéria orgânica, densidade e a relação carbono-nitrogênio foram associados a concentrações bacterianas na cama. A temperatura na camada profunda foi maior do que a da superfície, mas não o suficiente para diminuir substancialmente as concentrações de bactérias totais, estreptococos e coliformes na cama. Exceto em situações de erros de manejo, parâmetros como a temperatura, matéria orgânica, umidade, densidade e relação carbono-nitrogênio foram mantidas dentro de limites de controle durante o estudo. Coliformes e estreptococos ambientais foram os patógenos mais freqüentes isolados nos casos de mastite clínica. A prevalência de mastite causada por patógenos ambientais que podem causar surtos de mastite intratável não foi preocupante. Surtos de mastite ambiental não foram observados. Os resultados do presente estudo indicam que níveis baixos de umidade e densidade da cama estão associados a diminuição do risco de mastite clínica e melhora da limpeza dos animais, respectivamente. Os resultados desta pesquisa podem ser usados para reduzir o risco de mastite de animais alojados em sistema de compostagem.

Palavras-chave: Compostagem; Mastite bovina; Qualidade do leite.

ABSTRACT

FÁVERO, S. Bedding factors associated with milk quality, animal hygiene, and bedding bacterial concentrations in dairy herds housed on compost bedding. 2015. 107 f. Dissertação (Mestrado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, S.P.

The objectives of this study were to describe and identify bedding factors associated with bedding concentration of mastitis pathogens, mastitis epidemiologic indexes, cow hygiene, and concentration of selected bacterial populations found in bulk tank milk of herds housed on compost bedding. Three dairies were visited monthly during one year. Milk samples were collected from the bulk tank and mastitis cases for microbiological examination. Flank, leg, udder, and teat cleanliness were assessed. Bedding samples were collected to estimate bacterial concentrations and physical-chemical characteristics. Bedding organic matter, density and carbon-nitrogen ratio were associated with bedding specific bacterial concentrations. Temperature observed in the deep layer of the bedding was greater than that measured on the surface, but not enough to substantially decrease bedding concentrations of total bacteria, streptococci and coliforms. Except for situations of management errors, bedding characteristics such as temperature, organic matter, moisture, density, and carbon-nitrogen ratio were maintained within control limits during the study period. Coliforms and environmental streptococci were the most frequent pathogens isolated from clinical mastitis cases. The prevalence of mastitis caused by environmental pathogens that can cause outbreaks of untreatable mastitis was not concerning. No outbreaks of environmental mastitis were observed during the course of the study. Results of the present study indicate that bedding low moisture and wet density levels are associated with decreased risk of clinical mastitis and improvement of cow cleanliness, respectively. Results of this research can be used to manage compost bedded pack systems towards reducing the risk of mastitis.

Keywords: Compost bedding; Bovine mastitis; Milk quality.

CAPÍTULO 1

1. INTRODUÇÃO

O alojamento de vacas livres em camas manejadas pelo sistema de compostagem (“compost bedded pack”) tem crescido rapidamente ao redor do mundo. A cama composta é iniciada com uma camada de cerca de 40 cm de uma base orgânica como serragem ou maravalha espalhada em um galpão coberto. Uma vez que as vacas comecem a defecar e urinar sobre a cama a atividade das bactérias, composta o material, produzindo calor, o que provoca aquecimento da camada profunda. A cama é aerada com subsoladores e enxadas rotativas duas vezes ao dia para que ocorra incorporação dos dejetos animais e descompactação do material. Produtores reportaram que adicionavam cama nova à cama existente em intervalos de uma a quatro semanas para controle da umidade e adição de substrato para os microrganismos. Após um período de seis meses a um ano a cama pode ser substituída e utilizada ou comercializada como fertilizante.

Pesquisas realizadas na América do Norte indicaram que as principais razões para a adoção do sistema de compostagem foram o conforto dos animais, aumento da longevidade (diminuição de problemas de casco e lesões em geral), facilidade em completar tarefas diárias de manejo e menor custo de implantação quando comparado aos “freestalls” tradicionais. Uma das principais motivações para a implantação seria também a redução drástica na quantidade de dejetos animais eliminados no meio ambiente, o que poderia justificar a inclusão em programas de créditos de carbono e contribuir significantemente para a sustentabilidade da pecuária leiteira. Em condições brasileiras, o sistema também poderia ser uma alternativa a sistemas ineficientes como o semi-confinamento, onde animais são alojados em condições inadequadas de conforto e higiene.

Entretanto, há poucos estudos sobre o assunto até a presente data. Nessa revisão de literatura a maioria das pesquisas consultadas foram realizadas nos EUA e são de natureza descritiva. Nenhum trabalho brasileiro foi encontrado sobre o assunto. Devido à natureza orgânica da cama, estudos são necessários para validar a bioseguridade do

sistema e identificar características da cama que podem ser manejadas em nível de fazenda para melhorar a higiene animal e diminuir o risco de mastite.

2. REVISÃO DE LITERATURA

2.1. Sistemas utilizados para alojamento de vacas leiteiras em camas orgânicas

Nos meados do ano de 1950, produtores de leite desenvolveram a cama convencional orgânica (BP), um sistema de alojamento para vacas leiteiras onde os animais se deitavam em uma cama de palha na qual seus dejetos eram incorporados continuamente (BICKERT e LIGHT, 1982). A BP foi projetada para reduzir investimentos iniciais com as instalações e proporcionar um ambiente confortável aos animais (KAMMEL, 2004).

A área onde se encontrava depositada a cama era rebaixada em relação ao solo e permitia o acúmulo do material orgânico. Um muro de concreto ou de ripas de madeira separava a cama de comedouros e bebedouros que estavam em uma área de piso de concreto. Nos sistemas convencionais, havia uma grande área de cama com grande quantidade de material orgânico. A reposição frequente da base orgânica da cama (palha) se tornava necessária para manter a superfície da cama limpa e seca e dependia da lotação utilizada no galpão (BERRY, 1998; BLACK, 2013). Quanto maior a área de cama por vaca, menor era a necessidade de reposição de matéria orgânica, por ocorrer menor deposição de fezes e urina por área de cama. A área de cama recomendada era entre 4 a 6 m^2 por vaca leiteira, com uma reposição diária de matéria orgânica (palha) entre 9,7 a 13,2 kg por animal (BLACK, 2013).

A cama convencional agia como um depósito de armazenamento de fezes, embora fosse necessário um local adicional para armazenamento dos dejetos provenientes das áreas concretadas próximas aos cochos (KAMMEL, 2004). A cama geralmente era removida no verão em sistemas onde o gado ficava somente nesse alojamento na estação de inverno, e solto ao pasto nas outras estações do ano. Em criações onde o rebanho era confinado no BP o ano todo, a substituição da cama era feita a cada dois a quatro meses (KAMMEL, 2004).

Entretanto, o alto risco de infecções intramamárias observado em fazendas leiteiras que utilizavam a BP (BERRY, 1998; PEELER et al., 2000; FREGONESI e LEAVER, 2001) desestimulou os produtores a utiliza-la. Adicionalmente, Snell et al. (2003) relataram que houve uma maior emissão de gases como amônia e metano em sistemas de criação onde era usada a BP, quando comparados a três diferentes construções de “freestalls”.

Utilizando métodos de compostagem tradicional, produtores objetivaram solucionar problemas do BP como emissão de gases tóxicos aos animais e ao meio ambiente (metano, amônia e sulfeto de hidrogênio) e redução do risco de mastite. Dessa forma, o sistema de cama em compostagem (CBP) foi desenvolvido em fazendas leiteiras no estado de Virgínia, E.U.A., para melhorar o conforto e longevidade dos animais, fornecendo condições adequadas de espaço, conforto e bem estar (JANNI et al., 2007).

Resultados de estudos realizados em Minnesota e Kentucky, incluindo um total de 72 fazendas leiteiras que mudaram de outros sistemas de confinamento para o CBP, indicaram que as principais razões dos produtores para adoção do sistema foram o conforto animal, a longevidade dos animais no rebanho, facilidade de manejo e baixo custo econômico para sua implantação, quando comparados ao freestalls (BARBERG et al., 2007a; JANNI et al., 2007; SHANE et al., 2010; BLACK et al., 2014).

2.2. Compostagem na cama de vacas leiteiras confinadas em galpões

2.2.1. Instalações

As instalações no CBP são compostas de um galpão coberto, onde as vacas ficam confinadas, circundado por uma parede que tem em média 1,2 m de altura que contorna o galpão, tendo como finalidade conter o material da cama. Cortinas podem ser utilizadas para controlar as condições ambientais do galpão em diferentes estações do ano. Dentro do galpão, a área sob a cama é de terra compactada e pode haver um corredor de piso de concreto (corredor de alimentação) de livre acesso, anexo a área da cama, onde estão localizados os comedouros e bebedouros (BARBERG et al., 2007a).

2.2.2. Práticas de manejo

A cama é colocada inicialmente para formar uma camada inicial de 20 a 50 cm de altura, e depois se adiciona a cada duas a quatro semanas uma carga de material novo em toda a superfície, fazendo com que a altura da cama aumente de 10 a 20 cm (BARBERG et al., 2007a; JANNI et al., 2007). A frequência de reposição depende de fatores como época do ano, condições meteorológicas, densidade animal e tipo de material utilizado para compostagem. A reposição está relacionada intimamente à umidade da cama e a capacidade de aderência desta as tetas dos animais. Quando a cama começa a aderir ao corpo dos animais (principalmente na região das tetas), torna-se necessário a adição de mais material novo ao sistema para baixar a umidade total do composto (BARBERG et al., 2007a). Apesar dessas práticas de manejo terem disso recomendadas com base em observações de campo, não foram apresentados dados científicos para valida-las.

O revolvimento da cama deve ser realizado duas vezes ao dia para promover a oxigenação do material e é geralmente realizado quando as vacas vão para a sala de ordenha (BARBERG et al., 2007a; JANNI et al., 2007; SHANE et al., 2010). Os equipamentos mais utilizados para realizar esta operação são o subsolador e a enxada rotativa (SHANE et al., 2010).

O subsolador tem o papel de revolver as camadas mais profundas da cama, evitando dessa forma que as regiões profundas se tornem anaeróbicas. A enxada rotativa não revolve as partes mais profundas da cama, mas tem a função de quebrar os materiais que se tornam compactados. Essa descompactação de partículas agregadas facilita a oxigenação e aumenta a superfície de ação dos microrganismos presentes na cama, resultando em um aumento na eficiência de compostagem. No manejo da cama, torna-se necessário o uso alternado desses dois equipamentos. A profundidade de aeração observada na maioria dos CBP estudados nos EUA foi de 25 a 30 cm (JANNI et al., 2007).

A lotação no CBP deve ser adequada para manter um equilíbrio entre o influxo de dejetos animais necessários para a atividade microbiana, e o controle da umidade da cama, além de permitir que todas as vacas se deitem ao mesmo tempo e ainda possam transitar livremente para ir aos comedouros e bebedouros (JANNI et al., 2007). Em

estudos realizados em Minnesota (BARBERG et al., 2007a) e Kentucky (Black et al., 2014), a lotação média reportada foi de 8,6 e 9,0 m^2 por vaca, respectivamente. A lotação mínima recomendada foi de 7,4 m^2 por vaca pesando 540 kg de peso vivo (JANNI et al., 2007). A lotação animal foi identificada como um fator positivamente associado a concentração bacteriana na cama do CBP (BLACK et al., 2014).

A maioria dos produtores fizeram a retirada total da cama dos barracões uma vez ao ano, e alguns removeram a metade superior da cama na primavera para assegurar que haveria espaço para a reposição de material da cama no verão (BARBERG et al., 2007a). Em outro estudo, a cama foi substituída completamente duas vezes ao ano, na primavera e no outono (SHANE et al., 2010).

2.3. Princípios da compostagem

A compostagem, que ocorre no CBP, é uma adaptação do processo de compostagem tradicional, o qual é caracterizado pela decomposição biológica em condições de aerobiose controlada. Microrganismos reduzem a matéria orgânica heterogênea em compostos orgânicos químicos mais estáveis (compostos humificados), resultando na liberação de calor, vapor de água e dióxido de carbono (KIEHL, 1985). Além de trazer vantagens para a criação de vacas leiteiras quando comparados ao BP, o CBP pode trazer benefícios ao meio ambiente pela redução significativa na emissão de gases com potencial efeito de aquecimento global, e pela diminuição na contaminação ambiental devido a eliminação de dejetos animais (JANNI et al., 2007).

Alguns dos usos potenciais da cama, após a retirada dos galpões, seriam a aplicação como condicionadora de solos fazendo face à erosão, como fonte de matéria orgânica na agricultura (enriquecendo os solos em húmus e nutrientes), e como meio de cultura na horticultura. O decréscimo da eliminação de dejetos no ambiente resulta na diminuição da contaminação de nascentes, riachos, córregos e rios com resíduos de esterco líquido (fezes com água) que em outros sistemas são formados em grandes quantidades. O manejo de dejetos tem sido um problema crescente para os produtores, que são obrigados a fazer altos investimentos em instalações para submeter esses resíduos a um tratamento adequado antes de devolver a água para a natureza.

2.3.1. Características físicas e químicas dos substratos orgânicos utilizados no processo de compostagem

A matriz do material que forma a compostagem possui partículas sólidas, entre as quais é formado um espaço intersticial que confere a cama certa porosidade. A porosidade livre depende do conteúdo em umidade e do tamanho e estrutura física das partículas. Esses parâmetros são importantes para manter as condições aeróbicas durante a compostagem, pois refletem a permeabilidade da massa orgânica ao ar. No contexto da compostagem os macroporos podem ser definidos como os espaços vazios entre as partículas, enquanto os microporos podem ser definidos como os espaços vazios dentro das partículas. A umidade (água) no material compostado fica armazenada nos microporos por capilaridade, e nos macroporos (KIEHL, 1985). A porosidade total é a somatória da microporosidade e macroporosidade do material, sendo que, materiais com granulometria pequena apresentam maior proporção de relativa de microporos (em relação aos macroporos), enquanto que os mais grosseiros possuem maior proporção macroporos (KIEHL, 1985).

No espaço, entre as partículas sólidas, são encontrados ar e água, ou uma mistura de ambos, que são componentes essenciais e que devem apresentar seus níveis na cama dentro de um intervalo aceitável para que ocorra um processo microbiológico eficiente de compostagem. Com a evolução do processo de compostagem, ocorrem modificações das características físicas e químicas do material que foi colocado para formar a matriz orgânica inicial da cama, obtendo-se ao final o produto húmus (KIEHL,1985; NRAES-54, 1992).

Materiais com alto teor de celulose e lignina apresentam grande resistência a degradação microbiana e são mais duradouros no processo de compostagem (KIEHL,1985). Os materiais mais utilizados como cama no CBP foram o pó de serra ou a maravalha (BARBERG et al., 2007a), sendo que esses materiais apresentam maior facilidade de serem incorporados juntos aos dejetos das vacas. Além disso, favorece uma melhor relação entre área de superfície disponível para a ação dos microrganismos e o volume total do composto, quando comparados a materiais mais grosseiros como lascas de madeira e palhas (JANNI et al., 2007). Materiais alternativos como palha de

trigo, palha de linho, palha de soja e casca de aveia tem sido utilizados no CBP de acordo com a disponibilidade regional e custo-benefício (SHANE et al., 2010). Entretanto, dados científicos são necessários para avaliar a biosseguridade e custo-benefício de cada material utilizado no CBP, e identificar substratos que não promovam o desenvolvimento de bactérias patogênicas para as vacas leiteiras.

2.3.2. Microrganismos envolvidos no processo de compostagem

No processo de compostagem ocorre uma atividade combinada de uma ampla população de bactérias e fungos. Durante a evolução do processo, ocorre uma mudança nos tipos de populações de microrganismos agindo no material da cama em função de fatores como temperatura, nutrientes disponíveis, quantidade de água, oxigênio e pH (KIEHL,1985). A compostagem ocorre naturalmente, mas sistemas podem ser projetados e manejados para acelerar o processo. O produto final do composto consiste de biomassa de microrganismos vivos e mortos, e subprodutos estáveis não degradáveis (KIEHL, 1985; NRAES-54, 1992).

O composto produzido é o resultado de uma compostagem aeróbica, que tem como objetivo reduzir a concentração de microrganismos patogênicos, emissão de gases indesejáveis, e facilitar o manuseio e armazenamento da cama sem atrair insetos e vetores. Com a evolução do processo de compostagem e quando comparados aos parâmetros dos materiais iniciais, o composto possuirá uma menor quantidade de microrganismos, maior densidade, menor volume, menor porcentagem de matéria orgânica, menor porcentagem de umidade, menor relação carbono-nitrogênio (C/N) e aumento de pH. O grau de estabilização e destruição dos patógenos do composto dependerá da finalidade desejada para esse subproduto (KIEHL, 1985; NRAES-54, 1992).

Devido à natureza orgânica da cama, uma das preocupações no alojamento de vacas leiteiras no CBP é a concentração de patógenos que possam causar mastites clínicas e subclínicas nos animais. Para as camas convencionais como areia e pó de serra utilizadas em “freestalls”, a concentração bacteriana na cama apresentou uma correlação positiva com a quantidade de bactéria na pele dos tetos das vacas em

lactação (HOGAN e SMITH, 1997; ZDANOWICZ et al., 2004), e com a taxa de mastite (HOGAN et al., 1989).

Dessa forma, estudos são necessários para identificar práticas de manejo que minimizem a concentração de bactérias patogênicas causadoras de mastite, e que não comprometam o crescimento e desenvolvimento de bactérias não patogênicas necessárias para a manutenção do processo de compostagem no CBP. O papel competitivo de microrganismos não patogênicos no controle de patógenos precisa ser definido em sistemas de CBP, pois poderia ser base de estratégias de manejo utilizadas para minimizar o risco de mastite e outras doenças dos animais.

Resultados de estudos recentes (BARBERG et al., 2007b; BLACK et al., 2014) indicam que a cama no CBP apresenta uma alta concentração de bactérias causadoras de mastite, principalmente as coliformes porque o sistema utiliza dejetos dos animais como substrato para a compostagem (BLACK et al., 2014). Tem sido demostrado que o uso de camas de maravalha ou pó de serra resulta em um aumento da probabilidade de exposição dos tetos a *Klebsiela spp* e consequente risco de mastite (RENDOS et al., 1975). Entretanto, nenhum estudo foi realizado para identificar associações entre concentrações de patógenos causadores de mastite na cama e a incidência de mastite clínica ou subclínica em animais alojados no CBP.

Em um estudo realizado em Minnessotta (N = 12 fazendas), a concentração de bactérias nas amostras de superfície de cama de várias fazendas que utilizavam o CBP foi em média 9,1 x 10^6 ufc/cc (BARBERG et al., 2007a), sendo que a distribuição de grupos bacterianos de interesse foi: 10,7% de coliformes, 39,4% de estreptococos ambientais, 17,4% de estafilococos e 32,5% de *Bacillus* spp (BARBERG et al., 2007a). Em outro estudo em Kentucky (N = 47 fazendas), a concentração de bactérias nas amostras da cama foi em média 158 x 10^6 ufc/g de cama (BLACK et al., 2014), sendo que a distribuição de grupos de interesse bacterianos foi: 1,9% de coliformes, 20,6% de estreptococos ambientais, 52,3% de estafilococos e 25,3% de *Bacillus* spp (BLACK et al., 2014). Dessa forma, devido a alta concentração de bactérias causadoras de mastite na cama no CBP, tem-se recomendado uma maior eficiência no procedimento de limpeza e desinfecção dos tetos dos animais antes da ordenha (BARBERG et al., 2007a). Entretanto tais recomendações não foram baseadas em evidências cietíficas. Pesquisas

são necessárias para estudar a transferência de sujidade e microrganismos da cama para a pele das tetas das vacas alojadas no CBP.

2.3.3. Fatores físicos e químicos que interferem na compostagem

Em um processo de compostagem da cama vários fatores são importantes e necessários e devem apresentar-se dentro de um intervalo adequado de valores para que as populações de microrganismos possam decompor o material de forma eficiente. Entre esses fatores destacam-se: umidade, oxigenação, temperatura, presença de minerais, relação C/N e pH.

2.3.3.1. Umidade

A compostagem é um processo biológico de decomposição da matéria orgânica e dessa forma necessita de umidade para que aconteça, pois os microrganismos envolvidos no processo assimilam nutrientes através de suas paredes celulares semipermeáveis e realizam suas atividades fisiológicas apenas quando essas substâncias se encontram dissolvidas em meio aquoso. É importante determinar os limites mínimos e máximos de umidade que os diferentes resíduos e o material total do composto devem apresentar, para que este fator não seja limitante para o processo (KIEHL, 1985; NRAES-54, 1992).

A umidade deve estar associada a capacidade de arejamento do composto, sendo esta em função dos equipamentos utilizados para a aeração e da natureza estrutural do material que está sendo compostado. O oxigênio é fornecido para o sistema através dos espaços entre as partículas chamado de interstícios, que é determinado pelo tamanho das partículas que formam a matéria prima do composto (KIEHL, 1985; NRAES-54, 1992). Quando o interstício estiver repleto de água, a entrada de ar é dificultada e o suprimento de oxigênio fica comprometido, tornando o processo anaeróbico. Adicionalmente, o aumento de umidade resulta em um aumento da compactação e diminuição da estabilidade da estrutura do material (KIEHL, 1985; NRAES-54, 1992).

No processo de compostagem a umidade em um intervalo com valores entre 40 e 60% não é fator limitante no sistema (KIEHL,1985; NRAES-54, 1992), e valores de umidade abaixo do intervalo de 12 a 15% fazem com que a atividade microbiologica

diminua drasticamente. Quanto mais próxima desse valor chega a umidade do composto, mais lento se torna o processo (KIEHL, 1985; NRAES-54, 1992).

As fontes de umidade para a cama nos galpões que utilzam o CBP geralmente são os dejetos dos animais (fezes e urina), e a atividade microbiana (JANNI et al., 2007). Em um grupo de CBP em Minnesota, produtores relataram que a adesão da cama úmida a pele dos animais foi um indicativo para adição de material novo de cama (BARBERG et al., 2007a). Em estudos realizados em Minnesota e Kentucky, a umidade média aferida na cama foi de 54,4% (N = 12 galpões; BARBERG et al., 2007a), 63,4% (N = 7 galpões; JANNI et al., 2007), e 56,1% (N = 47 galpões; Black et al., 2013). A matéria seca da cama de seis galpões variou de 36 a 45,8% no verão, e de 29,6 a 41,9% no inverno (SHANE et al., 2010). Esses níveis de umidade encontrados pelos pesquisadores estão dentro do intevalo recomendado de 40% a 65% para o processo de compostagem tradicional (NRAES-54, 1992).

2.3.3.2. Aeração

Embora a decomposição da matéria orgânica possa ser realizada em ambientes anaeróbicos ou aeróbicos, a compostagem deve ser realizada em ambientes aeróbicos, pois com a abundância de ar, o processo de decomposição se torna mais eficiente, rápido e com a produção de um produto final mais homogêneo (KIEHL, 1985). Com fartura de oxigênio dentro do material que esta sendo compostado, evita-se a produção em excesso de gases indesejáveis como amônia, metano e sulfeto de hidrogênio (MISRA et al., de 2003; LOPEZ-BENAVIDES et al., 2007), odores fétidos, putrefação do composto, proliferação de insetos e vetores, que são caracteristicas desejadas no processo de compostagem na cama de vacas leiteiras.

A aeração do composto tem como objetivo fornecer oxigênio para os microrganismos aeróbicos realizarem seu metabolismo, remover umidade da massa em compostagem e remover calor evitando temperatura excessivas. O oxigênio é necessário para a decomposição da matéria orgânica, e a quantidade necessária a ser fornecida deve manter no interstício o suficiente para assegurar sua difusão até aos microporos dos partículas (KIEHL, 1985; NRAES-54, 1992). A necessidade de oxigênio no processo está correlacionada com a quantidade de oxigênio na composição química da matéria

prima do composto e do grau de degradação durante a compostagem. Concentrações de oxigênio no composto inferiores a 5% sugerem um ambiente anaerobico (NRAES-54, 1992).

Durante a compostagem o consumo máximo de oxigênio ocorre simultaneamente ao pico de atividade microbiana, o qual ocorre em temperaturas entre 30 e 50 ºC (KIEHL,1985). Após a penetração no composto, o ar se torna saturado com o vapor de água formado na parte interna devido as reações quimicas que ocorrem no interior da compostagem. Consequentemente, ocorre um aumento da temperatura seguido pela eliminação de gases e umidade do composto. Entretanto, um aumento excessivo da temperatura pode ser prejudicial ao sistema e pode ser evitado por meio do aumento da taxa de oxigenação do composto (revolvimento da cama). A remoção do calor portanto é ocasionada principalmente pelos gases umidos e quentes que abandonam o sistema por evaporação.

No CPB, a compactação da cama ocorre com maior frequencia devido aos animais se deitarem e caminharem sobre ela, reduzindo dessa forma o espaço vazio entre as particulas do material e consequente capacidade de armazenamento de oxigenio (KADER et al., 2007). A aeração da cama duas a três vezes ao dia com a utilização de equipamentos torna-se necessária para que a cama torne-se descompactada e ocorra a entrada de ar no material (JANNI et al., 2007). Em galpões onde a cama era descompactada, foram observados valores maiores de temperatura, o que indicou uma melhor atividade microbiana devido a uma melhor oxigenação do composto (BARBERG et al., 2007a). Quando a aeração da cama é feita de maneira superficial, as camadas mais profundas não são oxigenadas adequadamente de modo a favorecer uma decomposição anaeróbica do material (RUSSELLE et al., 2009). Como cosequência da atividade anaeróbica profunda, observa-se um resfriamento da cama e dificultade em manter a temperatura da cama em valores ideais para o processo de compostagem (GALAMA et al., 2011).

2.3.3.3. Temperatura

No processo de compostagem, as populações de microrganismos transformam a matéria orgânica menos estável e facilmente degradável em formas mais estáveis,

compostos húmicos e produtos inorgânicos (dióxido de carbono e água). O metabolismo dos microrganismos é exotérmico, e as reações químicas para a degradação do material inicial do composto ocorrem em um ambiente aeróbico com liberação de calor que vai ocasionar um aumento de temperatura do composto. Modificações químicas e físicas ocorrem no composto devido a produção de calor e produtos metabólicos gerados pela atividade dos microrganismos. Consequentemente, ocorrem mudanças ao longo do tempo de compostagem nos tipos de populações de microrganismos agindo no material (KIEHL, 1985; NRAES-54, 1992).

A capacidade de produção de calor do processo de compostagem esta relacionada a suscetibilidade dos diferentes materiais orgânicos ao ataque dos microrganismos, gerando mais ou menos calor no composto. Materiais ricos em proteína apresentam baixa C/N, e dessa forma se aquecem mais rapidamente quando comparados aos que apresentam alta C/N como os celulósicos (KIEHL,1985).

Com a evolução do processo de compostagem, o material compostado apresenta fases distintas com relação a temperatura. No início do processo, o material encontra-se na temperatura ambiente, mas quando o sistema oferece condições ideais, os microrganismos começam a multiplicar-se no composto e ocorre um rápido aquecimento. Essa fase recebe o nome de mesófila e a temperatura permanece constante ao redor de 45 ºC, durante a qual ocorre rápido crescimento e multiplicação microbiana.

A segunda fase caracteriza-se por um novo aumento de temperatura, onde o composto atinge uma temperatura em torno de 60 ºC, e recebe o nome de fase termófila, após a qual ocorre uma lenta queda de temperatura do material compostado. Durante a fase termófila, o material pode atingir temperaturas superiores a 60 ºC, que podem inibir o crescimento da maioria dos microrganismos resultando em uma menor taxa de degradação da matéria orgânica e remoção da umidade (KIEHL, 1985; NRAES-54, 1992).

A remoção do excesso de calor deve ser realizada na fase termofílica para que não ocorra auto inibição do sistema. No sistema a temperatura é um dos fatores mais críticos, e para cada grupo de microrganismo existe uma temperatura adequada e qualquer alteração pode alterar a atividade (diminuição da eficiência metabólica) e crescimento dos mesmos (KIEHL, 1985; NRAES-54, 1992). Pequenas variações na

temperatura do composto podem afetar os microrganismos mais do que pequenas variações em outros parâmetros do material compostado como umidade, pH e C/N. Com temperaturas acima de 65 ºC a maioria das espécies de microrganismos começa a perder a forma vegetativa e assumir a forma de esporos, e a maioria das espécies que não formam esporos morrem, e embora uma pequena quantidade de espécies ainda apresente atividade metabólicas, sua contribuição não é significante para o processo de compostagem (KIEHL, 1985; NRAES-54, 1992).

As temperaturas atingidas na fase termófila podem ocasionar a eliminação de microrganismos patogênicos do composto que são pouco resistentes a temperaturas entre 50 a 60 ºC (KIEHL, 1985). Barberg et al. (2007a) reportaram que as temperaturas das camas dos CBP em Minnesota não foram tão altas como em uma compostagem tradicional para promover a pasteurização ou esterilização do composto, mas indicaram atividades microbiológica nos mesmos. Dessa forma, as camas do CBP sofreram um processo de semi-compostagem.

Nos sistemas de CBP incluídos em estudos realizados em Minnesota, a temperatura média profunda (20 cm) reportada de 12 galpões foi de 42,5 ºC (BARBERG et al., 2007a). Shane et al. (2010) reportaram temperaturas profundas entre 31,8 e 48,1 Cº no verão, e de 13,8 a 40,6 Cº no inverno em um grupo de seis galpões. Em Kentucky (n = 47 galpões), a temperatura média foi de 10,5 ºC na camada superficial, e foram de 32,3 e 36,1ºC nas profundidades de 10 e 20 cm, respectivamente (BLACK et al., 2013). As temperaturas reportadas no CBP foram menores do que os valores recomendados (43 e 65 ºC) para o processo de compostagem tradicional (NRAES-54, 1992).

Barberg et al. (2007a) reportaram que as temperaturas de superfície da cama foram semelhantes as temperaturas do ambiente dentro dos galpões. Nos galpões onde eram utilizados materiais mais grosseiros e maiores do que a serragem, a temperatura da cama foi menor do que aquelas observadas em outras camas compostas exclusivamente de serragem. Temperaturas maiores também foram reportadas em fazendas onde a cama não era compactada.

2.3.3.4. Relação carbono-nitrogênio

Os microrganismos presentes no material de compostagem necessitam de nutrientes para assegurar suas funções metabólicas e se multiplicarem. No processo de compostagem, a C/N é um dos principais indicadores do processo de compostagem. O carbono é fonte de energia e de cadeias carbônicas, enquanto o nitrogênio é essencial para formação das proteínas microbianas. O carbono sempre deve estar em maior quantidade no composto quando comparado ao nitrogênio devido a sua alta proporção no material celular e porque ocorrem grandes perdas para a atmosfera na forma de CO_2. Embora o valor ideal para a C/N seja de 25, valores maiores podem ser recomendados devido a indisponibilidade de carbono de certos materiais mais resistentes ao ataque dos microrganismos (KIEHL,1985; NRAES-54, 1992).

No processo de compostagem, o carbono é incorporado ao material celular na proporção de 10 partes para cada 20 partes liberadas na forma de CO_2, enquanto o nitrogênio é incorporado na proporção de uma parte para cada 10 partes de carbono incorporado (KIEHL,1985). Quando a C/N não se encontra nos valores adequados, ocorrem maiores perdas para a atmosfera tanto de carbono (quando a relação apresenta valor acima do ideal, com maior produção de CO_2), como de nitrogênio (quando a relação apresenta valor abaixo do ideal com maior produção amônia). O excesso de perdas desses nutrientes ocorre até que a C/N alcance valores normais (KIEHL,1985).

Nos sistemas de CBP estudados em Minnesota e Kentucky, a C/N da cama foi 19,5 (N = 12 galpões; BARBEG et al., 2007a), 15,5 (N = 7 galpões; JANNI et al., 2007) e 26,7 (N = 47 galpões; BLACK et al., 2013). Shane et al. (2010) reportaram (N = 6 galpões) que a C/N variou entre 13 e 18 no verão, e 16 e 26 no inverno. Os valores reportados da C/N foram próximos ao limite inferior recomendado para a compostagem tradicional (entre 25 e 30; NRAES-54, 1992).

2.3.3.5. pH

No início do processo de compostagem ocorre uma diminuição do pH resultante da degradação dos materiais orgânicos complexos em ácidos orgânicos intermediários, seguida por um aumento gradativo até o ponto de alcalinidade. O pH influencia a

atividade microbiológica na matéria orgânica que forma o composto. Valores ótimos de pH são observados entre 6,5 e 8,0 (KIEHL,1985; NRAES-54, 1992). Valores de pH menores do que 5,5 inibem o crescimento de microrganismos termófilos e ocasionam uma deficiência na produção de temperatura, a qual aumenta mais lentamente do que em condições ideais de pH. Quando os valores de pH são maiores do que 10 são observados períodos de estagnação do processo de compostagem.

Em estudos realizados em Minnessota, valores médios do pH da cama do CBP foram de 8,4 (BARBERG et al., 2007a), 8,5 (JANNI et al., 2007), e variaram no verão entre 8,7 e 9,1 e no inverno entre 8,5 e 8,9 (SHANE et al., 2010). Os valores observados foram maiores do que os recomendados (6,5 a 8,0) para a compostagem tradicional (NRAES-54, 1992).

2.3.3.6. Produto final da compostagem

No processo de compostagem, resíduos de animais e vegetais sofrem decomposição através de atividade microbiológica, resultando na liberação de gases, água e formação de substâncias húmicas, que são caracterizadas como complexas e mais estáveis do que o material inicial (KIEHL,1985; NRAES-54, 1992). Os compostos iniciais da compostagem apresentam grande quantidade de matéria orgânica facilmente degradável, o que os torna instáveis quando comparado ao húmus, o produto final da compostagem. No início do processo são produzidas substâncias fitotóxicas como os ácidos orgânicos voláteis, as quais tornam o composto imaturo impróprio para o uso como fertilizante.

O húmus é caracterizado pela ausência de odores, riqueza em micro (ferro, boro, cobre, zinco, molibdênio, cloro) e macronutrientes (potássio, nitrogênio, fósforo). Diante dessas propriedades, o processo de formação do húmus (chamado humificação) o torna um excelente fertilizante devido a reposição de minerais, correção da debilidade de nutrientes, e manutenção da estabilidade do solo, tornando a terra mais estável e apropriada para as mais diversas culturas.

A qualidade de um composto para a agricultura está relacionada a sua estabilidade e maturação. O grau de estabilidade, por sua vez, está relacionado com a baixa atividade microbiológica no composto, enquanto a maturação está relacionada com

a capacidade do composto em propiciar o crescimento vegetal (KIEHL, 1985). As proteínas, lipídios e hidratos de carbono do composto são os principais grupos de matéria orgânica para a produção de substancias húmicas. Entretanto, não há estudos realizados para determinar o estágio máximo de maturação e a qualidade agronômica da cama processada no sistema de CBP. Esses dados seriam de importância para os produtores para a comercialização e utilização da cama na agricultura.

2.4. Fatores associados a concentração de bactérias causadoras de mastite na cama do CBP

Entre os artigos revisados sobre o CBP, apenas um estudo foi realizado com o objetivo de identificar fatores associados a concentração de bactérias na cama. Black et al. (2014) incluíram 47 propriedades leiteiras que utilizavam o CBP em Kentucky em um estudo transversal, no período de outubro de 2010 a março de 2011 (inverno e primavera). Nesse estudo, fatores como a lotação animal por área, CT (média de valores aferidos da temperatura de superfície e na profundidade de 10,2 cm), temperatura profunda (20 cm), umidade, C/N e a temperatura ambiente dentro do galpão foram associados à concentração de patógenos causadores de mastite na superfície da cama.

Black et al. (2014) reportaram que, dentre as variáveis estudadas, a temperatura ambiente foi a única variável que permaneceu associada (positivamente) à concentração de *Escherichia coli*, estafilococos e estreptococos nos modelos multivariados. No mesmo trabalho, os pesquisadores relataram que o manejo dos fatores da cama como temperatura ambiente, temperatura profunda, umidade, densidade animal e C/N no CBP não foram capazes de reduzir a níveis desejados as bactérias patogênicas causadoras de mastite na superfície da cama e sugeriram que os produtores mantivessem a cama seca para que os animais permecessem limpos. Entretanto, não houve evidências científicas para apoiar tais recomendações.

2.5. Saúde da glândula mamária e qualidade do leite em sistemas de CBP

2.5.1. Mastites

A mastite bovina é a doença mais prevalente em rebanhos leiteiros, e como outras doenças infecciosas, resulta de uma interação complexa entre os animais, os patógenos, e o ambiente. Muito progresso tem sido feito no controle de patógenos contagiosos causadores da mastite. Em fazendas leiteiras desenvolvidas ao redor do mundo, a prevalência de patógenos contagiosos como *Staphylococus aureus* e *Streptococcus agalactiae* tem sido reduzida drasticamente devido à implementação de medidas de controle e o uso extensivo de testes diagnósticos para a detecção dessa enfermidade (SCHUKKEN et al., 1992, RODRIGUES et al., 2005).

Consequentemente, o controle do ambiente dos animais tem se tornado um dos maiores desafios para os produtores leiteiros na atualidade porque a incidência de mastite clínica causada por bactérias de origem ambiental como os coliformes fecais e *Streptococcus uberis* tem aumentado em rebanhos que conseguiram erradicar ou controlar a mastite contagiosa. Em uma revisão recente de estudos realizados em vários países e continentes, a proporção de casos de mastite causada por bactérias coliformes e estreptococos ambientais variou entre 26 e 52% (RUEGG, 2012). No Brasil, a mesma tendência pôde ser observada em regiões leiteiras desenvolvidas. Jobim et al. (2010) reportaram que aproximadamente 50% de 628 de casos de mastite clínica provenientes da região sul do Brasil foram causados por patógenos ambientais.

Nesse contexto, a qualidade da cama usada para acomodar vacas leiteiras confinadas é um dos principais fatores de risco para: 1) novas infecções intramamárias; 2) contaminação bacteriana do leite a granel e 3) doenças associadas ao conforto e bem estar dos animais. A contagem bacteriana na cama apresenta uma correlação positiva com a quantidade de bactéria na pele dos tetos (HOGAN e SMITH, 1997; ZDANOWICZ et al., 2004) e com a incidência de mastite das vacas em lactação (HOGAN et al.,1989).

Quando são usados materiais inorgânicos como areia, as contagens bacterianas na cama dos animais são menores do que quando comparados a materiais orgânicos como serragem ou fezes desidratadas. A areia tem sito utilizada como principal forma de

cama em fazendas de alta produção ao redor do mundo (HOGAN et al., 1989; ZDANOWICZ et al., 2004).

Dessa forma, animais sujos e altos índices de mastite foram observados em animais alojados em BP (BERRY, 1998; PEELER et al., 2000; WARD et al., 2002). Um correto manejo da cama, para propiciar a higiene adequada das vacas em lactação pode diminuir o risco de mastite (NEAVE et al.,1969; RENEAU et al., 2005; SCHREINER e RUEGG, 2003). A prevalência de mastite subclínica foi 1,5 vezes maior quando os animais apresentaram escore de limpeza entre 3 a 4 (sujos e muito sujos), quando comparados aos que apresentaram escore entre 1 a 2 (limpos e pouco sujos) (SCHREINER e RUEGG, 2003).

Ótimas condições de higiene animal têm sido reportadas para os animais confinados no CBP. Em dois estudos em Minnesota, o escore médio em uma escala de 1 a 5 (1 = limpo e 5 = muito sujo) foi de 2,6 (BARBERG et al., 2007b) e 3,1 (SHANE et al., 2010). Em Kentucky (N = 47 galpões), foi observado um escore médio de 2,2 em uma escala de 1 a 4 em um estudo realizado por Black et al. (2013). Em um estudo comparando três sistemas de criação, os escores de higiene (escala de 1 a 5) foram maiores para os animais mantidos no CBP (3,2), quando comparados aos animais mantidos em “freestall” com camas de areia e ventilação cruzada (2,8) ou “freestall” com cama de areia e ventilação natural (2,8) (LOBECK et al., 2011).

BLACK et al. (2013) reportaram que, independentemente da temperatura ambiente, a umidade da cama foi positivamente associada aos escores de limpeza animal. Fazendas nas quais a umidade da cama era mais baixa (35%) mantiveram animais mais limpos do que aquelas nas quais a umidade da cama era mais alta (70%). Black et al. (2013) supuseram que a menor aderência de material da cama seca aos animais resultaria em melhores condições de higiene. Entretanto, estudos são necessários para validação desta hipótese científica.

Apenas um estudo revisado reporta uma comparação na taxa de incidência de mastite clínica e subclínica entre animais mantidos no CBP e outro sistema. Na Universidade de Copenhagen, Dinamarca, no período de dezembro de 2012 a maio de 2013, a incidência de mastite em vacas leiteiras foi comparada entre grupos de animais alojados aleatoriamente no CBP ou em freestall de areia. Entretanto, o material utilizado

na cama do CBP apresentava alto teor de umidade (65 a 70%) e era um composto formado por raízes picadas, maravalha de madeira e resíduos de jardim (SVENNESEN et al., 2014). Esse trabalho apresentou características diferentes em relação ao tipo de material e umidade de cama quando comparado aos estudos dos CBP que foram realizados no EUA. Svennesen et al. (2014) relataram que houve um aumento de 72.000 células/mL na média da contagem de celulas somáticas (CCS) dos animais alojados no CBP, em comparação ao grupo que permaneceu no freestal. Entretanto não houve diferença entre os grupos no risco de mastite clinica, proporções de vacas com quartos secos, ou tipos de patógenos causadores de mastite. Esses resultados indicam que o alojamento de vacas sobre uma cama que apresenta um alto teor de umidade (65%) pode resultar em aumento do risco de mastite subclinica.

2.5.2. O sistema de compostagem e a qualidade do leite do tanque

Em um estudo realizado em Minnesota (SHANE et al., 2010), a CCS do leite do tanque de seis fazendas variou entre 224.000 e 729.000 células/ml. Para outro grupo de 12 galpões a CCS média foi de 325.000 células/ml, menor do que a média do estado no mesmo período (357.000 células/ml; BARBERG et al., 2007b). Black et al. (2013) reportaram que CCS média no leite do tanque de 246.500 células/ml em 47 rebanhos no estado de Kentucky, também foi menor do que a média do estado no mesmo período (313.000 células/ml).

Em dois estudos, a média da CCS mensal do tanque foi comparada entre os períodos anteriores e posteriores a mudança para o CBP. Das sete fazendas estudadas desta forma por Barberg et al. (2007b), uma redução de CCS foi observada em cinco rebanhos após a mudança para o CBP (média mensal dos dois últimos anos anteriores a mudança comparada à média mensal do ano subsequente a mudança). Black et al. (2013) reportaram que a média da CCS mensal do leite do tanque de oito rebanhos em Kentucky diminui de 411.000 no ano anterior a mudança, para 305.000 e 275.000 células/mL no primeiro e segundo ano após a mudança para o CBP, respectivamente.

É importante notar que, devido à natureza observacional e longitudinal desses estudos (ausência de um grupo controle), os resultados devem ser interpretados com

cuidado porque parte das melhoras observadas nos índices qualidade do leite poderiam ser resultado de outras mudanças implementadas no processo de transição.

Em conclusão, a maioria dos estudos realizados para avaliar a qualidade microbiológica da cama do CBP e o impacto do sistema na saúde da glândula mamária foi de natureza descritiva. Estudos epidemiológicos analíticos são necessários para determinar características da flora bacteriana presente no leite a granel, na dinâmica de infeções intramamárias, e dos patógenos causadores de mastite em vacas alojadas no CBP. Adicionalmente, devido à natureza orgânica da cama, o risco de surtos de mastites causadas por patógenos ambientais refratários ao tratamento (tais como *Nocardia* spp, *Pseudomonas* spp e *Prototheca* spp) precisa ser avaliado como parte do processo de validação da bioseguridade do sistema.

2.6. Conclusões

Resultados de estudos descritivos realizados em fazendas em que foram implantados sistemas de CBP na América do Norte sugerem que animais alojados no sistema permanecem em condições de higiene (BARBERG et al., 2007a, 2007b; LOBECK et al., 2011; BLACK et al., 2014) comparáveis aquelas reportadas em sistemas padrão de confinamento como freestalls de areia. Entretanto, resultados sobre o impacto do sistema na saúde da glândula mamária e qualidade do leite parecem ser contraditórios.

Características reportadas da cama indicam que no processo de compostagem do CBP não ocorre destruição substancial dos microrganismos patogênicos porque as temperaturas observadas não foram altas como em uma compostagem tradicional, sugerindo um processo de semi-compostagem. Dessa forma, altas concentrações bacterianas foram observadas na cama oferecida aos animais.

O manejo de fatores da cama (umidade, temperatura, C/N e densidade animal) não foi capaz de reduzir a níveis desejados as bactérias patogênicas causadoras de mastite. Portanto, autores sugerem que a cama deveria ser manejada para propiciar uma superfície seca para que as vacas se mantivessem limpas e confortáveis. Entretanto,

essas recomendações foram baseadas em estudos descritivos e não foram validadas com base em evidência científica.

Estudos analíticos de natureza experimental e longitudinal serão úteis não somente para identificar fatores associados à concentração bacteriana na cama, higiene animal e ocorrência de mastite, mas também para identificar características da cama que sofrem maior variação e, portanto, requerem maior atenção em nível de fazenda.

CAPÍTULO 2 – TRABALHO CIENTÍFICO

Trabalho enviado a revista Livestock Science.

(Normas de publicação da revista – ANEXO I).

Longitudinal variation and associations between compost bedding characteristics and bedding bacterial concentrations

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ABSTRACT

The objectives of this study were to quantify bedding concentrations of total bacteria and selected groups of mastitis pathogens (coliforms and streptococci), identify bedding factors associated with these bacterial populations, and describe longitudinal variation of bedding characteristics. Bedding samples from the superficial and deep (20 cm) layers were collected biweekly during 1 yr from 3 compost bedded pack (**CBP**) dairies. Bedding bacterial concentrations (total bacteria, coliforms and streptococci) and physical-chemical characteristics (moisture, organic matter, carbon-nitrogen ratio (**C/N**), pH, and density) were determined. Mixed models were used to identify predictors for bedding bacterial concentrations. Shewhart control charts were produced to describe longitudinal variation of bedding characteristics and define alerts of out-of-control variation. Bedding temperature was greater in the deep layer than on the surface (difference = 27.0, 12.1, and 14.4 °C for farms A, B and C, respectively). Overall means for bacterial concentrations on the bedding surface of farms A, B, and C were 8.8 ± 0.5, 8.4 ± 0.4, and 8.9 ± 0.5 for total bacteria, 6.7 ± 1.1, 5.6 ± 0.9, and 6.8 ± 0.6 for streptococci, and 6.7 ± 0.7, 6.0 ± 0.7, and 6.7 ± 0.8 log10 cfu/g for coliforms. Except for farm B, bacterial concentrations were greater on the surface than in the deep layer. Bedding organic matter and dry density were associated with the concentration of total bacteria and coliforms. For all farms, C/N (positive association) and dry density (negative association) were associated with bedding concentration of streptococci. Mean C/N and moisture decreased as bedding aged (farms B and C), whereas dry density increased with age (farm C). Bedding moisture was negatively associated with bedding deep temperature on all farms, and decreased as bedding aged (farms B and C). Bedding deep temperature and moisture remained within the control limits defined for farms A and B during most of the period. Only 2 alerts for temperature and 1 alert for moisture

were detected on farm B. At the beginning of the study, farm C had more difficulty managing bedding, which resulted in 7 and 9 alerts for temperature and moisture, respectively. For all farms, organic matter, C/N, and pH exhibited more variation, resulting in several out-of-control alerts. Results of this study can be used to manage the CBP towards reducing cows' exposure to mastitis pathogens.

Keywords: compost bedding; bacterial count; mastitis; process control analysis

INTRODUCTION

The compost bedded pack system (**CBP**) has been increasingly used worldwide to house dairy cows. The CBP is based on principles of traditional composting, in which organic materials are degraded by means of aerobic microbiological decomposition (Janni et al., 2007). The CBP barns are bedded with an organic substrate (such as sawdust), to which feces and urine are constantly added by cows. Bedding is aerated twice a day between milking time to incorporate animal waste and ensure that the composting process continues aerobic and results in a comfortable, dry, and clean bedding surface to the animals. New bedding material is added systematically and after periods as long as 1 year, bedding can be entirely replaced and used as fertilizer (Barberg et al., 2007; Janni et al., 2007).

Results of North American cross-sectional surveys indicate that the main reasons why farmers shifted from other housing systems to the CBP were cow comfort and its consequences such as increase in milk production, longevity, and decrease in the occurrence of hock and foot lesions, easier management, less investment costs, decrease in environmental contamination by disposal of animal waste, and use of bedding as fertilizer (Barberg et al., 2007; Black et al., 2014). Reduction of animal waste disposal into the environment is of special interest to farmers and government agencies because waste management has been an increasing problem that has limited the expansion of the dairy industry.

Due to the organic nature of the bedding, one of the main concerns for housing cows in the CBP is the potential increase in exposure to environmental mastitis pathogens. It has been consistently demonstrated that bedding bacterial concentration is positively associated with teat skin bacterial contamination and risk of intramammary infection in lactating cows (Hogan et al., 1989; Hogan and Smith, 1997; Zdanowicz et al., 2004).

As a result of the traditional composting process, which is aimed to degrade organic material and produce humus, the concentration of pathogenic bacteria can be greatly minimized due to the high temperature (> 60 °C) reached during the process, and biological unavailability of the material (NRAS-54, 1992). Nonetheless, results of recent studies have indicated that temperatures observed in the deep layers of the CBP (< 48.1 °C) are not high enough to substantially decrease bacterial concentrations in the material (Barberg et al., 2007; Shane et al., 2010; Black et al., 2014). Despite using different laboratory methodologies, results of recent studies have demonstrated that compost bedding contains high bacterial concentrations. Barberg et al. (2007) and Black et al. (2014) reported that the concentration of total bacteria on the CBP bedding surface was 9.1 x 10^6 cfu/cc and 158 x 10^6 cfu/g, respectively. Lobeck et al. (2012) reported that mean concentration of coliforms, *Klebsiella* spp, and streptococci were 14000, 280, and 3 x 10^6 cfu/mL of bedding solution, respectively.

In this context, little research has been conducted to identify factors that could be managed by farmers to minimize bedding contamination, and consequently reduce cow's exposure to mastitis pathogens. Black et al. (2014) conducted a cross-sectional study and reported that ambient temperature was the only variable associated with bedding concentration of *Escherichia coli*, streptococci, and staphylococci in multivariable models.

Most studies published to date were performed in the United States and were cross-sectional. Longitudinal studies designed to follow the behavior of bedding variables over a period of time that encompasses all seasons of the year could be valuable not only to identify factors associated with bedding bacterial concentrations, but also to describe and identify bedding characteristics that are more difficult to control. Such studies are necessary to assess the

biosecurity of the CBP and could be used to suggest management practices towards minimizing bacterial exposure to cows.

The objectives of this study were to quantify bedding concentrations of total bacteria and selected groups of mastitis pathogens (coliforms and streptococci), identify bedding characteristics associated with these bacterial populations, and describe longitudinal variation of bedding manageable characteristics.

MATERIALS AND METHODS

Farm selection and sampling strategy

At the beginning of the study, there was supposedly 4 farms in Brazil that had adopted the CBP as a sole system to confine lactating cows. Three of these farms were conveniently selected to participate in the study based on the distance to the university, participation in a dairy herd improvement (**DHI**) testing program, and willingness to comply with the study protocol.

Farms A, B and C had 33, 53 and 145 lactating cows (milked twice a day) and used peanut shell, sawdust and wood shavings as bedding, respectively. The CBP area on farm A was 290 m^2 (11 m^2/cow) and 0.24 m^3 of new bedding were added monthly per m^2 of bedding area. There was a concrete feeding alley from which cows had free access to the bedding area. Bedding was tilled twice a day between milkings by use of a deep cultivator. There were 4 fans installed over the bedding area.

The bedding area on farm B was 1000 m^2 (19 m^2/cow) and 0.03 m^3 of new bedding were added monthly per m^2 of bedding area. Bedding was tilled twice a day between milkings by use of a deep cultivator and there were no fans installed over the bedding area. There was no paved alley and cows were fed on a feed bunk located on the longer side of the barn.

The CBP area on farm C was 1580 m^2 (12 m^2/cow) and 0.04 m^3 of new bedding were added monthly per m^2 of bedding area. Bedding was tilled twice a day between milkings by use of a deep cultivator and a rototiller was used approximately twice a week to further break bedding clusters. Twenty-four fans were installed over the bedding area (equally distributed) at the 7th month of the study. Cows were fed in a separate concrete-paved shed, outside the CBP.

Farms were visited monthly between May 2013 and June 2014 for data and sample collection, and verification of compliance with the study protocol. Bedding samples were collected biweekly by trained farm personnel, as described by Barberg et al. (2007). All samples were collected immediately before milking time (approximately 10 hours after bedding was last tilled) and kept frozen until monthly pick up. The bedding area was divided into 12 equal squares, from which 1 sample was collected from the superficial and deep (20 cm) layers. All samples from each layer were then mixed to create composite superficial and deep samples, which were used for microbiological analysis. Subsequently, the superficial and deep bedding samples were mixed to create a composite sample that represented the bedding as a whole. This sample was used to determine bedding physical-chemical characteristics. Bedding temperature was measured at each square and both depths by use of a spear tipped digital thermometer.

Bedding characteristics based on visual observations (such as moisture and presence of compacted areas) and management events (such as bedding replacement or addition) were recorded at each visit by study personnel.

Microbiological examination of bedding

Bedding samples were processed in the Sao Paulo State University´s Mastitis Research Laboratory within 2 weeks from each farm visit. Microbiological analyses of bedding was

performed by adding 90 mL of 0.1% peptone water to 10 g of bedding (Zdanowicz et al., 2004). Samples were mixed thoroughly for 1 minute and let settle for 2 minutes. One hundred µL of diluted samples (10^{-1} to 10^{-6}) were plated on blood agar, MacConkey, and Edward´s media and incubated for 24 hours to determine concentrations (expressed as log10 cfu/g of bedding) of total bacteria, coliforms and streptococci, respectively.

Analysis of bedding physical-chemical characteristics

Bedding samples were sent to the Sao Paulo State University´s Fertilizers and Correctives Laboratory for determination of moisture (%), organic matter (%), carbon (%), nitrogen (%), carbon-nitrogen ratio (**C/N**), pH, and wet and dry densities (kg/m^3). All analysis were performed according to official methods (Brazil, 2007).

Statistical analysis

Process control charts have been increasingly used for agricultural applications, such as monitoring of bulk tank milk somatic cell count (Reneau and Kinsel, 2000), and are a useful tool to study longitudinal variation of time series. Shewhart control charts for individual measurements (Montgomery, 2007) were produced to describe longitudinal variation of bedding physical-chemical characteristics and identify difficulties in maintaining variation under control. The process variability of each farm was estimated based on the moving range of two successive observations. The central line of the charts represents the average of the measurements and the upper and lower control limits were defined as follows (Montgomery, 2007):

Control limits = $\bar{X} \pm 3\sigma$,

where $\bar{X}$ is the average of the individuals measurements, $\sigma = \left(\frac{\overline{MR}}{\mathrm{d2}}\right)$, $\overline{MR}$ is the average moving range for 2 successive observations, and d_2 is an anti-biasing constant (1.128) for n = 2 successive observations.

Two tests of interest were used to identify out-of-control variation, according to the following definitions: Test 1 (**T1**) = 1 point outside the control limits ($\bar{X} \pm 3\sigma$), and Test 2 (**T2**) = 2 out of 3 consecutive points beyond the limits determined by $\bar{X} \pm 2\sigma$. Control charts were produced with Prism 6 (GraphPad Software Inc, La Jolla, CA, USA) using limits and tests derived from PROC SHEWHART (SAS Institute, 2011).

The distribution of study variables was examined to identify departures from a normal distribution. All bacterial counts were not normally distributed and were transformed to a log10 scale for analysis. For each farm, a paired T-test was used to compare mean bedding temperature (°C) and bacterial concentrations (log10 cfu/g) between the superficial and deep layers (Table 1). Subsequently, linear mixed models (Littell et al., 2006) were used to compare the means of the bedding variables listed in Table 2 among seasons of the year (summer = December, January, and February; fall = March, Abril, and May; winter = June, July, and August; and spring = September, October, and November). Farm was included in the models as a random term to model the correlation between repeated observations within the same farm. Bedding age was forced in these models as a covariate because it was associated with most physical-chemical bedding characteristics, and could, therefore, confound seasonal effects. Bedding age was a categorical variable based on the time interval between a given visit day and the last bedding

total replacement. Levels were defined as 1 (≤ 4), 2 (5-8), and 3 (≥ 9 months) from the last total bedding replacement).

As an exploratory analysis, linear mixed models (Littell et al., 2006) were used to identify unconditional associations between each explanatory variable and study outcomes Explanatory variables were bedding moisture (%), organic matter (%), C/N, pH, wet density (kg/m^3), dry density (kg/m^3), surface temperature (°C), and deep temperature (°C). Outcome variables were bedding concentrations of total bacteria, coliforms or streptococci (log10 cfu/g). Variables associated with each outcome at a significance level of 0.15, as well as interactions with farm, were considered for multivariable analyses.

Multivariable linear mixed models were then constructed to identify conditional associations between explanatory variables and bedding concentration of total bacteria, coliform and streptococci. A backward model selection procedure was used to select a final multivariable model for each study outcome. Interaction terms between farm and each explanatory variable were included in the models and remained if significant. Farm was included in the models as a random term to model the correlation between repeated observations within the same farm. Bedding age was not included in the multivariable models described above because it was highly associated with most bedding physical and chemical characteristics (collinearity).

To assess changes in bedding characteristics resulting from the maturation of the composting process, separate linear mixed models were constructed to compare mean bedding organic matter, density, moisture, and C/N among bedding age categories. Statistical analyses were performed with SAS 9.3 (SAS Institute, 2011) at a significance level of 0.05.

RESULTS

Seven, 5 and 1 biweekly bedding samples from farms A, B, and C, respectively, could not be collected by farm personnel (Table 1).

Bedding changes during the study period

During the study period, the most frequent management issues reported by farmers were improper aeration of the bedding deep layer (tilling too shallow), bedding compaction, and difficulties to maintain moisture under control. Compacted bedding was observed by study personnel on 25, 20, and 18% of the visits to farms A, B and C, respectively. Moist bedding was recorded on 38, 30, and 9% of the visits to farms A, B and C, respectively.

The bedding of farm B was entirely replaced during the last month of the study, when the farmer reported the material as compacted, moist, and more difficult to manage. The bedding of farm C was replaced once in the 1st month of the study due to lack of cultivation. Before replacement, bedding was reported as compacted, moist, rotten (blackened with foul odor), and cold at the deep layer. For farms B and C, bedding replacements were characterized by a drop followed by a gradual increase in deep temperature, a decrease in pH, and a sharp increase in moisture, C/N, organic matter (only for farm C), and the concentrations of total bacteria, streptococci, and coliforms (Figures 1 and 2).

Bedding temperature

For all farms studied, temperature measured in the deep layer (20 cm) of the bedding was greater than that measured on the surface (Table 1). Nonetheless, the difference between the superficial and deep layer was greater for farm A (27.0 °C), as compared with farms B (12.1 °C)

or C (14.4 °C), due to the greater deep temperature found on farm A (Table 1 and Figure 1). For all farms, bedding temperature at both layers varied little during the study period (coefficient of variation (**CV**) by farm ranged from 5.0 to 13.8%) and the temperature on the surface was not different among seasons of the year. Bedding superficial temperature of farm B was 5.8 and 4.7 °C greater than those observed on farms A and C, respectively (Table 1). Deep layer temperature was greater in spring than in winter for all farms (Table 2). No alerts of out-of-control variation occurred for bedding deep temperature of farm A. Two T1 alerts (temperature less than the lower limit) were signaled on farm B, coinciding with major bedding replacements, and 7 alerts (4 T1 and 2 T2) occurred on farm C during the study period (Figure 2).

Bedding moisture

Although mean bedding moisture only varied between 35.5% (farm C) and 40.4% (farm B) among farms, the within farm variation (as estimated by the CV) was 2.3 and 1.5 greater for farm C, as compared with farms A and B, respectively (Table 1 and Figure 2). Bedding moisture was not different among seasons of the year (Table 2). No alerts of out-of-control variation occurred for bedding moisture of farm A, and 1 T2 alert was observed at the beginning of the study for farm B. Farm C had more difficulty maintaining moisture within the control limits and experienced 9 alerts during the study period (5 T1 and 4 T2 alerts, Figure 2).

For all farms, bedding moisture was negatively associated with bedding deep temperature ($P < 0.01$). A one-unit increase in deep temperature was associated with a decrease of 0.5 units (percentage points) in bedding moisture (coefficient = -0.54, standard error = 0.16). For farms B and C, moisture decreased as bedding became older ($P < 0.01$ for the interaction between farm and bedding age, Figure 4).

Bedding bacterial concentrations

Except for farm B, concentrations of total bacteria, coliforms and streptococci were greater on the surface than in the deep layer of the bedding (Table 1 and Figure 1). Concentrations of total bacteria, coliforms and streptococci were less on the bedding surface of farm B than those observed on farms A e C, between which the concentrations were not different (Table 1). For all farms, concentration of total bacteria was less in winter than in spring or summer (Table 2), whereas no differences were found for the concentrations of streptococci and coliforms among seasons of the year (Table 2).

Of all explanatory variables, bedding organic matter and dry density were associated with the concentration of total bacteria on the bedding surface in the final multivariate model (Table 3). Nonetheless, both associations were farm dependent (there was a significant interaction between each variable and farm). There was a positive association between bedding organic matter and the concentration of total bacteria of farm A. A one-unit increase in organic matter was associated with a 4.8 % increase in the concentration of total bacteria. This association was not evident on farms B and C (Table 3). A negative association between dry density and the concentration of total bacteria was identified for farms B and C (wood-based bedding), whereas a positive association was found on farm A (peanut shell-based bedding) (Table 3).

Organic matter and dry density were associated with bedding concentration of coliforms in the final model. A one-unit increase in organic matter was associated with a 6.2 and 6.8 % increase in the concentration of coliforms on farms A and B, respectively. In contrast, a negative association was found on farm C (Table 3). A negative association between dry density and the concentration of coliforms was identified for farms B and C, whereas a positive association was found on farm A (Table 3).

Bedding C/N and dry density were both associated with the concentration of streptococci in the final model. For all farms, C/N was positively associated with the concentration of streptococci. A one-unit increase in C/N was associated with a 3.5 % increase in the concentration of streptococci. A negative association between dry density and the concentration of streptococci was identified for all farms (Table 3).

Bedding physical-chemical characteristics

Mean bedding organic matter, C and N were greater for farm A (peanut shell-based bedding), as compared with farms B and C (wood-based bedding, Table 1). Bedding organic matter was greater in summer than in fall or winter (Table 2). Bedding C/N and pH were not different among farms. For all farms, mean pH was greater in summer than in fall or winter and C/N was not different among seasons (Table 2). Bedding wet density was less in summer than in spring and dry density was not different among seasons of the year. Both wet and dry densities were greater for farm B (sawdust bedding), as compared with farms A (peanut shell) or C (wood shavings, Table 1). For all farms, several alerts of out-of-control variation for bedding organic matter, C/N, pH, were detected (Figures 2 and 3). Opposite longitudinal trends were observed for pH (increasing) and C/N (decreasing) during the study period (Figure 3).

Bedding organic matter, dry density and C/N were associated with bedding age, but these associations depended on the farm studied ($P < 0.01$ for the interaction between farm and organic matter, density, or C/N). For farms, B and C, C/N decreased with bedding age and there was a decrease in organic matter over time (which was significant only on farm C, Figure 4). For farm C, dry density of old bedding was greater than that of new bedding (Figure 4).

DISCUSSION

Little research has been conducted towards validating management practices that could be used to minimize CBP bacterial concentrations. Most studies were cross-sectional, in which bedding characteristics and bacterial concentrations were determined at a fixed point in time from a population of farms. While cross-sectional studies are useful for capturing variation among farms, longitudinal designs also allow capturing of within farm variation, which can be used to identify seasonal trends and bedding characteristics that are easier or more difficult to control. In addition, longitudinal studies are more valid in determining causal associations because a temporal relationship between explanatory and response variables can be determined. Because the CBP system had been recently adopted by all farms at the beginning of the study, we could study bedding characteristics at various stages of bedding maturity.

Monitoring of key indicators such as organic matter, moisture, deep temperature, C/N, density, and pH is important to assess the quality of the composting process. Results of the present study indicate that variation in the bedding characteristics studied greatly depended on the bedding management of each farm. A greater number of out-of-control alerts were observed on farm C (as compared with farms A and B) for all indicators studied. Most alerts occurred at the beginning of the study when bedding was neglected and had to be entirely replaced. Once the managers learned more about CBP management, the bedding of farm C was consistently maintained within the farm goals (little moisture, loose, deep layer temperature > 40 °C, and little adhesion to cows) until the end of the study. Except for such extreme situations, bedding characteristics that can be measured and managed on the farms (moisture, temperature, and density) varied little during the course of the study. For all farms, the several alerts of out-of-

control variation observed for bedding organic matter and C/N ratio could be explained by the periodic addition of new bedding to the CBP.

Factors associated with bedding bacterial concentrations

One hypothesis frequently raised by CBP users is that the increase in bedding temperature resulting from the composting process would substantially decrease bedding bacterial concentrations. However, mean concentration of total bacteria on the bedding surface was mostly > 8.0 log10 cfu/g for all farms during the course of the study. Likewise, Barberg et al. (2007) and Black et al. (2014) reported that mean concentration of total bacteria on the CBP bedding surface was 7.0 log10 cfu/cc and 8.2 log10 cfu/g, respectively.

Results of this study indicate that cows housed in the CBP are exposed to a contaminated surface, which might increase the pressure of intramammary infection if there is transfer of pathogens to the teat skin (Rendos et al., 1975; Hogan et al., 1989). Bedding characteristics such as moisture and particle size, which could influence bedding adhesion to cows, need to be studied to assess the risk of mastitis in the CBP.

Bedding organic matter was positively associated with bedding concentration of total bacteria (Farm A) and coliforms (Farms A and B), which indicates that an increase in available nutrients favors the growth of bacterial populations in the CBP. Main sources of organic matter to the CBP are new bedding and animal waste, which are frequently added to the CBP. Two farmers reported during the study that addition of large amounts of new bedding resulted in a temporary increase in clinical mastitis incidence, but this hypothesis should be formally investigated.

Bedding dry and wet densities are estimated by measuring the amount of bedding that can be placed into a fixed-size container and is mostly influenced by particle size and physical properties of the material. For farms that used wood-based bedding, density was negatively associated with bacterial concentrations. Denser bedding can result in less oxygen penetration and impairment of aerobic conditions. In addition, as bedding becomes mature due to composting, microbiological decomposition results in decreased particle size and bioavailability of nutrients. Thus, older bedding becomes denser and less bioavailable, which may decrease microbial growth. Other characteristics of mature bedding such as increased water retention might result in difficulties to manage the bedding. Studies are necessaries to determine whether management of new and mature bedding should be different and identify the ideal time for total bedding replacement. Composting characteristics of peanut shell seems to differ from wood-based materials and need to be further studied. For instance, bedding density of farm A was less than those measured on farms B and C, which might explain the higher composting temperatures observed on farm A. Less dense and more degradable materials allow rapid composting and may favor microbial growth due to greater nutrient availability and bedding aeration.

For all farms, a positive correlation was found between C/N and the concentration of streptococci. Carbon is a limiting factor for bacterial growth and its ratio relative to nitrogen has been used to monitor composting efficiency. Because nitrogen is constantly added to the CBP by animal waste, and the main source of carbon is new bedding, carbon availability to microbes becomes more limited and C/N decreases as bedding becomes older (Figure 3). During the course of the study, C/N decreased steadily on farms B and C, which used wood-based bedding.

Bedding pH was not associated with bedding bacterial concentrations but an increasing trend was observed for all farms as bedding became older (Figure 3). pH increases throughout

the composting process because acids are neutralized by bases released from organic matter (Kiehl, 1985). Because pH levels greater than 9 were observed on all farms during several consecutive months, possible detrimental effects on teat skin might deserve further investigation.

Bedding temperature

In agreement with previous studies conducted in the United States (Barberg et al., 2007; Shane et al., 2010; Black et al., 2014), mean deep layer temperature was greater than that measured on the surface, indicating microbiological activity. Barberg et al. (2007) and Black et al. (2014) reported mean deep temperatures of 42.5 and 36.1 ºC for populations of 12 and 47 CBP in Minnessota and Kentucky, respectively. Shane et al. (2010) studied a group of 6 CBP and found deep layer temperatures ranging from 31.8 to 48.1 ºC in summer, and 13.8 to 40.6 ºC in winter. Nonetheless, deep temperatures reported in the present and previous studies did not reach values (> 55 ºC) capable of substantially reducing bedding bacterial populations.

Although this study was not designed to make comparisons among farms, they can be useful to generate new hypotheses. It was interesting to note that the composting process seemed to depend on the type of organic material. The greater deep temperature observed on farm A (as compared to farms B and C) could be explained by the use of peanut shell, which is less dense than wood and more rapidly degraded by microorganisms. Despite the greater bedding temperature maintained on farm A, bacterial concentrations on the surface were not different among farms. Due to regional availability of materials such as wood, further research is necessary to study the use of alternative bedding types for the CBP.

Bedding surface temperature is a point of concern because it could be detrimental to cow comfort and health. The greater surface temperature observed on farm B (5.8 and 4.7 °C greater

than those observed on farms A and C, respectively) can be explained by the lack of fans and suggests that, for the environmental conditions observed during the study, proper ventilation in the CBP area is integral for the well-being of the cows. Both deep and surface temperature varied little during the study. Deep temperature was greater in spring than in winter, suggesting that weather conditions can influence bedding temperature. One limitation of this study is that associations between weather conditions (air temperature and humidity) and bedding factors could not be studied because these data were not collected.

Bedding deep temperature of farm C decreased to < 25 °C at the beginning of the study, when bedding was neglected. Cooling of the bedding was associated with an increase in bedding density, moisture, and bacterial concentrations, and a decrease in C/N. After bedding was replaced and management was corrected, deep temperature increased rapidly and remained stable until the end of the study. These observations agree with previous reports, suggesting that monitoring temperature is an inexpensive means to assess bedding quality and the efficiency of the composting process (Barberg et al., 2007).

Bedding moisture

Moisture has been considered one of the critical points for the management of the CBP (Barberg et al., 2007; Lobeck et al., 2012). Although moisture is necessary for the composting process, its excess can result in negative consequences such as bedding compacting, decrease in temperature and aeration, development of an anaerobic environment, and possible particle adherence to cows (NRAS-54, 1992; Barberg et al., 2007).

Except for farm C at the beginning of the study, moisture was maintained within the control limits for most of the study period. Moisture was negatively associated with bedding

deep temperature, suggesting that excessive levels can result in a series of events (compaction, less penetration of air, and impairment of aerobic conditions) that lead to decreased microbial activity in the deep layer and cooling of the bedding. The decrease in moisture observed during the process of bedding aging agrees with previous knowledge of traditional composting (Kiehl, 1985), and can be explained by the high moisture levels found in new bedding (Figure 4), and evaporation due to factors such as composting temperatures, bedding aeration, and ventilation.

Results of this study indicate that low moisture levels can be maintained without impairing bedding deep temperature. The low moisture levels (< 35 %) maintained on farm C´s bedding (Figure 2) were associated with deep temperatures > 40 ºC. In these conditions, there was little adherence to cows, which remained in excellent hygienic conditions (data shown in the companion paper). Therefore, moisture levels recommended for traditional composting (40 - 65%; NRAES-54, 1992) may not be ideal for the CBP. The moisture content (> 55 %) observed at the initial phase of the study on farm C was associated with bedding compaction and bacterial counts > 9.5 log10 cfu/g of bedding (Figures 1 and 2). Further studies are necessary to determine minimum moisture levels that could be targeted without compromising the composting process.

CONCLUSIONS

Results indicated that bedding organic matter, density and C/N were associated with bedding specific bacterial concentrations. Although the temperature observed in the deep layer of the bedding was greater than that measured on the surface, bedding temperatures were not great enough (> 55 ºC) to substantially decrease bedding concentrations of total bacteria, streptococci and coliforms.

In agreement with previous knowledge of traditional composting, bedding aging was characterized by changes in several physical-chemical characteristics. For farms that used wood

as bedding, moisture, C/N, and organic matter decreased, whereas density and pH increased over time.

Extreme variation of bedding characteristics occurred during the initial phase of the study. Once farmers learned the principles of composting, bedding manageable characteristics such as temperature, organic matter, moisture, density, and C/N were maintained within control limits during the study period. Results of this research can be used by farmers and consultants to manage the CBP towards reducing cows exposure to mastitis pathogens.

ACKNOWLEDGEMENTS

We are thankful to Dr. Leonardo Dantas and the farmers and collaborators who cooperated with the study. This research was funded by the Sao Paulo Research Foundation (FAPESP), grant 2013/00517-3.

REFERENCES

Barberg, A., Endres, M., Janni, K. 2007. Compost dairy barns in Minnesota: A descriptive study. Appl. Eng. Agric. 23:231-238.

Black, R.A., Taraba, J., Day, G., Damasceno, F., Newman, M., Akers, K.A., Wood, C.L., McQuerry, K.J., Bewley, J.M. 2014. The relationship between compost bedded pack performance, management, and bacterial counts. J. Dairy Sci. 97:2669-2679.

Brasil. 2007. Ministério da Agricultura, Pecuária e Abastecimento. Instrução normativa Nº 28, de 27 de Julho de 2007. Manual de métodos analíticos oficiais para fertilizantes minerais,

orgânicos, organominerais e corretivos. Available on-line at: http://sistemasweb.agricultura.gov.br/arquivosislegis/anexos/tm/INM28_2007_MAPA.pdf Accessed february 2, 2015.

Hogan, J., Smith, K., Hoblet, K., Todhunter, D., Schoenberger, P., Hueston, W., Pritchard, D., Bowman, G., Heider, L.E., Brockett, B. 1989. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72:250-258.

Hogan, J., Smith, K. 1997. Bacteria counts in sawdust bedding. J. Dairy Sci. 80:1600-1605.

Janni, K., Endres, M., Reneau, J., Schoper W. 2007. Compost dairy barn layout and management recommendations. Appl. Eng. Agric. 23:97-102.

Kiehl, E. J. 1985. Compostagem. Pages 229-310 in Fertilizantes orgânicos. Agronômica Ceres, Piracicaba, São Paulo, Brazil.

Littell, R., Milliken, G., Stroup, W., Wolfinger, R., Schabenberger, O. 2006. SAS for mixed models. 2nd ed. SAS institute Inc., Cary, NC.

Lobeck, K., Endres, M., Janni, K., Godden, S., Fetrow, J. 2012. Environmental characteristics and bacterial counts in bedding and milk bulk tank of low profile cross-ventilated, naturally ventilated, and compost bedded pack dairy barns. Appl. Eng. Agric. 28:117-128.

Montgomery, D. C. 2007. Control Charts for Variables. Pages 226-287 in Introduction to statistical quality control. 5th ed. John Wiley & Sons Inc, NY.

Northeast Resource Agriculture and Engineering Service (NRAES-54). 1992. On-farm composting handbook. Ithaca, NY.

Rendos, J., Eberhart, R., Kesler, E. 1975. Microbial populations of teat ends of dairy cows, and bedding materials. J. Dairy Sci. 58:1492-1500.

Reneau, J., Kinsel, M. 2001. Record systems and herd monitoring in production-oriented health and management programs in food producing animals. Pages 107-141 in Herd Health. 4th ed. WB Saunders Company, Philadelphia, PA.

SAS Institute. 2011. SAS/STAT 9.3 User's Guide. SAS Institute Inc., Cary, NC.

Shane, E., Endres, M., Janni, K. 2010. Alternative bedding materials for compost bedded pack barns in Minnesota: A descriptive study. Appl. Eng. Agric. 26:465.

Zdanowicz, M., Shelford, J., Tucker, C., Weary, D., Von Keyserlingk, M. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694-1701.

Table 2.1. Descriptive statistics for bedding variables by farm and bedding layer.

Variable	FARM A				FARM B				FARM C			
	N	MEAN[1]	SD	CV	N	MEAN	SD	CV	N	MEAN	SD	CV
Temperature (°C)												
Surface[2]	17	26.9 ^{Aa}	3.0	11.1	25	32.7 ^{Ba}	2.2	6.8	29	28.0 ^{Aa}	2.2	8.0
Deep layer[2]	17	53.9 ^{Ab}	2.7	5.0	25	44.8 ^{Bb}	3.2	7.2	29	42.4 ^{Bb}	5.9	13.8
Concentration of total bacteria (log10 cfu/g)												
Surface	18	8.8 ^{Aa}	0.5	6.0	24	8.4 ^{Ba}	0.4	4.6	28	8.9 ^{Aa}	0.5	8.4
Deep layer	18	8.0 ^{Ab}	0.7	8.4	24	8.3 ^{Aa}	0.4	5.2	28	8.3 ^{Ab}	0.7	5.4
Concentration of coliforms (log10 cfu/g)												
Surface	18	6.7 ^{Aa}	0.7	10.7	24	6.0 ^{Ba}	0.7	10.7	28	6.7 ^{Aa}	0.8	13.9
Deep layer	18	5.4 ^{Ab}	0.8	15.2	24	6.0 ^{Ba}	0.5	7.9	28	5.9 ^{ABb}	0.8	12.5
Concentraton of streptococci (log10 cfu/g)												
Surface	18	6.7 ^{Aa}	1.1	16.9	24	5.6 ^{Ba}	0.9	15.7	28	6.8 ^{Aa}	0.6	12.1
Deep layer	18	5.6 ^{ABb}	1.6	27.9	24	5.3 ^{Aa}	1.3	25.1	28	6.2 ^{Bb}	0.7	8.4
Organic matter[3] (%)	18	41.8 ^{A}	7.5	18.0	24	31.4 ^{B}	3.4	10.8	28	35.1 ^{B}	8.4	24.0
Carbon (%)	18	23.2 ^{A}	4.1	17.8	24	17.5 ^{B}	1.9	11.0	28	19.1 ^{B}	3.9	20.2
Nitrogen (%)	18	1.8 ^{A}	2.6	148.0	24	0.7 ^{B}	0.2	30.0	28	0.9 ^{B}	0.3	28.5
Carbon-nitrogen ratio	18	26.6 ^{A}	4.0	15.1	24	26.6 ^{A}	9.4	35.4	28	22.9 ^{A}	8.8	38.4
Moisture (%)	18	37.4 ^{AB}	4.0	10.6	24	40.4 ^{A}	6.4	15.8	28	35.5 ^{B}	8.4	23.7
pH	18	9.0 ^{A}	0.5	5.7	24	8.8 ^{A}	0.5	6.0	28	8.9 ^{A}	0.4	4.6
Wet density (kg/m^3)	18	389.4 ^{A}	158.6	40.7	24	539.3 ^{B}	52.1	10.0	28	458.9 ^{A}	103.9	22.6
Dry density (kg/m^3)	18	240.7 ^{A}	93.8	39.0	24	324.4 ^{B}	56.5	17.4	28	298.0 ^{B}	90.3	30.3

[1] Different upper case letters within the same row indicates a significant difference ($P < 0.05$) between means. Different lower case letters within the same column indicates a significant different between means ($P < 0.05$).

[2] Bedding samples were collected from 12 equally divided areas of the bedding and mixed to create composite superficial and deep (20 cm) samples.

[3] Bedding physical-chemical characteristics were estimated on composite samples created by mixing the superficial and deep bedding samples.

Table 2.2. Least square means for bedding characteristics by season of the year, adjusted by bedding age.

Variable	Fall			Winter			Spring			Summer		
	N	Mean[1]	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Concentration of total bacteria[2] (log10 cfu/g)	16	8.68^{ab}	0.21	21	8.38^{a}	0.21	16	8.97^{b}	0.21	17	8.92^{b}	0.21
Concentration of coliforms[2] (log10 cfu/g)	16	6.33^{a}	0.22	21	6.37^{a}	0.31	16	6.54^{a}	0.32	17	6.77^{a}	0.33
Concentration of streptococci[2] (log10 cfu/g)	16	6.74^{a}	0.46	21	6.30^{a}	0.45	16	6.35^{a}	0.46	17	6.17^{a}	0.46
pH[3]	16	8.68^{a}	0.09	21	8.71^{a}	0.08	14	8.87^{ab}	0.09	19	9.18^{b}	0.09
Carbon-nitrogen ratio	16	27.55^{a}	1.68	21	25.39^{a}	1.42	14	22.90^{a}	1.72	19	26.98^{a}	1.63
Organic matter (%)	16	35.83^{a}	3.62	21	31.66^{a}	3.50	14	37.10^{ab}	3.63	19	40.75^{b}	3.60
Wet density (kg/m^3)	16	436.11^{ab}	55.03	21	471.03^{ab}	52.58	14	528.86^{a}	55.33	19	402.38^{b}	54.70
Dry density (kg/m^3)	16	267.73^{a}	33.52	21	281.78^{a}	31.46	14	338.30^{a}	33.78	19	253.30^{a}	33.24
Moisture (%)	16	38.83^{a}	1.80	21	40.44^{a}	1.56	14	34.89^{a}	1.83	19	37.46^{a}	1.76
Surface temperature (°C)	15	29.04^{a}	1.90	21	28.31^{a}	1.86	16	29.19^{a}	1.89	19	30.13^{a}	1.89
Deep temperature (°C)	15	47.63^{ab}	3.74	21	44.09^{a}	3.68	16	48.75^{b}	3.72	19	48.28^{ab}	3.73

[1] Different upper case letters within the same row indicates a significant difference ($P < 0.05$) between means.

[2] Bacterial concentrations on the bedding surface. Bedding samples were collected from 12 equally divided areas of the bedding and mixed to create a composite sample.

[3] Bedding physical-chemical characteristics were estimated on composite samples created by mixing the superficial and deep bedding samples.

Table 2.3. Multivariate mixed models used to identify bedding factors associated with bedding bacterial concentrations (log10 cfu/g)

Explanatory variables	Coefficient	SE	P-value
Total bacteria			
Intercept	8.458	1.325	
Organic matter (%)	-0.002	0.024	0.15
Organic matter*farm			< 0.01
Organic matter*farm A	0.050	0.026	
Organic matter*farm C	-0.003	0.026	
Organic matter*farm B	0	Reference	
Dry density (kg/m^3)	-0.001		0.11
Dry density*farm			< 0.01
Dry density*farm A	0.002	0.002	
Dry density*farm C	-0.004	0.002	
Dry density*farm B	0	Reference	
Coliforms			
Intercept	6.092	2.196	
Dry density (kg/m^3)	-0.002	0.003	0.05
Dry density*farm			0.01
Dry density*farm A	0.003	0.003	
Dry density*farm C	-0.005	0.003	
Dry density*farm B	0	Reference	
Organic matter (%)	0.068	0.042	0.07
Organic matter*farm			< 0.01
Organic matter*farm A	-0.006	0.046	
Organic matter*farm C	-0.101	0.046	
Organic matter*farm B	0	Reference	
Streptococci			
Intercept	6.229	0.625	
C/N ratio	0.035	0.014	< 0.01
Dry density (kg/m^3)	-0.005	0.001	0.06
Dry density*farm			< 0.01
Dry density*farm A	0.003	0.001	
Dry density*farm C	0.004	0.001	
Dry density*farm B	0	Reference	

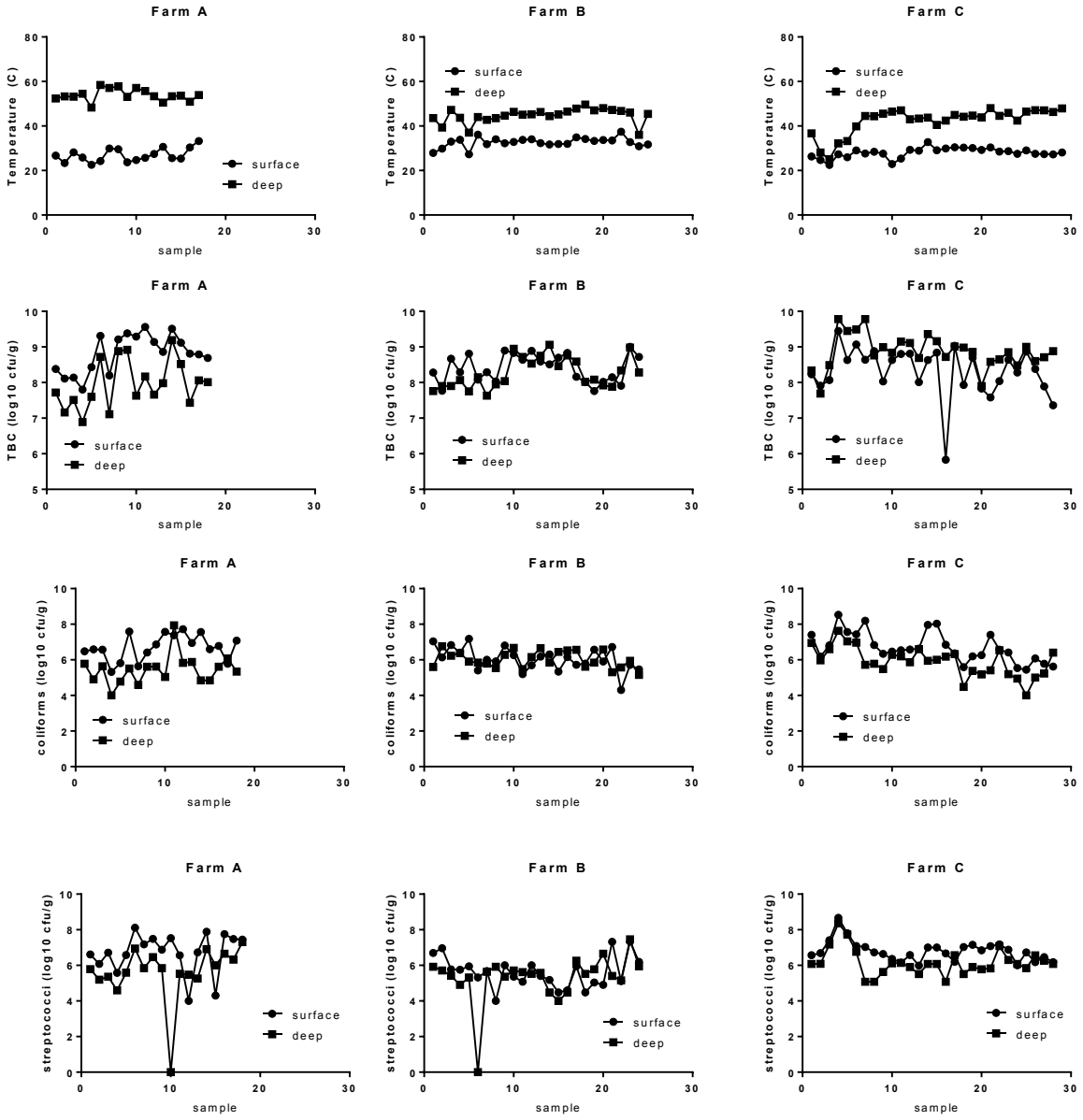

Figura 2.1. Longitudinal variation of bedding concentrations of total bacteria. coliforms. streptococci. and temperature. by bedding layer. Samples were collected biweekly from the surface and deep layer (20 cm) of the bedding.

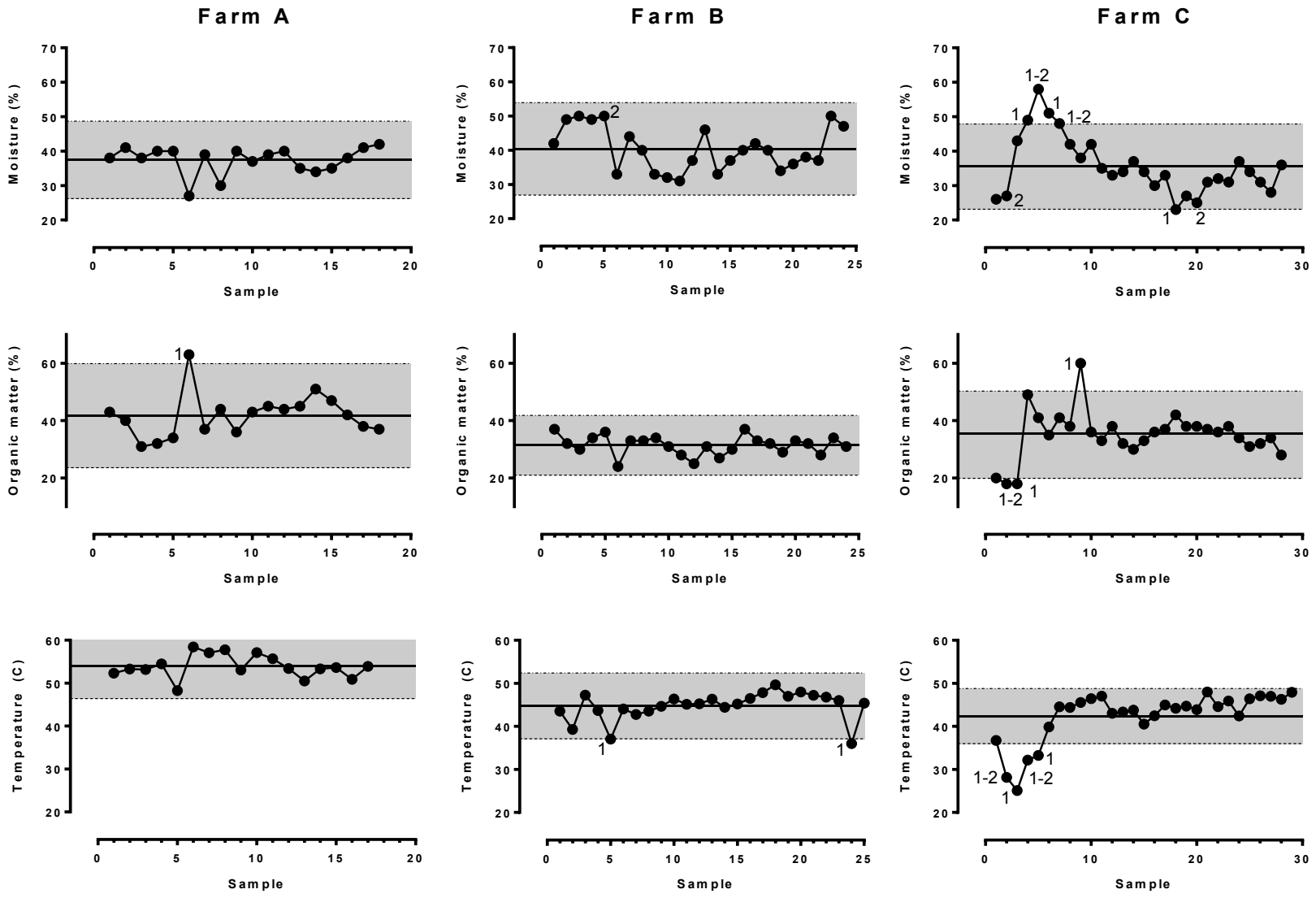
Farm A
Farm B
Farm C
Moisture (%)
Organic matter (%)
Temperature (C)
Sample

Figure 2.2. Shewhart control charts for individual measurements of bedding manageable characteristics. Bedding samples were collected biweekly. The central line indicates the mean of the observations. The upper and lower horizontal lines indicate the upper and lower control limits ($\overline{X} \pm 3\sigma$). Test 1 = 1 point beyond the mean ± 3σ (control limits); Test 2 = 2 of 3 consecutive points beyond the mean ± 2σ. Temperature was measured at the deep layer (20 cm) of the bedding.

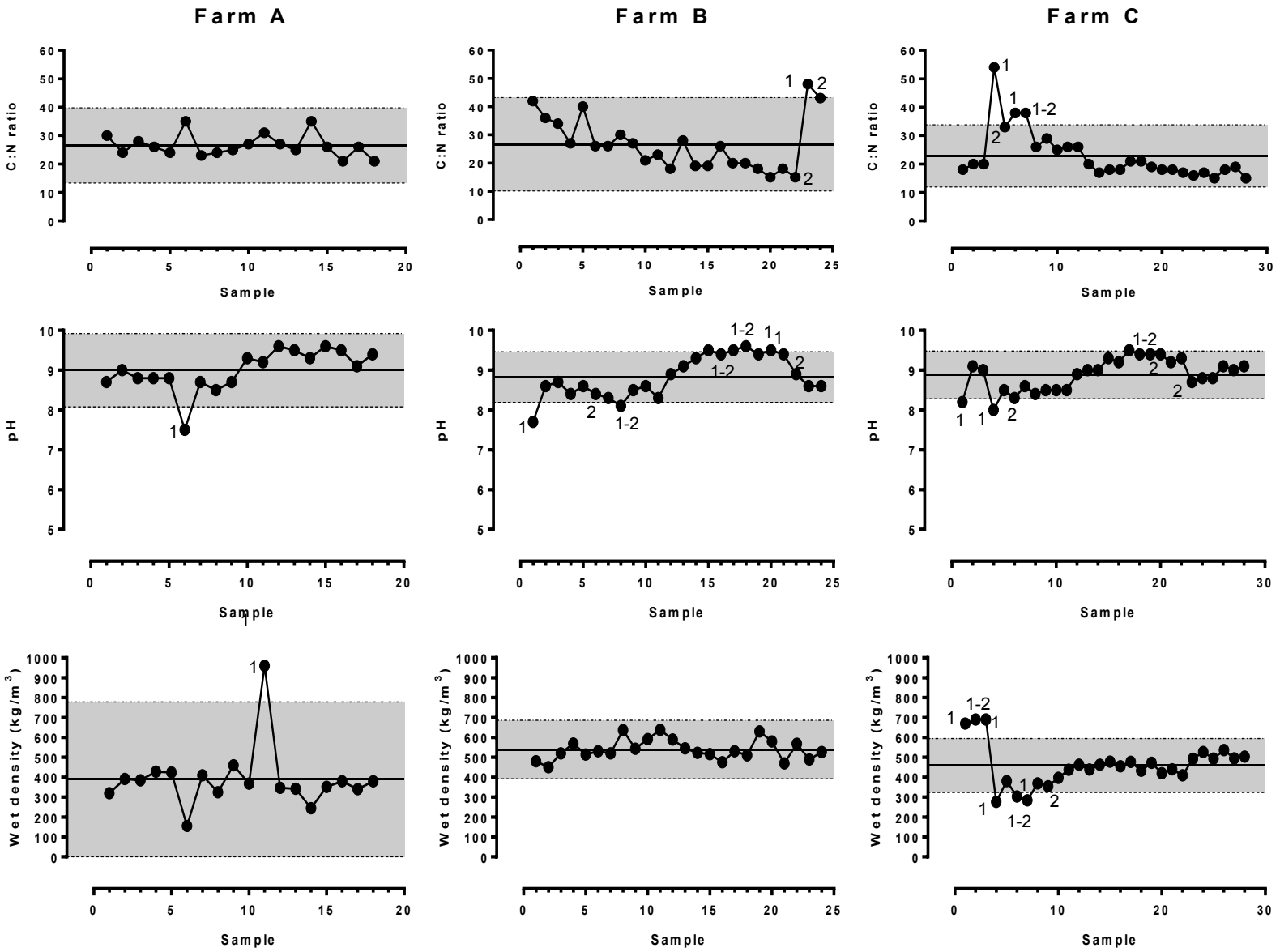
Farm A
Farm B
Farm C
C:N ratio
pH
Wet density (kg/m3)
Sample

Figure 2.3. Shewhart control charts for individual measurements of bedding manageable characteristics. Bedding samples were collected biweekly. The central line indicates the mean of the observations. The upper and lower horizontal lines indicate the upper and lower control limits ($\overline{X} \pm 3\sigma$). Test 1 = 1 point beyond the mean ± 3σ (control limits); Test 2 = 2 of 3 consecutive points beyond the mean ± 2σ. Temperature was measured at the deep layer (20 cm) of the bedding.

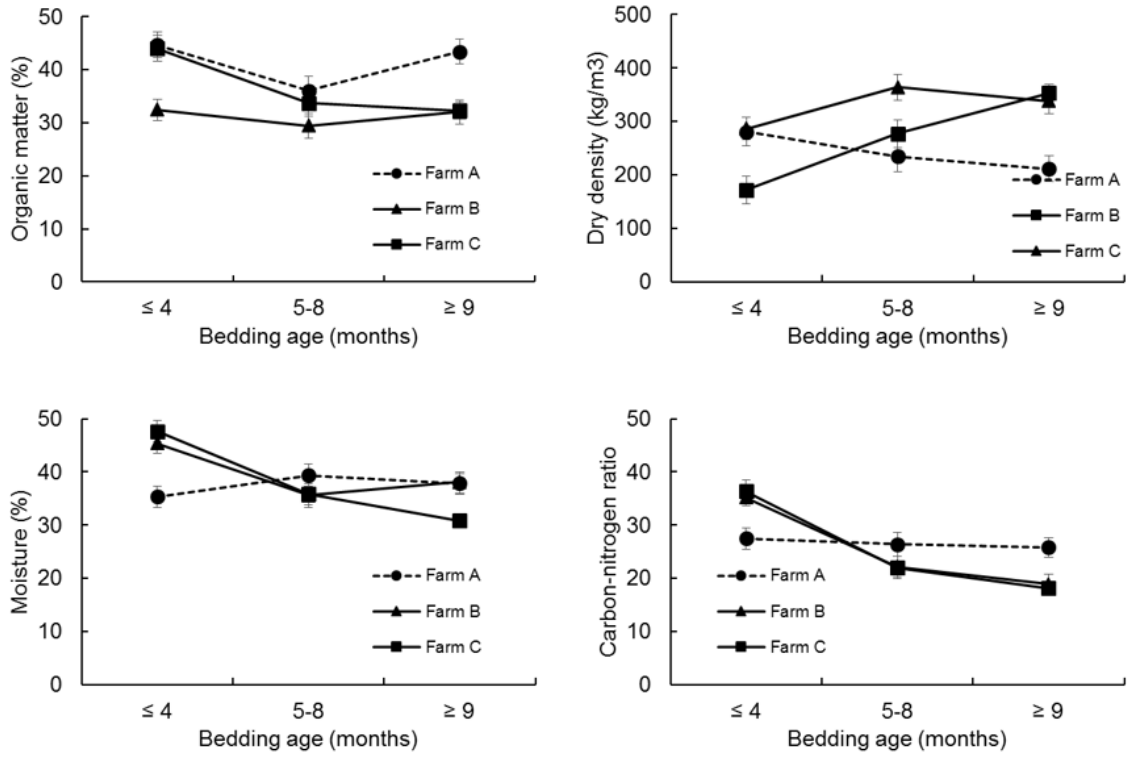

Figure 2.4. Mean bedding organic matter, dry density, moisture, and carbon-nitrogen ratio, by bedding age. Significant differences ($P < 0.05$) between means were found for the following contrasts: Organic matter and dry density: ≤ 4 x ≥ 9 on farm C. Moisture: ≤ 4 x 5-8 on farms B and C, and ≤ 4 x ≥ 9 on farm C. Carbon-nitrogen ratio: ≤ 4 x 5-8 on farms B and C, and ≤ 4 x ≥ 9 months on farm C.

CAPÍTULO 3 – TRABALHO CIENTÍFICO

Trabalho enviado para revista Livestock Science.

(Normas de publicação da revista – ANEXO I).

Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding

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ABSTRACT

The objectives of this study were to describe the profile of pathogens isolated from clinical and subclinical mastitis cases, describe hygienic conditions of cows, and identify bedding characteristics associated with mastitis epidemiologic indexes, cow hygiene, and concentration of selected bacterial populations found in bulk tank milk of herds housed on compost bedding. Three dairies were visited monthly during 1 year. On each visit day, milk samples were collected from the bulk tank and from a sample of mammary quarters for microbiological examination. Milk samples were collected from all cases of clinical mastitis. Flank, leg, udder, and teat cleanliness were assessed by use of a score chart based on a 4-point scale (1 = clean to 4 = very dirty). Bedding samples were collected to estimate concentrations of total bacteria, streptococci, and coliforms, moisture, organic matter, carbon-nitrogen ratio, pH, and density. Mixed models were used to identify factors associated with incidence and prevalence of mastitis, and cow cleanliness. Except for farm A, on which contagious pathogens caused most cases, *Escherichia coli,* coagulase-negative staphylococci, and environmental streptococci were the most frequent pathogens isolated from clinical mastitis cases. *Corynebacterium bovis* was the most frequent pathogen isolated from subclinical cases of farms B (17.6) and C (26.0%). Environmental pathogens were isolated from 17.2, 10.1, and 14.8% of all subclinical cases of farms, A, B, and C, respectively. No outbreaks of environmental mastitis were observed during the course of the study. Bedding moisture, carbon-nitrogen ratio, pH, and dry density were unconditionally associated with the incidence of environmental clinical mastitis. Nonetheless, bedding moisture remained as a sole predictor in the final model. The odds of a case of environmental clinical mastitis increased 5.7% for each one-unit increase in

bedding moisture. The odds of a new case of subclinical mastitis (incidence) and of a cow being infected (SCC > 200000 cells/mL, prevalence) increased 32% and 16% for each one-unit increase in leg cleanliness score, respectively. Overall means for udder, teat, flank, and leg hygiene scores were less than 2.1 for all farms and did not vary among seasons of the year. Bedding wet density was positively associated with all cleanliness scores and bulk milk concentration of total bacteria. Results suggest that managing bedding to remain dry and loose will result in cleaner animals with decreased risk of mastitis.

Keywords: compost bedding, mastitis, milk quality, cow hygiene

INTRODUCTION

The compost bedded pack system (**CBP**) has been increasingly used worldwide to confine dairy cows. The CPB is characterized by a process of microbiological decomposition of an organic substrate (such as wood shavings or sawdust), to which feces and urine are constantly added by cows. Bedding is tilled twice a day to incorporate animal waste, facilitate aerobic composting, and provide a comfortable and dry surface to cows (Barberg et al., 2007; Janni et al., 2007; Black et al., 2013).

Because compost bedding is mostly organic, one of the potential hazards for udder health is the concentration of environmental mastitis pathogens. Coliforms (such as *Escherichia coli* and *Klebsiella* spp) and environmental streptococci (such as *Streptococcus uberis* and *Streptococcus dysgalactiae*) are the most prevalent pathogens causing clinical mastitis on farms that have successfully controlled contagious mastitis (Jobim et al., 2010; Lago et al., 2011; Oliveira and Ruegg, 2013). Environmental streptococci are also one of the main causes of subclinical mastitis in herds worldwide (Jobim et al., 2010; Oliveira and Ruegg, 2014).

Barberg et al. (2007) reported that mean bedding concentration of total bacteria was 9.1×10^6 cfu/cc for a group of 12 CBP in Minnesota. Lobeck et al. (2012) reported bedding concentrations of 1.4×10^3, 280, and 3×10^6 cfu/mL for coliforms, *Klebsiella* spp, and environmental streptococci, respectively. Bedding concentration of environmental streptococci was not different between CBP and sand-bedded freestalls, but concentrations of coliforms and *Klebsiella* spp in CBP bedding were 47 and 14 times greater than those observed in naturally ventilated sand-bedded freestalls, respectively (Lobeck et al., 2012).

Other environmental pathogens that might be present in compost bedding, such as

Nocardia spp, *Pseudomonas* spp, and *Prototheca* spp, have been associated with herd outbreaks of clinical and subclinical mastitis, and are capable of causing chronic, untreatable mastitis (Janosi et al., 2001; Condas et al., 2013). The occurrence of such pathogens in herds housed on compost bedding has not been studied.

In this context, little research has been conducted to describe the profile of pathogens causing clinical and subclinical mastitis, assess the risk of intramammary infections (**IMI**) caused by environmental pathogens, and characterize the quality of milk produced in CBP systems. Two studies were conducted to describe longitudinal changes in bulk tank milk SCC from herds that shifted from other systems to the CBP. Barberg et al. (2007) observed SCC reduction in 5 of 7 herds by comparing mean monthly dairy herd improvement (**DHI**) somatic cell count (**SCC**) before (2 years) and after (1 year) the change. Likewise, Black et al. (2013) reported that mean SCC of 8 herds decreased from 411000 cells/ml (12-month mean prior to the change) to 305000 (first year) and 275000 cells/ml (second year) after the change. Nonetheless, a causal relationship between the use of CBP and bulk tank milk SCC should not be established solely on these data because control groups were not used for comparison.

Contrarily to the results aforementioned, Svennesen et al. (2014) performed an experimental study and reported a herd SCC increase of 72.000 cells/mL for animals randomly allocated to a CBP of high moisture content (65-70%, bedded with chopped roots, wood shavings and garden organic residues), as compared to a group of cows that remained in a sand-bedded freestall. Therefore, conflicting results about the impact of the CBP on the occurrence of mastitis suggest that bedding management plays a major role in minimizing the risk of IMI.

Among bedding-related indicators of animal health, cow cleanliness has been universally used as an indicator of udder health. Results of cross-sectional studies conducted at the herd level have consistently demonstrated that herds in which most cows were scored "clean" were more likely to have less bulk tank milk SCC than herds in which most cows were scored "dirty" (Barkema et al., 1998; Ellis et al., 2007; Dufour et al., 2011). Although results of North American studies (Barberg et al., 2007; Shane et al., 2010; Black et al., 2013) have demonstrated that cows housed in the CBP are maintained in good hygienic conditions (comparable to those found in well managed sand-bedded freestalls), researchers reported difficulties maintaining clean cows during humid and rainy weather (Lobeck et al., 2011). In those studies, visual observations suggested that cow cleanliness was dependent on bedding moisture (especially during winter) and density of the CBP, but these associations have not been scientifically demonstrated. Identification of CBP bedding factors associated with animal hygiene is integral for developing management practices towards maintaining clean cows and minimizing the risk of mastitis.

The objectives of this longitudinal study were to: 1) describe the profile of pathogens isolated from clinical and subclinical mastitis cases; 2) describe hygienic conditions of cows; and 3) identify bedding characteristics associated with mastitis epidemiologic indexes, cow hygiene, and concentration of selected bacterial populations found in bulk tank milk of herds housed in the CBP.

MATERIALS AND METHODS

Farm selection and sampling strategy

A convenience sample of 3 CBP dairies was used for the study. Inclusion criteria were location in Sao Paulo state, Brazil, adoption of compost bedding as sole system to confine lactating cows, participation in a monthly DHI testing program, and willingness to comply with the study protocol.

Initially farms were visited to explain the study protocol and provide training for collection of bedding and milk samples. Farms were then visited monthly between May 2013 and June 2014 for data collection and sampling.

Milk sampling

On each visit day, cows whose most recent DHI composite SCC was > 200.000 cells/mL were tested with the California Mastitis Test (**CMT**) for identification of possibly infected quarters. Aseptic milk samples were collected from a random sample of CMT-positive quarters (1 quarter per cow) for microbiological examination. Sampling of 50, 50, and 30% of the high SCC cows was attempted on farms A (< 100 cows), B (< 100 cows), and C (> 100 cows), respectively. The number of cows sampled was calculated based on herd size, to provide a representative sample of mastitis pathogens, and not disrupt the milking routines.

Bulk tank milk samples were collected on each visit day by use of sterile uterine infusion pipettes attached to 60-mL syringes, after milk was agitated for at least 5 minutes. All milk samples were refrigerated and frozen on the same day.

Aseptic quarter milk samples were also collected and frozen by trained farm personnel before treatment (or at detection for cases that were not treated) from all cases of clinical mastitis that occurred during the study. Clinical mastitis was identified using a strip cup and was defined as the presence of milk abnormalities such as flakes, pus, and changes in color. Severity of the cases was recorded as mild, moderate, or severe, according to the scale proposed by Wenz et al. (2001).

Cow cleanliness assessment

On each visit day, cleanliness of the flank, leg, and udder was assessed before milking (within the CBP area) by use of a score chart based on a 4-point scale ranging from 1 (clean) to 4 (very dirty) (Canadian Bovine Mastitis Research Network; Montreal, Canada). All lactating cows were scored on farms A and B (< 100 cows), and 50% of the cows were scored on farm C (> 100 cows), according to chart instructions.

On each visit day, teat swabs were collected during milking (before any milking procedure was performed) to assess teat cleanliness and estimate the population of total bacteria, coliforms, and streptococci on teat skin. Eight sterile gauze pads were placed into a sterile 50-mL plastic container and 25 mL of 0.1% peptone water were added to moisten swabs and preserve bacteria. To collect swabs, teats were scrubbed with 1 circular movement around the teat barrel, finishing with a pinch of the teat end. For all farms, swabs were collected from a random sample of 30 cows (1 teat per cow), alternating the swabbed teat between cows in a clockwise manner. Swabs were returned to sterile containers and refrigerated until processing. Teat cleanliness was assessed by use of a score card based on a 4-point scale (GEA Farm Technologies, Inc.; Naperville, IL, USA). For each visit, results

of each cleanliness score type were reported as herd weighted mean score (weight = 1, 2, 3, or 4).

Bedding sampling

Bedding samples were collected biweekly by trained farm personnel, as described by Barberg et al. (2007). For the present study, a subset of monthly bedding samples collected on the visit days was used for analysis. In brief, the bedding area of each farm was divided into 12 equal squares, from which 1 sample was collected from the superficial and deep (20 cm) layers. All samples from each layer were mixed to create a composite sample for each layer, which were used to determine bedding bacterial concentrations. The composite samples of each layer were then mixed to create a composite sample that was used to determine bedding physical-chemical characteristics.

Microbiological examination of milk, bedding, and teat swabs

Bedding samples were processed in the Sao Paulo State University´s Mastitis Research Laboratory. Microbiological analyses of bedding was performed by adding 90 mL of 0.1% peptone water to 10 g of bedding (Zdanowicz et al., 2004). Samples were mixed for 1 minute and let settle for 2 minutes. One hundred µL of diluted samples (10^{-2} to 10^{-5}) were spread onto blood agar, MacConkey, and Edward´s medium and incubated for 24 hours to determine the concentration (log10 cfu/g of bedding) of total bacteria, coliforms and streptococci, respectively. Bedding samples were sent to the Sao Paulo State University´s Soil Science Laboratory for determination of moisture (%), organic matter (%), Carbon-nitrogen ratio, pH, and wet and dry densities (kg/m^3).

Milk samples from clinical and subclinical cases were processed in the Sao Paulo State University´s Mastitis Research Laboratory and cultivated according to the NMC recommendations (NMC, 1999). In brief, 10 µL of each milk sample were streaked onto blood agar and McConkey plates. Plates were incubated at 37°C and read at 24, 48, and 72 hours. Mastitis pathogens were diagnosed based on morphology (Gram staining) and biochemical reactions. Staphylococcus aureus was differentiated from other staphylococci by means of mannitol and tube coagulase reactions. *Streptococcus* spp were identified with the Christie-Atkins-Munch-Petersen (CAMP) test, esculin, and bile-esculin reactions. Gram-negative bacteria were identified by growth on McConkeky agar, lactose production, and reactions on MIO (motility-indole-ornithine), Citrate, and TSI (triple sugar iron) agar slants.

An intramammary infection was defined as the presence of 3 or more colonies of the same type. Non-significant growth (< 3 colonies of the same type) was considered negative for analysis and samples were contaminated when there were 3 or more colony types on plates.

Bulk tank milk concentrations of total bacteria, coliforms, and streptococci were estimated by inoculating 100 µL of milk (undiluted to 10^{-4}) onto Blood agar, McConkey, and Edward´s medium, respectively. Plates were incubated at 37°C and read at 24 hours. Results were expressed as log10 cfu/mL.

Upon arrival to the laboratory, teat swabs were placed into a sterile plastic container and weighted. Twice as much peptone water was added to the plastic bag, which was stomached for 2 minutes. One mL of the solution was then used to create serial dilutions (10^{-1} to 10^{-3}). Concentrations of total bacteria, coliforms, and streptococci were estimated by inoculating 100 µL of the swab solutions onto Blood agar, McConkey, and Edward´s

medium, respectively, as previously described. Results were expressed as log10 cfu/mL of solution.

Statistical analysis

Definitions

Monthly DHI SCC was used to estimate IMI at the cow level. The SCC threshold used by the DHI association to define a cow as subclinically infected was 200000 cells/mL. Prevalence of subclinical mastitis was defined as the percentage of cows with SCC > 200000 cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk).

Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd on the DHI test day. For cows that experienced repeated episodes of clinical mastitis (regardless of the quarter), only cases that occurred after 14 days from a previous case were considered new.

Mastitis pathogens isolated during the study (Table 1) were also grouped as environmental (coliforms, *Bacillus* spp, Lactose-negative Gram-negative rods, environmental streptococci, yeast, *Prototheca* spp, *Trueperella pyogenes*, and *Pseudomonas* spp), contagious (*Streptococcus agalactiae*, *Staphylococcus aureus*, and *Corynebacterium bovis*), and opportunistic (Coagulase-negative staphylococci).

Bedding age was defined as the time interval between the last bedding total replacement and a given visit day. Bedding age was categorized into 1 (≤ 4), 2 (5-8), and 3 (≥ 9 months old).

Analytical procedures

Initially the distribution of variables was analyzed to assess normality. All bacterial counts (bedding, milk, and teat swabs) were not normally distributed and therefore transformed to a log10 scale for analysis. Descriptive statistics were produced to generate reference values for the variables studied.

Outcome variables were mastitis epidemiologic indexes (incidence of clinical mastitis, incidence of environmental clinical mastitis, incidence of subclinical mastitis, and prevalence of subclinical mastitis) and cow cleanliness scores (udder, teat, flank, and leg). Explanatory variables for mastitis epidemiologic indexes were teat, udder, leg, and flank cleanliness, season (summer, fall, winter, and spring), bedding age, and all bedding characteristics presented in Table 2. Explanatory variables for cleanliness scores were season, bedding age, and all bedding characteristics presented in Table 2.

Preliminarily, bivariate analysis was used to identify unconditional associations between each explanatory variable and study outcomes. Variables associated with a given outcome at a significance level of 0.15, and interaction terms between farm and explanatory variables were included in a stepwise model selection procedure to select final models.

All epidemiologic indexes were initially modeled as binomial outcomes by use of logistic regression, according to the following structure (Littell et al., 2006; PROC GLIMMIX, SAS Institute, 2011):

$$\text{Logit}(Y) = \alpha + \beta_i (X_i) + \delta_{j,}$$

where Y was prevalence or incidence of mastitis, α was the intercept, $\beta_i (X_i)$ was the i^{th} coefficient for the i^{th} explanatory variable, and δ_j was a random term to model repeated measurements within the j^{th} farm. When overdispersion was detected, a negative binomial distribution was used for modeling (Palta, 2003), according to the following structure:

$$\text{Log}(Y) = \alpha + \beta_i (X_i) + \text{offset} + \delta_{j,}$$

were Y was the number of new infections in a one-month period (incidence), or number of infected cows (prevalence) at a given test day, α was the intercept, $\beta_i (X_i)$ was the i^{th} coefficient for the i^{th} explanatory variable, δ_j was a random term to model repeated measurements within the j^{th} farm, and offset was the log of the number of cows included in the denominator of the incidence or prevalence calculations.

For continuous outcomes, linear mixed models (Littell et al., 2006) were construced with PROC MIXED (SAS Institute, 2011) to identify predictors for each cleanliness score. Farm was considered a random effect to model the correlation between repeated observations within the same farm. Statistical analyses were performed at a significance level of 0.05.

RESULTS

Farm characteristics

Farm A had 33 lactating Holsteins that were milked twice a day. The CBP was approximately 6 months old at the beginning of the study and consisted of an area of 290 m^2 (11 m^2 /cow) bedded with peanut shells. Seventy m^3 of new bedding were added monthly to the CBP. There was a concrete feeding alley from which cows had free access to the bedding area. Bedding was tilled twice a day between milkings by use of a deep cultivator. Fans were installed throughout the barn over the bedding area. Cows were machine-milked on the concrete feeding alley and milk was stored within a bulk tank. The milking routine consisted of examination of the first milk streams on a strip cup, pre-milking teat disinfection with a chlorine-based solution, drying of teats with single paper towels, and use of a barrier post-milking teat dip (1% iodine).

Farm B had 53 lactating Holsteins that were milked twice a day. The CBP was approximately 2 months old at the beginning of the study and was bedded with sawdust. There was no paved alley and cows were fed on a bunk located along the barn side. The CBP area on farm B was 1000 m^2 (19 m^2 /cow) and 34 m^3 of new bedding were added monthly. Bedding was tilled twice a day between milkings by use of a deep cultivator, and a rototiller was used occasionally to loosen the material and decrease particle size. No fans were installed over the bedding area. Cows were machine-milked in a Herringbone pit parlor and milk was stored in a bulk tank. The milking routine consisted of examination of the first milk streams on a streak cup, pre-milking teat disinfection with sodium hypochlorite, drying of teats with single paper towels, and use of a barrier acid lactic-based post-milking teat dip.

Farm C had 145 Simmental lactating cows that remained on a bedding area of 1580 m^2 (12 m^2/cow). The CBP was approximately 2 months old at the beginning of the study. Wood shavings were used as bedding material and 38 m^3 of new bedding were added monthly to the CBP. Bedding was tilled twice a day between milkings by use of a deep cultivator and a rototiller was used approximately twice a week to further break bedding clusters. Fans were installed over the bedding area at the 7th month of the study. Cows were fed in a separate concrete-paved shed, outside the CBP. Cows were machine-milked in a Herringbone pit parlor and milk was stored in a bulk tank. The milking routine consisted of examination of the first milk streams on a streak cup, pre-milking teat disinfection with iodine 0.5%, drying of teats with single paper towels, and use of a lactic acid-based post-milking teat dip.

Although the study was observational, farmers asked for milk quality advice during the study. Therefore, at the beginning of the study, the authors recommended management practices based on the NMC´s 10-point mastitis control plan (NMC; Verona, WI). Farms complied with proposed changes at different levels, according to their interest in improving milk quality.

Missing data

The study was interrupted 3 months before the attempted endpoint for farm A, due to difficulties in complying with the study protocol. On the 7th visit to farm C, teat swabbing and teat hygiene scoring could not be performed.

Profile of pathogens isolated from clinical and subclinical mastitis cases

Environmental pathogens were the most frequent cause of clinical mastitis on farms B (36.7) and C (33.8%). *Escherichia coli* was the most prevalent environmental pathogen isolated from clinical cases of farms B (8.3%) and C (16.9%, Table 1). Contagious pathogens were the most frequent cause of clinical mastitis on farm A (52.2%), and were isolated from 6.7 and 16.9% of the cases of farms B and C, respectively. Opportunistic pathogens were isolated from 2.2, 11.7, and 8.5% of the clinical cases of farms A, B, and C, respectively (Table 1).

Most subclinical mastitis cases were caused by contagious pathogens (farm A = 35.2, farm B = 20.7, and farm C = 37.8% of all cases). *Corynebacterium bovis* was the most frequent pathogen isolated from subclinical cases of farms B (17.6) and C (26.0%). For farms, A, B, and C, environmental pathogens were isolated from 17.2, 10.1, and 14.8% of all cases, and opportunistic pathogens were isolated from 7.0, 10.8, and 7.6% of all cases, respectively. Farm A experienced an outbreak of *Streptococcus agalactiae* during the study, which was the most frequent pathogen isolated from clinical and subclinical mastitis cases (Table 1).

The prevalence of environmental pathogens that have been associated with outbreaks of untreatable mastitis, such as *Nocardia* spp, *Pseudomonas* spp, *Serratia* spp, and *Prototheca* spp was low. Only 1 case of *Prototheca* spp (subclinical) and 2 cases of *Serratia* spp (subclinical) were diagnosed during the course of the study.

Mastitis severity was recorded for 108 clinical cases with positive culture results and 46 cases with a “no growth” result. Of the 108 cases from which pathogens were isolated, the distribution of severity by pathogen group was: 1) environmental (N = 35):

5.7% severe, 22.9 % moderate, and 71.4% mild; 2) contagious (N = 17): 0% severe, 23.5% moderate, and 76.5% mild; 3) opportunistic (N = 10 cases): 0% severe, 10% moderate, and 90% mild. Of the 46 cases with a "no growth" result, none were severe, 23.9% were moderate, and 76.1% were mild.

Mastitis epidemiology

Mean prevalence and incidence of subclinical mastitis (based on DHI SCC) during the study period were 40.9 and 20.6% for farm A, 45.7 and 10.1% for farm B, and 41.1 and 23% for farm C, respectively (Table 3). Mean incidence of environmental clinical mastitis was 3.0, 6.4, and 2.3% for farms A, B, and C, respectively, and was not different among seasons of the year ($P = 0.68$). No interaction was found between season and farm ($P = 0.12$).

Both incidences of clinical and subclinical mastitis varied substantially on farm A during the study period. Subclinical mastitis incidence peaked at 44% during the fifth month and prevalence reached 53% at the 8th month of the study (Figure 1). Except for the first month of the study (incidence = 7%), farm A´s incidence of environmental clinical mastitis ranged from 0 to 3% during the study period.

For farm B, no apparent trends were observed for the incidence of subclinical mastitis, which ranged from 10 to 26% during the study (Figure 1). Prevalence of subclinical mastitis increased steadly from the 4th (36%) to the 9th month of the study (60%), and decreased to 40% at the end of the period. Incidence of environmental clinical mastitis ranged from 2 to 12% during the study (Figure 1).

For farm C, decreasing trends were observed for the prevalence and incidence of subclinical mastitis during most of the study period (Figure 1). Similar decreasing trends were observed for the incidences of clinical (all pathogens) and environmental clinical mastitis. None of the herds experienced outbreaks of environmental clinical mastitis during the course of the study.

Bedding moisture ($P < 0.01$), carbon-nitrogen ratio ($P < 0.01$), pH ($P < 0.01$), and dry density were unconditionally associated with the incidence of environmental clinical mastitis (Table 4). Nonetheless, bedding moisture ($P < 0.01$) remained as a sole significant predictor in the final model (Table 5 and Figure 2). The odds of a case of environmental clinical mastitis increased 5.7% for each one-unit increase in bedding moisture (Table 5).

Bedding moisture ($P < 0.01$), dry density ($P = 0.04$), and carbon-nitrogen ratio ($P = 0.04$) were unconditionally associated with the incidence of clinical mastitis (Table 4). Moisture ($P < 0.01$) remained as the only predictor in the final model. The odds of a case of clinical mastitis increased 5.8% for each one-unit increase in bedding moisture (Table 5 and Figure 2).

Leg cleanliness score was the only variable associated with the prevalence and incidence of subclinical mastitis in both bivariate and multivariable analysis. The odds of a new case of subclinical mastitis (incidence) and of a cow being infected (SCC > 200000 cels/mL, prevalence) increased 32% and 16% for each one-unit increase in leg cleanliness score, respectively (Table 5, Figure 2).

Cow cleanliness

For all farms, most cows remained clean (score 1) or slightly dirty (score 2) during the study period. Overall means for udder, teat, flank, and leg hygiene scores were less than

2.1 for all farms and varied little during the study (Table 3 and Figure 3). Mean udder (P = 0.07), teat (P = 0.32), flank (P = 0.17), and leg (P = 0.21) scores were not different among seasons of the year.

Bedding wet density was unconditionally associated with udder (P = 0.05), leg (P < 0.04), teat (P < 0.01), and flank (P = 0.03) cleanliness, and bedding dry density was positively associated with udder (P = 0.02) and teat (P = 0.02) cleanliness. Bedding organic matter was negatively associated with teat (P = 0.03) and flank (P = 0.04) cleanliness. Mean udder cleanliness increased across bedding age categories (P < 0.01, Table 4).

Bedding wet density remained as a sole predictor of all cleanliness scores studied (Table 4). All associations were positive, but of small magnitude (Table 5 and Figure 4).

Bulk tank milk bacterial concentrations

For farms A and C, bulk milk concentration of total bacteria ranged from 2.6 to 4.6 log10 cfu/mL and remained below the Brazilian legal limit (3 x 10^5 cfu/mL) during the entire study period (Figure 5). For farm B, bulk milk concentration of total bacteria increased steadly from the fourth month (1.9) and reached 6.4 log10 cfu/mL at the sixth month of the study. Subsequetly, bulk milk concentration of total bacteria decreased to levels similar to those observed at the beginning of the study. A total of 2 monthly counts were greater than the official regulatory limit (Figure 5).

Longitudinal trends of bulk milk concentration of streptococci were similar to those observed for total bacteria (Figure 5). Trends of bulk milk concentration of coliforms were similar for farms B and C, and showed great variability during the study (Figure 5). Farm A´s bulk milk concentration of coliforms was below the detection limit of the culturing

method throughout the study. Bulk milk concentrations of total bacteria ($P = 0.36$), streptococci ($P = 0.12$), and coliforms ($P = 0.91$) were not different among seasons of the year.

Bedding dry density ($P < 0.01$), wet density ($P = 0.02$) and organic matter ($P = 0.02$) were unconditionally associated with bulk milk concentration of total bacteria, but only wet density remained in the final model (positive association, Table 5). Bedding dry density was the only variable associated (positive association) with bulk milk concentration of streptococci ($P = 0.02$) and organic matter was the sole predictor (negative association) for the concentration of coliforms ($P = 0.049$, Table 5).

DISCUSSION

Because the CBP is a recent system to confine dairy cows, most studies published to date were descriptive. Only one cross-sectional study has been conducted to identify factors associated with bedding microbial quality (Black et al., 2014), and to our knowledge, no peer-reviewed studies have been published to determine the influence of bedding characteristics on mastitis epidemiologic indexes or animal hygiene.

As compared to cross-sectional studies, longitudinal designs allow capturing of within-farm variation, longitudinal monitoring of study outcomes, and establishment of temporal relationships between explanatory variables and outcomes (Dohoo et al., 2010). Epidemiological surveillance is important to assess the biosecurity of the CBP (e.g., occurrence of mastitis outbreaks). It has been hypothesized that housing cows in the CBP would increase the risk of environmental mastitis because compost bedding is highly organic and feces are constantly added by cows. In this environment, the risk of outbreaks caused by environmental mastitis pathogens that are refractory to conventional treatments

(such as *Nocardia* spp) also needed to be assessed. We observed that the prevalence of such pathogens was very low and no outbreaks of environmental mastitis were observed during the course of the study. The outbreak of contagious mastitis observed on Farm A was linked to the introduction of animals infected with *Streptococcus agalactiae*.

The distribution of environmental pathogens isolated from clinical and subclinical mastitis cases was similar to those reported from different countries and housing systems (Olde Riekerink et al., 2008; Jobim et al., 2010; Lago et al., 2011; Oliveira and Ruegg, 2014). In agreement with those studies, "no growth" was the most frequent culture result (33.3% of all cases), and *Escherichia coli* (9.6%) and environmental streptococci (17.5% of all cases) were the most frequent pathogens isolated from clinical mastitis cases.

The distribution of pathogens isolated from subclinical mastitis was characterized by a high prevalence of contagious pathogens. *Corynebacterium bovis*, *Streptococcus agalactiae*, and *Staphylococcus aureus* are still highly prevalent on Brazilian dairies (Bueno et al., 2008) due to the lack of adoption of mastitis control programs. Except for contagious pathogens, coagulase-negative staphylococci and environmental streptococci were the most prevalent pathogens isolated form subclinical mastitis cases, as previously reported (Wallace et al., 2004; Pol and Ruegg, 2007; Gianneechini et al., 2008).

It is important to emphasize that the present study was not designed to assess whether shifting from other systems to the CBP would improve milk quality and mastitis control. Thus, the longitudinal trends in mastitis epidemiologic indexes observed on the 3 farms could have been affected by several factors not related to compost bedding, such as milking machine, milking management, and profile of pathogens found on each herd. Moreover, because bedding materials were different among farms, assessment of the effect of bedding type (e.g., sawdust versus peanut shell) on mastitis epidemiologic indexes or

animal hygiene should not be encouraged because one farm might not be representative of a greater population of farms that use the same bedding type.

Because of the high bacterial concentrations found in compost bedding (Barberg et al., 2007; Black et al., 2014), researchers have recommended adoption of excellent pre-milking hygienic procedures, and that bedding be maintained dry and not adherent to cows (Barberg et al., 2007; Black et al., 2013). Nonetheless, these associations had not been scientifically demonstrated. Results of the present study indicate that bedding factors such as density and moisture are associated with mastitis epidemiologic indexes and cow cleanliness. Wet density is related to particle size and moisture. As particle size decreases and moisture increases, bedding becomes denser and more compacted. As a result, bedding is probably more likely to adhere to cows, which become dirtier. Bedding wet density was positively associated with all cleanliness scores studied here.

Another consequence of denser bedding is the decrease in bedding aeration. An aerobic composting process is important to maintain an efficient microbiological decomposition of the organic material. When bedding is loose and aerated, temperature in the deep layer (20 cm deep) increases to approximately 40-55 °C as a consequence of microbial activity (Barberg et al., 2007; Black et al., 2014). Thus, a combination of effective aeration and high deep layer temperature facilitates moisture loss and maintenance of a dry environment to cows.

In the present study, bedding moisture was the main predictor for the incidence of environmental clinical mastitis. Bedding moisture has been reported as one of most difficult characteristics to control in CBP systems (Lobeck et al., 2011) because it can be greatly influenced by bedding management and weather conditions. Inadequate aeration, high animal density, and lack of ventilation can lead to increased moisture levels (Janni et al.,

2007; Black et al., 2014). Weather factors include air humidity and rainwater entering the CBP.

There could be different mechanisms by which bedding moisture is associated with increased risk of environmental mastitis. Moisture is essential for bacterial growth and increase in moisture levels can favor multiplication of microorganisms and therefore increase exposure to cows. Another factor that needs to be studied is the transfer of bacteria to the teat skin. It can be hypothesized that moisten bedding particles adhere to cows and facilitate the transfer of bacteria to the skin. Conversely, if bedding is maintained dry and loose, transferring of bacteria to the skin may be greatly minimized. Further research should be conducted to study physical characteristics of bedding particles (such as particle size and water retention) that can affect the transfer of bacteria to the teat skin.

It was interesting to observe that bedding moisture decreased dramatically on farm C after installation of fans over the bedding area (data shown in the companion paper). The bedding became dry and not adherent to cows, which remained in excellent hygienic conditions. Even after reaching moisture levels as low as 30%, bedding deep temperature (20 cm deep) was maintained > 40 °C, indicating that it is possible to maintain dry bedding without compromising microbiological activity.

A negative association was found between bedding pH and the incidence of environmental clinical mastitis. Is has been consistently demonstrated that, during the composting process, pH increases with time. Perhaps the alkaline pH levels found in this study (overall mean > 8.8 for all farms) inhibit growth of environmental mastitis pathogens. The effect of pH on bedding bacterial populations needs to be further studied because it could be managed on the farms.

Other changes that occur along the composting process are decrease in density and organic matter, and increase in the water retention capacity of the material (Khiel, 1985). As a result of the bivariate analysis, we observed that the risk of environmental mastitis decreased progressively as bedding became older. This finding agrees with the farmer´s observations that increases in mastitis occurrence were observed after bedding was totally replaced. This increase in risk could be explained by some characteristics of new bedding material such as high moisture content, organic matter, and carbon-nitrogen ratio. All of these factors have been known to support bacterial growth and were positively associated with the risk of environmental mastitis.

Dairy consultants and researchers have routinely used several cow cleanliness score systems as indicators of udder health. Cow cleanliness have been associated with milk quality outcomes at the cow (prevalence of mastitis; Schreiner and Ruegg, 2003) and herd (bulk tank milk SCC; Barkema et al., 1998; Ellis et al., 2007; Dufour et al., 2011) levels.

Results of the present study corroborate with those of North American studies that have demonstrated that cows housed in the CBP are maintained in good hygienic conditions, comparable to standard systems such as well managed sand-bedded freestalls (Lobeck et al., 2011). Mean cow cleanliness score (ranging from 1 = clean to 5 = very dirty) was 2.6 (Barberg et al., 2007) e 3.1 (Shane et al., 2010) for a population of Minnesota CBP. Likewise, Black et al. (2013) reported mean cleanliness score of 2.2 (scale ranging from 1 = clean to 4 = very dirty) for a group of Kentucky CBP. Researchers found significant seasonal variation and reported difficulties controlling cow cleanliness during humid and rainy weather. In Brazilian conditions, where weather differences among seasons are not as extreme as in North America, cows remained in excellent hygienic conditions throughout the year studied.

One important observation during the study was the milking technicians´ satisfaction about teat cleanliness. Although these data were not recorded during the study, technicians frequently reported that teats were very clean and how it facilitated their daily work. Housing cows in well-managed CBP could be an interesting alternative to one of the most prevalent systems used in Brazil, the semi-confinement (dry lot), in which cows are usually maintained in poor hygienic conditions.

CONCLUSIONS

Coliforms and environmental streptococci were the most frequent pathogens isolated from clinical mastitis cases. The prevalence of IMI caused by *Nocardia* spp, yeast, *Prototheca* spp, *Serratia* spp, *Pseudomonas* spp, and other environmental pathogens that can cause outbreaks of untreatable mastitis was not concerning. No outbreaks of environmental mastitis were observed during the course of the study. Results of the present study indicate that bedding low moisture and wet density levels are associated with decreased risk of clinical mastitis and improvement of cow cleanliness, respectively. Cow cleanliness scoring can be useful to aid bedding management and asses the risk of subclinical mastitis.

CONFLICT OF INTEREST STATEMENT

None

ACKNOWLEDGEMENTS

We are thankful to Dr. Leonardo Dantas and the farmers and collaborators who cooperated with the study. This research was funded by the Sao Paulo Research Foundation (FAPESP), grant 2013/00517-3.

REFERENCES

Barberg, A.E., Endres, M.I., Janni, K. 2007a. Compost dairy barns in Minnesota: A descriptive study. Appl. Eng. Agric. 23:231-238.

Barberg, A.E., Endres, M.I., Salfer J.A., Reneau, J.K. 2007b. Performance and welfare of dairy cows in an alternative housing system in Minnesota. J. Dairy Sci. 90:1575-1583.

Barkema, H.W., Schukken, Y.H., Lam, T.J., Beiboer, M.L., Benedictus, G., Brand, A. 1998. Management practices associated with low, medium, and high somatic cell counts in bulk milk. J. Dairy Sci. 81:1917–1927.

Black, R.A., Taraba, J., Day, G., Damasceno, F., Newman, M., Akers, K.A., Wood, C.L., McQuerry, K.J., Bewley, J.M. 2014. The relationship between compost bedded pack performance, management, and bacterial counts. J. Dairy Sci. 97:2669-2679.

Bueno, V.F.F., Nicolau, E.S., Mesquita, A.J., Ribeiro, A.R., Silva, J.A.B., Costa, E.O., Couto, D.V. 2008. Etiologia e suscetibilidade à antimicrobianos dos agentes da mastite bovina isolados na região de Pirassununga-SP-Brasil. Rev. patol. trop, 32: 33-43.

Condas, L.A., Ribeiro, M.G., Yazawa, K., Vargas, A.P.C., Salerno, T., Giuffrida, R., Langoni, H., Melville, P.A., Biesdorf, S., Matsuzawa, T., Gonoi, T., Kastelic, J.P., Barkema, H.W. 2013. Molecular identification and antimicrobial susceptibility of *Nocardia* spp. isolated from bovine mastitis in Brazil. Vet. microbiol., 167: 708-712.

Dohoo, I., Martin, W., Stryhn, H. 2010. Veterinary Epidemiologic Research. 2nd Ed. VER Inc., Charlottetown, Prince Edward Island, Canada.

Dufour, S., Fréchette, A., Barkema, H.W., Mussell, A., Scholl, D.T. 2011. Invited review: Effect of udder health management practices on herd somatic cell count. J. Dairy Sci. 94: 563-579.

Ellis, K.A., Innocent, G.T., Mihm, M., Cripps, P., McLean, W.G., Howard, C.V., Grove-White, D. 2007. Dairy cow cleanliness and milk quality on organic and conventional farms in the UK. J. Dairy Res., 74: 302-310.

Gianneechini, R., Concha, C., Rivero, Delucci, R.,I., Moreno López J. 2002. Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. Acta Vet. Scand., 43: 221-230.

Janni, K., Endres, M., Reneau, J., Schoper, W. 2007. Compost dairy barn layout and management recommendations. Appl. Eng. Agric. 23:97-102.

Janosi, S., Ratz, F., Szigeti, G., Kulcsar, M., Kerenyi, J., Lauko, T., Huszenicza, G. 2001. Review of the microbiological, pathological, and clinical aspects of bovine mastitis caused by the alga Prototheca zopfii. Vet. Quart. 23: 58-61.

Jobim, M.B., Lopes, M.A., Costa, G.M.D., Demeu, F.A. 2010. Pathogens associated with bovine mastitis in dairy herds in the south region of Brazil. Bol. Ind. Anim. 67:175-181.

Kiehl, E.J. 1985. Compostagem. Pages 229-310 in Fertilizantes orgânicos. Agronômica Ceres, Piracicaba, Brasil.

Lago, A., Godden, S.M., Bey, R., Ruegg, P.L., Leslie, K. 2011. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. J. Dairy Sci. 94:4441-4456.

Littell, R., Milliken, G., Stroup, W., Wolfinger, R., Schabenberger, O. 2006. SAS for mixed models. 2nd ed. SAS institute Inc., Cary, NC.

Lobeck, K.M., Endres, M.I., Shane, E.M., Godden, S.M., Fetrow, J. 2011. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. J. Dairy Sci. 94: 5469-5479.

Lobeck, K., Endres, M., Janni, K., Godden, S., Fetrow, J. 2012. Environmental characteristics and bacterial counts in bedding and milk bulk tank of low profile cross-

ventilated, naturally ventilated, and compost bedded pack dairy barns. Appl. Eng. Agric. 28:117-128.

NMC (National Mastitis Council). 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI, USA.

Olde Riekerink, R.G.M., Barkema, H.W., Kelton, D. F., Scholl, D. T. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. J. Dairy Sci. 91: 1366-1377.

Oliveira, L., Ruegg, P.L. 2014. Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. J. Dairy Sci. 97: 5426-5436.

Palta, M. 2003. Quantitative Methods on Population Health: Extensions of Ordinary Regression. John Wiley and Sons, Hoboken, NJ, USA.

Pol, M., Ruegg, P.L. 2007. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin J. Dairy Sci. 90: 249-261

SAS Institute. 2011. SAS/STAT User's Guide. Version 9.3, SAS Institute Inc., Cary, NC.

Schreiner, D.A., Ruegg, P.L. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci. 86: 3460-3465.

Shane, E., Endres, M., Janni, K. 2010. Alternative bedding materials for compost bedded pack barns in Minnesota: A descriptive study. Appl. Eng. Agric. 26: 465-473.

Svennesen, L., Enevoldsen, C., Bjerg, B., Klaas, I.C. 2014. Udder health in a Danish compost bedded pack barn. Page 154 in Natl. Mastitis Counc. Reg. Mtg. Proc., Ghent, Belgium. Natl. Mastitis Counc. Inc., Madison, WI, USA.

Wallace, J.A., Stipetic, K., Schukken, Y.H., Dingwell, R.T., Baillargeon, P., Bacic, G., Leslie, K.E. 2004. An evaluation of a treatment protocol for intramammary infections in early postpartum of dairy cows based on a positive california mastitis test result. Bovine Practicioner 38: 72-78.

Wenz, J.R., Barrington, G.M., Garry, F.B., Dinsmore, R.P., Callan, R.J. 2001. Use of systemic disease signs to assess disease severity in dairy cows with acute coliform mastitis. J. Am. Vet. Med. Assoc. 218: 567-572.

Zdanowicz, M., Shelford, J., Tucker, C., Weary, D., Von Keyserlingk, M. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87:1694-1701.

Table 3.1. Results of microbiological examination of milk, by farm and mastitis type

		Farm B		Farm A		Farm C	
Mastitis type	Result	N	%	N	%	N	%
Clinical	Bacillus spp					1	1.4
	Citrobacter spp	1	1.7			2	2.8
	Coagulase-negative staphylococci	7	11.7	1	2.2	6	8.5
	Corynebacterium bovis	3	5.0	4	8.7	7	9.9
	Enterobacter spp	2	3.3	1	2.2	1	1.4
	Enterococcus spp			2	4.3	1	1.4
	Escherichia coli	5	8.3			12	16.9
	Gram-negative rods	3	5.0			3	4.2
	Klebsiella spp	3	5.0	4	3.1	1	1.4
	Yeast	3	5.0				
	Serratia spp	1	1.7				
	Staphylococcus aureus					5	7.0
	Streptococcus agalactiae	1	1.7	20	43.5		
	Streptococcus dysgalactiae	2	3.3				
	Streptococcus spp			1	2.2	3	4.2
	Streptococcus uberis	1	1.7				
	Trueperella pyogenes	1	1.7				
	No growth	25	41.7	10	21.7	24	33.8
	Contaminated	2	3.3	7	15.2	5	7.0
	TOTAL	60	100	50	103	71	100
Subclinical	Citrobacter spp	2	0.7				
	Coagulase-negative staphylococci	30	10.8	9	7.0	23	7.6
	Corynebacterium bovis	49	17.6	16	12.5	79	26.0
	Enterobacter spp	1	0.4				
	Enterococcus spp	3	1.1	1	0.8	10	3.3
	Escherichia coli					2	0.7
	Gram-negative rods	3	1.1				
	Klebsiella spp	3	1.1	4	3.1	4	1.3
	Yeast	1	0.4			4	1.3
	Prototheca spp					1	0.3
	Pseudomonas spp	2	0.7	1	0.8		
	Serratia spp	1	0.4			1	0.3
	Staphylococcus aureus	3	1.1			36	11.8
	Streptococcus agalactiae	6	2.2	29	22.7		
	Streptococcus dysgalactiae	4	1.4	1	0.8	7	2.3
	Streptococcus spp	8	2.9	11	8.6	13	4.3
	Streptococcus uberis			4	3.1	3	1.0
	No growth	160	57.6	42	32.8	106	34.9
	Contaminated	2	0.7	10	7.8	15	4.9
	TOTAL	278	100	128	100	304	100

Table 3.2. Descriptive statistics for bedding characteristics, by farm

	Farm A					Farm B					Farm C				
Bedding variable	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Concentration of total bacteria[1] (log10 cfu/g)	9	8.74	0.47	8.14	9.38	12	8.50	0.31	8.01	8.89	12	8.98	0.41	8.34	9.78
Concentration of coliforms[1] (log10 cfu/g)	9	6.58	0.58	5.64	7.57	12	6.16	0.63	5.34	7.18	12	6.77	0.86	5.45	8.20
Concentration of streptococci[1] (log10 cfu/g)	9	6.70	0.99	4.30	7.75	12	5.83	0.81	4.48	7.32	12	6.85	0.50	6.18	7.80
Organic matter[2] (%)	9	40.56	5.50	31.00	47.00	11	31.18	3.12	27.00	37.00	12	31.92	7.18	18.00	41.00
Carbon-nitrogen ratio[2]	9	25.56	2.60	21.00	30.00	12	27.17	10.15	15.00	43.00	12	21.83	6.51	15.00	33.00
Moisture[2] (%)	9	36.78	3.11	30.00	40.00	12	40.75	6.02	32.00	50.00	12	37.08	9.40	26.00	58.00
pH[2]	9	9.04	0.42	8.50	9.60	12	8.81	0.52	7.70	9.60	12	8.85	0.38	8.20	9.40
Wet density[2] (kg/m^3)	9	372.67	46.07	320.00	460.00	12	526.17	39.64	470.00	596.00	12	477.33	103.12	368.00	690.00
Dry density[2] (kg/m^3)	9	234.67	21.50	198.00	276.00	12	313.17	48.08	257.00	402.00	12	298.42	91.21	160.00	496.00

[1] Estimated from samples collected from the bedding surface.

[2] Estimated from composite samples of the bedding superficial and deep (20 cm) layers.

Table 3.3. Descriptive statistics for mastitis epidemiologic indexes, bulk milk and teat swab concentration of selected bacterial groups, and cow cleanliness scores, by farm

	Farm A					Farm B					Farm C				
Variable	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Incidence of subclinical mastitis[1] (%)	9	20.56	14.35	0.00	44.00	12	19.08	5.21	10.00	26.00	12	23.00	6.13	16.00	37.00
Prevalence of subclinical mastitis[2] (%)	9	44.68	6.69	34.21	53.49	12	45.72	6.75	35.71	60.00	12	41.13	8.11	31.00	59.86
Incidence of clinical mastitis[3] (%)	7	15.04	8.89	2.86	25.00	11	8.84	3.93	1.85	17.24	11	4.24	3.71	0.74	14.29
Incidence of environmental clinical mastitis (%)	8	2.95	1.97	0.00	7.14	11	6.36	3.50	1.85	12.07	11	2.33	2.17	0.00	8.07
Bulk milk concentration of total bacteria (log10 cfu/mL)	9	3.19	0.53	2.63	4.36	12	3.92	1.43	1.90	6.40	12	3.52	0.52	2.67	4.63
Bulk milk concentration of coliforms (log10 cfu/mL)	9	0.00	0.00	0.00	0.00	12	1.95	1.51	0.00	4.45	12	1.45	0.99	0.00	2.51
Bulk milk concentration of streptococci (log10 cfu/mL)	9	1.95	0.99	0.00	3.08	12	2.97	1.26	1.00	4.70	12	2.92	0.50	2.23	3.78
Teat swab concentration of total bacteria[4] (log10 cfu/mL)	9	4.69	0.61	3.59	5.56	12	4.43	0.54	3.38	5.63	11	4.74	0.62	3.49	5.79
Teat swab concentration of coliforms[4] (log10 cfu/mL)	9	1.41	1.51	0.00	3.83	12	0.72	0.83	0.00	2.40	11	0.87	1.10	0.00	2.62
Teat swab concentration of sreptococci[4] (log10 cfu/mL)	9	2.46	0.78	1.30	3.90	12	1.73	0.97	0.00	2.90	11	1.44	0.88	0.00	2.54
Udder cleanliness score[5] (herd weighted mean)	9	1.26	0.18	1.09	1.61	12	1.30	0.17	1.03	1.59	12	1.29	0.27	1.05	2.04
Leg cleanliness score[5] (herd weighted mean)	9	1.60	0.41	1.18	2.53	12	1.77	0.38	1.24	2.40	12	1.66	0.45	1.19	2.63
Flank cleanliness score[5] (herd weighted mean)	9	1.36	0.21	1.10	1.67	12	1.43	0.32	1.03	2.23	12	1.40	0.30	1.10	2.11
Teat cleanliness score[5] (herd weighted mean)	9	1.86	0.26	1.63	2.44	12	2.12	0.27	1.74	2.62	11	1.72	0.42	1.13	2.58

[1] Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day.

[2] Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk).

[3] Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.

[4] Teat swabs were collected from a random sample of 30 quarters at each farm visit.

[5] Cleanliness scoring was performed before milking, within the CBP area, sampling 100, 100, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

Table 3.4. Unconditional associations between explanatory variables and study outcomes

Outcome (bold letters) and explanatory variables	Coefficient	SE	P-value
Incidence of clinical mastitis – environmental pathogens[1]			
Bedding moisture (%)	0.055	0.013	< 0.01
Bedding dry density (kg/m^3)	-0.006	0.002	< 0.01
Bedding organic matter (%)	0.036	0.024	0.15
Bedding carbon-nitrogen ratio	0.050	0.014	< 0.01
Bedding pH	-0.948	0.332	< 0.01
Bedding age (months)			0.01
≤ 4	0.869	0.273	
5 – 8	0.271	0.308	
≥ 9	Reference		
Incidence of clinical mastitis – all pathogens[1]			
Bedding moisture (%)	0.050	0.013	< 0.01
Bedding dry density (kg/m^3)	-0.004	0.002	0.04
Bedding carbon-nitrogen ratio	0.038	0.016	0.03
Leg cleanliness score (herd weighted mean)	0.623	0.311	< 0.06
Prevalence of subclinical mastitis[2]			
Bedding moisture (%)	0.007	0.004	0.08
Bedding concentration of streptococci	-0.071	0.038	0.07
Leg cleanliness score (herd weighted mean)	0.148	0.071	0.05
Incidence of subclinical mastitis[3]			
Leg cleanliness score (herd weighted mean)	0.279	0.129	0.04
Udder cleanliness score[4]			
Bedding wet density (kg/m^3)	0.001	0.000	0.05
Bedding dry density (kg/m^3)	0.001	0.001	0.02
Bedding age (months)			< 0.01
Intercept	1.41	0.05	
≤ 4	-0.18	0.08	
5 – 8	-0.26	0.08	
≥ 9	Reference		
Teat cleanliness score[4]			
Bedding wet density (kg/m^3)	0.002	0.001	< 0.01
Bedding dry density (kg/m^3)	0.002	0.001	0.03
Bedding organic matter (%)	-0.02	0.01	0.03
Bedding concentration of total bacteria (log10 cfu/g)	-0.37	0.14	0.01
Flank cleanliness score[4]			
Bedding wet density (kg/m^3)	0.001	0.001	0.03
Bedding organic matter (%)	-0.02	0.01	0.04
Leg cleanliness score[4]			
Bedding wet density (kg/m^3)	0.002	0.001	0.04
Bulk tank milk - concentration of total bacteria[5]			
Bedding wet density (kg/m^3)	0.004	0.002	0.02
Bedding dry density (kg/m^3)	0.006	0.002	< 0.01
Bedding organic matter (%)	-0.06	0.02	0.02

Table 3.4. continuation

Bulk tank milk - concentration of streptococci[3]			
Bedding dry density (kg/m^3)	0.006	0.003	0.02
Bulk tank milk - concentration of coliforms[3]			
Bedding organic matter (%)	-0.07	0.03	0.05

[1]Associations were derived from generalized linear mixed models based on a binomial distribution. Model coefficients are log (odds ratio). Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.

[2]Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day.

[3]Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk).

[4] Associations were derived from linear mixed models based on a normal distribution. Cleanliness scoring was performed before milking, within the CBP area, sampling 100, 100, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

[5] Associations were derived from linear mixed models based on a normal distribution.

Table 3.5. Associations between explanatory variables and study outcomes derived from multivariable analyses.

Outcome (bold letters) and explanatory variables	Coefficient	SE	P-value
Incidence of clinical mastitis – environmental pathogens[1]			
Intercept	-5.457	0.638	
Bedding moisture (%)	0.055	0.013	< 0.01
Incidence of clinical mastitis – all pathogens[1]			
Intercept	-4.596	0.635	
Bedding moisture (%)	0.056	0.010	< 0.01
Prevalence of subclinical mastitis[2]			
Intercept	-1.101	0.126	
Leg cleanliness score (herd weighted mean)	0.148	0.071	0.05
Incidence of subclinical mastitis[3]			
Intercept	-2.004	0.232	
Leg cleanliness score (herd weighted mean)	0.279	0.129	0.04
Udder cleanliness score[4]			
Intercept	1.40	0.67	
Bedding wet density (kg/m^3)	0.002	0.001	0.74
Wet density x Farm			< 0.01
Farm A	0.001	0.002	
Farm C	0.004	0.001	
Farm B	Reference		
Teat cleanliness score[4]			
Intercept	0.89	0.34	
Bedding wet density (kg/m^3)	0.002	0.001	< 0.01
Flank cleanliness score[4]			
Intercept	0.87	0.24	
Bedding wet density (kg/m^3)	0.001	0.001	0.03
Leg cleanliness score[4]			
Intercept	0.95	0.35	
Bedding wet density (kg/m^3)	0.002	0.001	0.04
Bulk tank milk - concentration of total bacteria[5]			
Intercept	1.53	0.82	
Bedding wet density (kg/m^3)	0.004	0.002	0.02
Bulk tank milk - concentration of streptococci[5]			
Intercept	0.96	0.73	
Bedding dry density (kg/m^3)	0.006	0.25	0.02
Bulk tank milk - concentration of coliforms[5]			
Intercept	3.55	1.22	
Bedding organic matter (%)	-0.07	0.03	0.05

[1]Associations were derived from generalized linear mixed models based on a binomial distribution. Model coefficients are log (odds ratio). Incidence of clinical mastitis was

defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day.

[2]Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day.

[3]Associations were derived from generalized linear mixed models based on a negative binomial distribution. Model coefficients are log (risk ratio). Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk).

[4] Associations were derived from linear mixed models based on a normal distribution. Cleanliness scoring was performed before milking, within the CBP area, sampling 100, 100, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

[5] Associations were derived from linear mixed models based on a normal distribution.

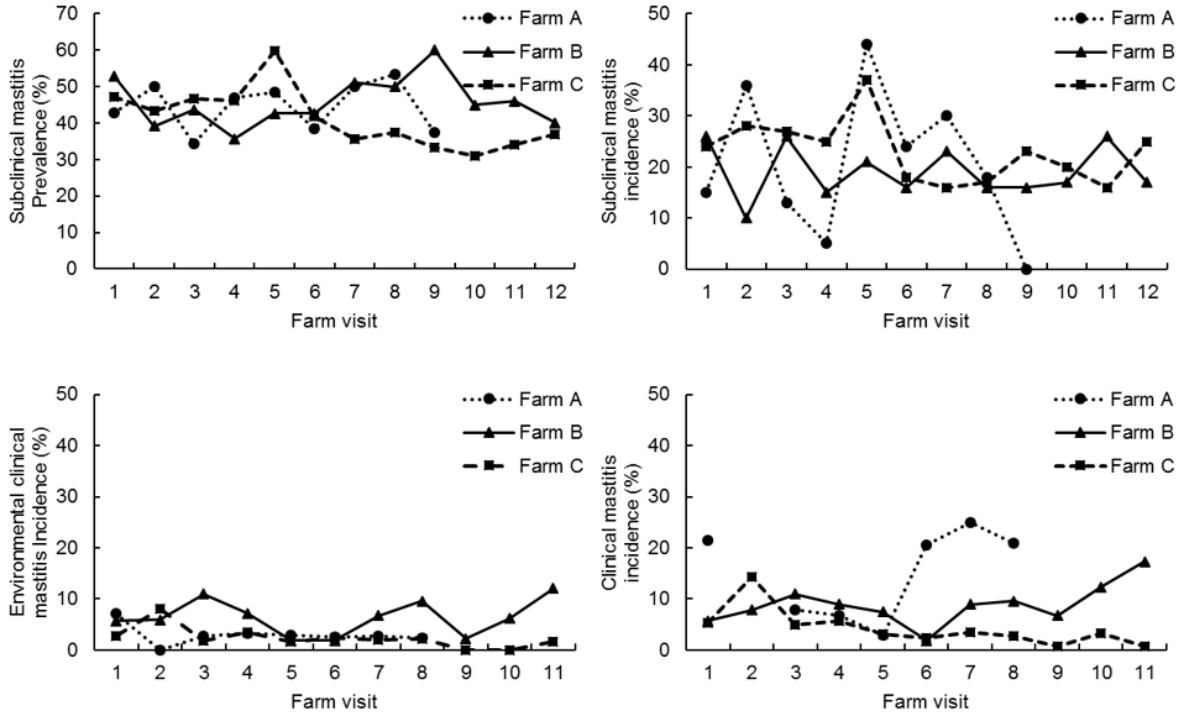

Figure 3.1. Monthly mastitis epidemiologic indexes, by farm. Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day. Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk).

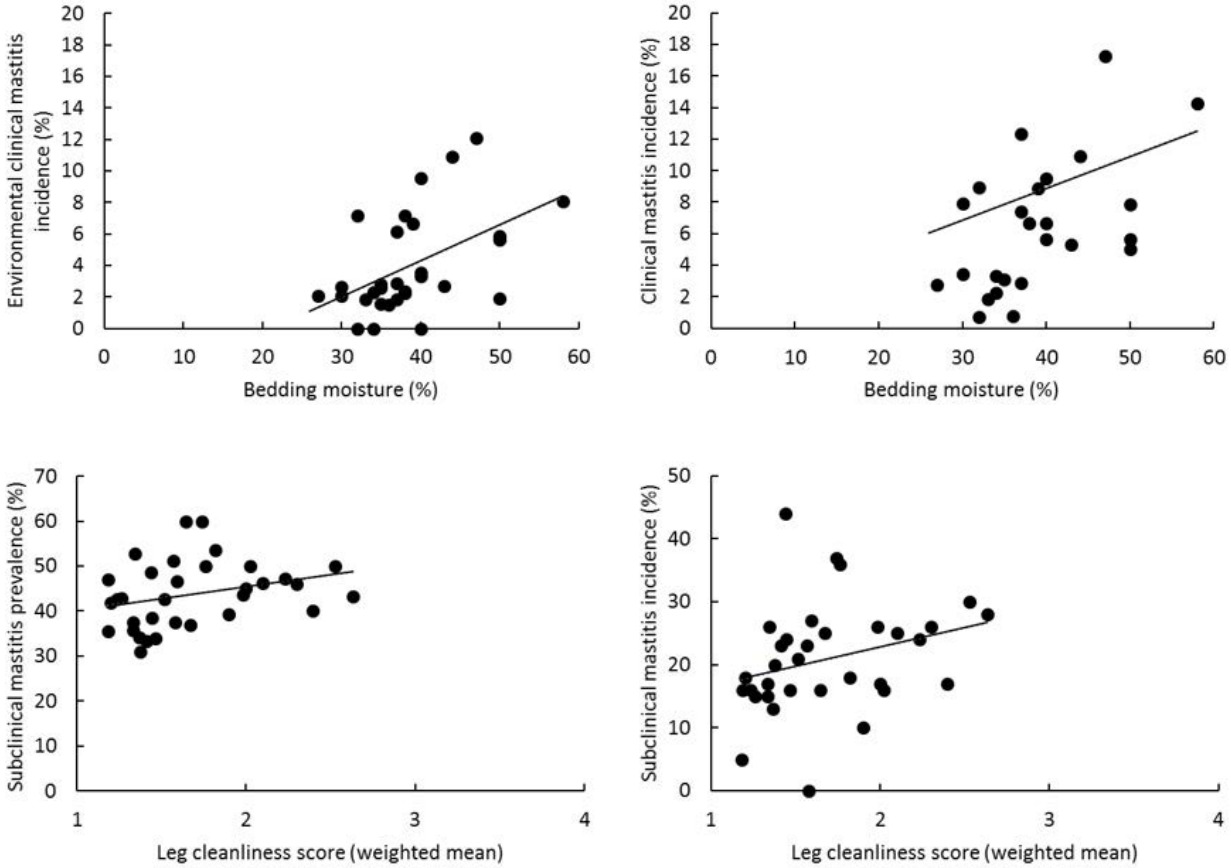

Figure 3.2. Associations between mastitis epidemiologic outcomes and bedding moisture or leg cleanliness score. Incidence of clinical mastitis was defined as the number of cows who experienced mastitis during a one-month period, divided by the number of lactating cows in the herd at the DHI test day. Prevalence of subclinical mastitis was defined as the percentage of cows with DHI SCC > 200000 cells/mL at a given test day. Incidence of subclinical mastitis was defined as the number of cows whose SCC increased from < 200000 to ≥ 200000 cells/mL on 2 consecutive test days (cows that became infected), divided by the number of cows whose SCC was < 200000 cells/mL on the previous test day (cows at risk). Results of cleanliness scoring were reported as weighted means, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

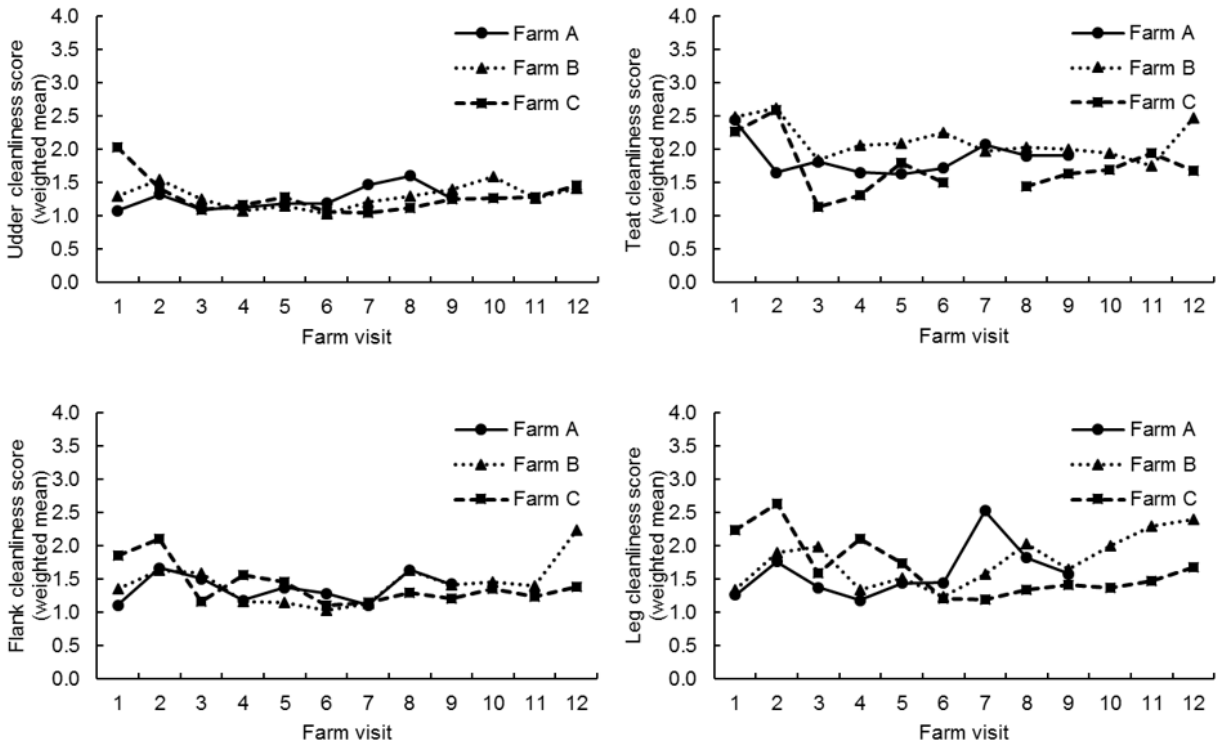

Figure 3.3. Monthly udder, teat, flank and leg cleanliness scores, by farm. Cleanliness scoring was performed before milking, within the CBP area, sampling 100, 100, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty).

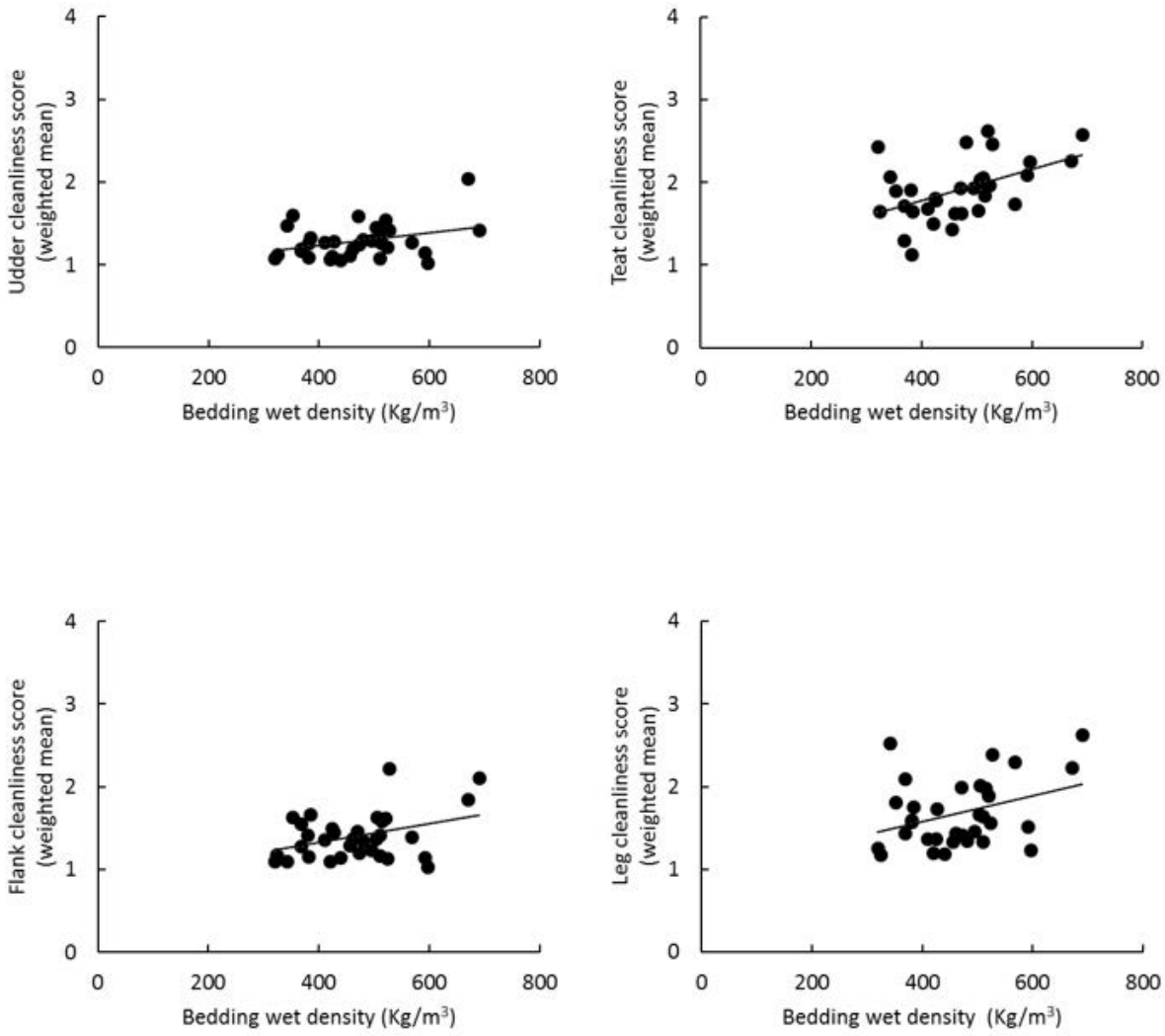

Figure 3.4. Associations between bedding wet density and cow cleanliness scores. Cleanliness scoring was performed before milking, within the CBP area, sampling 100, 100, and 50% of the lactating cows of farms A, B, and C, respectively. Results were reported as weighted mean score, where weights were 1 (clean), 2 (slightly dirty), 3 (dirty), or 4 (very dirty). Wet density was estimated using composite bedding samples from 12 areas of the CBP, collected from the surface and deep (20 cm) layers.

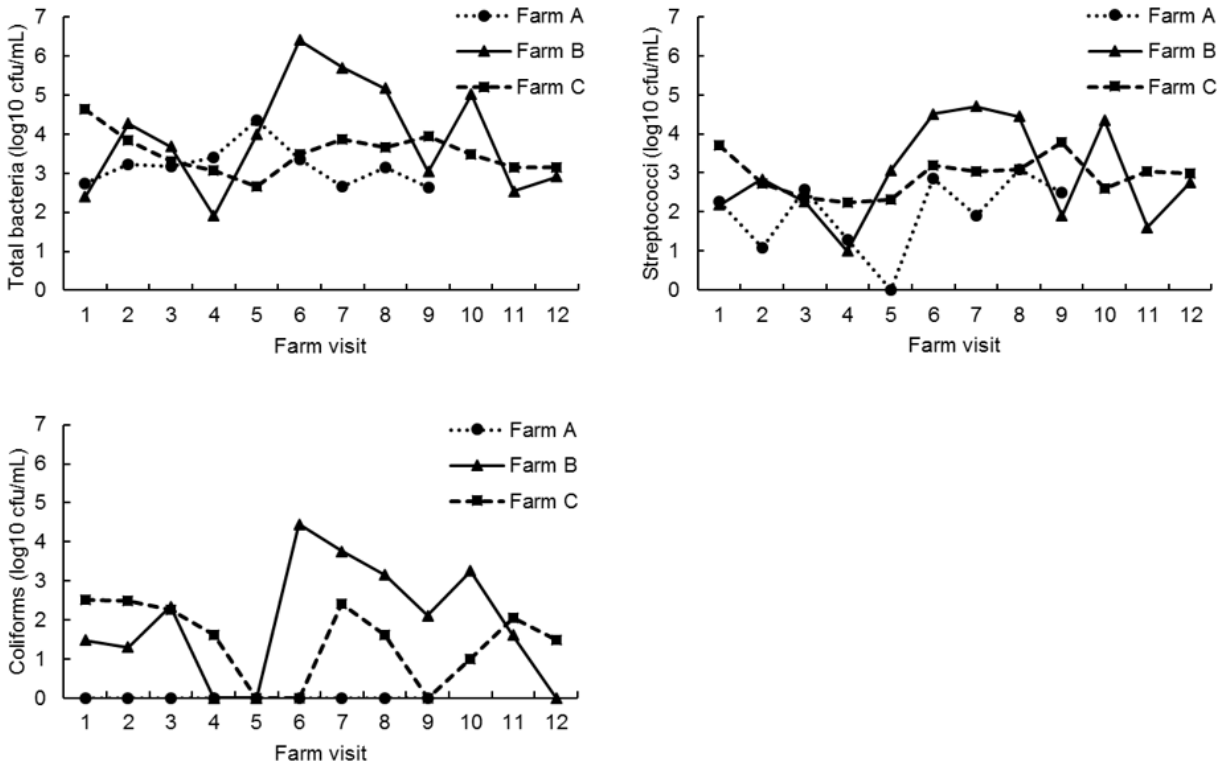

Figure 3.5. Monthly bulk milk concentration of total bacteria, streptococci, and coliforms by farm

CAPÍTULO 4

DISCUSSÃO GERAL

O uso do sistema CBP para o confinamento de vacas leiteiras é recente e tem crescido rapidamente ao redor do mundo. Produtores que adotaram o sistema relataram que vantagens quando comparados a outros sistemas tradicionais, como o freestall, foram melhora nas condições de conforto e produtividade animal, redução do custo de instalações, diminuição na quantidade de dejetos animais eliminados no meio ambiente e a obtenção de um produto final (semi-composto) que pode ser utilizado na agricultura. Em um contexto atual, no qual o bem estar animal é cada vez mais enfatizado, e o desenvolvimento da pecuária leiteira tem sido limitado pelo seu impacto ambiental, o CBP seria uma alternativa de interesse aos sistemas tradicionais de criação de gado leiteiro. No Brasil, o sistema seria uma alternativa especialmente para substituição do sistema de semi-confinamento, no qual os animais permanecem geralmente em condições inadequadas de higiene e conforto.

Entretanto, o fato da cama orgânica usada no CBP conter altas concentrações de microrganismos tem gerado preocupações relativas ao risco de doenças aos animais mantidos no sistema. Dessa forma, o presente estudo oferece uma contribuição relevante ao processo de avaliação da bioseguridade do sistema.

Como em outros estudos realizados na América do Norte, verificou-se que as temperaturas mais elevadas na camada profunda da cama não foram suficientes para esterelizar o material ou diminuir substancialmente a concentração de bactérias na cama. Uma alta concentração bacteriana foi constantemente observada ao longo do estudo na superfície da cama. Resultados desse estudo sugerem que ocorre um processo de semi-compostagem da cama, diferente de um processo de compostagem tradicional.

Nessas condições, é importante identificar características da cama que possam ser manejadas em nível de fazenda, voltadas a melhora da higiene animal e saúde da glândula mamária. Por meio da análise dos dados coletados na cama foram identificados parâmetros como a matéria orgânica, densidade seca e relação

C/N que foram associadas a concentração de bactérias na superfície da cama. Estudos complementares serão importantes para validar práticas de manejo que controlem esses parâmetros e diminuam a concentração das bactérias patogênicas na superfície da cama.

Mesmo com a alta concentração de patógenos causadores de mastite ambiental na superfície da cama (principalmente coliformes e estreptococos), a distribuição de patógenos isolados de casos de mastite clínica e subclínica foi similar àquelas reportadas anteriormente em diferentes sistemas de criação e regiões do mundo. Adicionalmente, não foram identificados surtos de mastite causados por patógenos ambientais refratários ao tratamento (tais como *Nocardia* spp, *Pseudomonas* spp e *Prototheca* spp) durante o decorrer da pesquisa.

Foram correlacionados positivamente a umidade da cama com a incidência de mastite clínica e clínica ambiental, e escores de higiene de perna com a incidência e prevalência de mastites subclínicas. Dessa forma, devem ser adotados medidas de manejo que visem a manutenção da cama seca e aerada (como o uso de ventiladores de teto e cultivo adequado), e pouco aderente ao corpo dos animais.

Os animais apresentaram pouca sujidade no decorrer do estudo, e o parâmetro da cama mais significantemente correlacionado positivamente aos escores de higiene foi a densidade úmida. Dessa forma medidas de manejo que evitem a compactação e umedecimento da cama resultarão em animais mais limpos e com menor risco de mastite.

Nas fazendas do estudo, com exceção de problemas pontuais como dificuldade de limpeza do equipamento de ordenha, a concentração média de bactérias totais permaneceu ao longo do estudo abaixo dos limites máximos especificados pela legislação brasileira e de outros países como Estados Unidos, Canadá e Europa. As concentrações de estreptococos e coliformes se mantiveram dentro de padrões internacionais preconizados para o leite não pasteurizado.

A densidade úmida da cama foi associada positivamente à concentração de bactérias totais no leite, como também ocorreu com os escores de higiene. Dessa forma, vacas e tetas permaneceram em ótimas condições de higiene no sistema, o

que provavelmente tornou mais eficiente o trabalho de higienização das tetas realizado pelos ordenadores.

Como resultado desse estudo exploratório, várias hipóteses científicas puderam ser geradas. É importante definir a dinâmica entre as populações bacterianas da cama em compostagem. Ainda não é claro se práticas de manejo visando a diminuição da população bacteriana da cama seriam indicadas, pois o papel das bactérias não patogênicas no controle de patógenos ainda não é definido.

Embora a cama contenha altas concentrações de bactéria, características da cama como a umidade e densidade parecem influenciar a transferência efetiva de patógenos da cama para a pele das tetas. Portanto, fatores que influenciam tais mecanismos de transferência precisam ser estudados.

Estudos adicionais são necessários para estabelecer a concentração de nutrientes desse composto que estão disponíveis aos vegetais e se esse material está pronto para ser usado ou deve ser submetido a outros processos de compostagem antes de sua utilização na agricultura. Isso será importante para a valorização do material e viabilidade econômica do sistema.

CONCLUSÕES GERAIS

Resultados do presente estudo indicam que a matéria orgânica, densidade e C/N foram associados às concentrações bacterianas estudadas. Temperaturas observadas na camada profunda da cama foram maiores do que aquelas medidas na superfície. Entretanto, as temperaturas encontradas não foram suficientemente altas para minimizar as concentrações bacterianas na superfície da cama.

De acordo com conhecimentos prévios de compostagem tradicional, o envelhecimento da cama foi caracterizado por mudanças em várias características. Nas fazendas que utilizavam camas a base de madeira, umidade, C/N, matéria orgânica decresceram ao longo do tempo, enquanto a densidade e o pH aumentaram.

Coliformes e estreptococos ambientais foram os patógenos mais frequentemente isolados de casos de mastite clínica e subclínica. A prevalência de patógenos ambientais refratários ao tratamento (tais como *Nocardia* spp, *Pseudomonas* spp e *Prototheca* spp) não foi preocupante e surtos causados por mastites ambientais não foram observados.

Os resultados indicam que a manutenção da cama com baixos níveis de umidade e densidade úmida resulta em diminuição do risco de mastite ambiental e melhora nas condições de higiene dos animais, respectivamente. A avaliação dos escores de limpeza animal é uma ferramenta útil para determinar o manejo da cama e avaliar o risco de mastite.

BIBLIOGRAFIA

BARBERG, A.E.; ENDRES, M.I.; JANNI, K.A. Compost dairy barns in Minnesota: A descriptive study. Appl. Eng. Agric., v.23, n.2, p.231-238, 2007a.

BARBERG, A.E.; ENDRES, M.I.; SALFER, J.A.; RENEAU, J.K. Performance and welfare of dairy cows in an alternative housing system in Minnesota. J. Dairy Sci., v.90, n.3, p.1575-1583, 2007b.

BERRY, E.A. Mastitis incidence in straw yards and cubicles. Vet. Rec., v.142, n.19, p.517-518, 1998.

BICKERT, W.G.; LIGHT, R.G. Housing systems. J. Dairy Sci., v.65, n.3, p.502-508, 1982.

BLACK, R.A. Compost Bedded Pack Barns: Management Practices and Economic Implications. 2013. 206f. Dissertação (MESTRADO) – Animal and Food Science, University of Kentucky, Kentucky.

BLACK, R.A.; TARABA, J.L.; DAY, G.B.; DAMASCENO, F.A.; NEWMAN, M.C.; AKERS, K.A.; WOOD, C.L.; MCQUERRY, K.J.; BEWLEW, J.M. The relationship between compost bedded pack performance, management, and bacterial counts. J. Dairy Sci., v.97, p.1-11, 2014.

FREGONESI, J.A.; LEAVER, J.D. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livest. Prod. Sci., v.68, n.2-3, p.205-216, 2001.

GALAMA, P.J.; BOKMA, S.; H. DOOREN, J.van; OUWELTJES, W.; SMITS, M.; DRIEHUIS, F.van. Prospects for bedded pack barns for dairy cattle. Lelystad, The Netherlands: Wageningen UR Livestock Research, 2011. 71p.

HOGAN, J.S.; SMITH, K.L. Bacteria counts in sawdust bedding. J. Dairy Sci., v.80, n.8, p.1600-1605, 1997.

HOGAN, J.S.; SMITH; K.L.; HOBLET, K.H.; TODHUNTER, D.A.; SCHOENBERGER, P.S.; HUESTON, W.D.; PRITCHARD, D.E.; BOWMAN, G.L.; HEIDER, L.E.; BROCKETT, B.L. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci., v.72, n.1, p.250-258, 1989.

JANNI, K.A.; ENDRES, M.I.; RENEAU, J.K.; SCHOPER, W.W. Compost dairy barn layout and management recommendations. Appl. Eng. Agric., v.23, n.1, p.97-102, 2007.

JOBIM, M.B.; LOPES, M.A.; COSTA, G.M.D; DEMEU, F.A. Pathogens associated with bovine mastitis in dairy herds in the south region of Brazil. Bol. Ind. Anim., v.67, p.175-181, 2010.

KADER, N.A.E.; ROBIN, P.; PAILLAT, J.M.; LETERME, P. Turning, compacting and the addition of water as factors affecting gaseous emissions in farm manure composting. Bioresour. Technol., v.98, n.14, p.2619-2628, 2007.

KAMMEL, D.W. Design and maintenance of a bedded pen (Pack) housing system. In: MIDWEST HERD HEALTH CONFERENCE. Dairy Team UW Extension. UW Madison. 2004.

KIEHL, E.J. Compostagem. In: Fertilizantes orgânicos. Piracicaba: Editora Agronômica Ceres, 1985. Chap.7, p.229-310.

LOBECK, K.M.; ENDRES, M.I.; SHANE, E.M.; GODDEN, S.M.; FETROW, J. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. J. Dairy Sci., v.94, n.11, p.:5469-5479, 2011.

LOPEZ-BENAVIDES, M.G.; WILLIAMSON, J.H.; PULLINGER, G.D.; LACY-HULBERT, S.J.; CURSONS, R.T.; LEIGH, J.A. Field observations on the variation of Streptococcus uberis populations in a pasture-based dairy farm. J. Dairy Sci., v.90, n.12, p.5558-5566, 2007.

MISRA, R.V.; ROY, R.N.; HIRAOKA, H. FOOD AND AGRICULTURE ORGANIZATION, UNITED NATIONS. On-farm composting methods. Rome, Italy, 2003. 26p.

NEAVE, F.K.; DODD, F.H.; KINGWILL, R.G.; WESTGARTH, D.R. Control of mastitis in the dairy herd by hygiene and management. J. Dairy Sci., v.52, n.5, p.696-707, 1969.

NORTHEAST RESOURCE, AGRICULTURE AND ENGINEERING SERVICE (NRAES). On-farm composting handbook. NRAES-54. Ithaca, NY, 1992. 187p.

PEELER, E.J.; GREEN, M.J.; FITZPATRICK, J.L.; MORGAN, K.L.; GREEN, L.E. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. J. Dairy Sci., v.83, n.11, p.2464-2472, 2000.

RENDOS, J. J.; EBERHART, R. J.; KESLER, E. M. Microbial-populations of teat ends of dairy-cows, and bedding materials. J. Dairy Sci. v.58, p1492-1500, 1975.

RENEAU, J.K.; SEYKORA, A.J.; HEINS, B.J.; ENDRES, M.I.; FARNSWORTH, R.J.; BEY, R.F. Association between hygiene scores and somatic cell scores in dairy cattle. J. Am. Vet. Med. Assoc., v.227, n.8, p.1297-1301, 2005.

RODRIGUES, A.C.O.; CARAVIELLO, D.Z.; RUEGG, P.L. Management of Wisconsin dairy herds enrolled in milk quality teams. J. Dairy Sci., v.88, n.2660-2651, 2005.

RUEGG, P.L. New Perspectives in Udder Health Management. Vet. Clin. North Am. Food Anim. Pract., v.28, p149-163, 2012.

RUSSELLE, M.P.; BLANCHET, K.M.; RANDALL, G.W.; EVERETT, L.A. Characteristics and nitrogen value of stratified bedded pack dairy manure. Crop. Mgmt. 2009. Disponível em: < http://naldc.nal.usda.gov/naldc/download.xhtml?id=46651&content=PDF>. Acesso em: 12 jan. 2015.

SCHREINER, D.A.; RUEGG, P.L. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci., v.86, n.11, p.3460-3465, 2003.

SCHUKKEN, Y.; LESLIE, K.; WEERSINK, A.; MARTIN, S. Ontario Bulk Milk Somatic Cell Count Reduction Program. 1. Impact on Somatic Cell Counts and Milk Quality. J. Dairy Sci., v.75, p.3352-3358, 1992.

SHANE, E.M.; ENDRES; M.I.; JANNI, K.A. Alternative bedding materials for compost bedded pack barns in Minnesota: A descriptive study. Appl. Eng. Agric., v.26, n.3, p.465, 2010.

SVENNESEN, L.; ENEVOLDSEN, C.; BJERG, B.S.; KLAAS, I.C. Udder health in a Danish compost bedded pack barn. In: NMC REGIONAL MEETING, 2014, Ghent, Belgium.

WAGNER, P.E. 2002. Bedded pack shelters. Accessed June 4, 2012. http://crbh.psu.edu/das/research-extension/dairy/dairy-digest/articles/beddedpack-shelters

WARD, W.R.; HUGHES, J.W.; FAULL, W.B.; CRIPPS, P.J.; SUTHERLAND, J.P.; SUTHERST, J.E. Observational study of temperature, moisture, pH and bacteria in

straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. Vet. Rec., v.151, n.7, p.199-206, 2002.

WENZ, J.R.; JENSEN, S.M.; LOMBARD, J.E.; WAGNER, B.A.; DINSMORE, R.P. Herd management practices and their association with bulk tank somatic cell count on united states dairy operations. J. Dairy Sci., v.90, p.3652-3659, 2007.

ZDANOWICZ, M.; SHELFORD, J.A.; TUCKER; C.B.; WEARY, D.M.; KEYSERLINGK, M. A. G. von. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci., v.87, p.1694-1701, 2004.

ANEXO I – Normas de publicação da Livestock Science