UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

ACESSO À INFORMAÇÃO SOBRE BOAS PRÁTICAS DE BEM-ESTAR ANIMAL: EFEITOS SOBRE A QUALIDADE DO MANEJO, TEMPERAMENTO E BEM-ESTAR DE BOVINOS DE CORTE

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Zootecnia.

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Jaboticabal, 02 de outubro de 2017

DADOS CURRICULARES DA AUTORA

MARIA CAMILA CEBALLOS BETANCOURT – Nascida em 01 de novembro de 1986 na cidade de Medellín – Antioquia – Colômbia. Formada em Zootecnia pela Universidad Nacional de Colombia – Sede Medellín no ano de 2010. De 2010 a 2012 trabalhou como pesquisadora no grupo de pesquisa Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV) na Colômbia, sendo bolsista do programa Jóvenes Investigadores de COLCIENCIAS. Em março de 2012 ingressou no curso de Mestrado do Programa de Pós-Graduação em Zootecnia na Universidade Estadual Paulista – Campus de Jaboticabal sob orientação do Prof. Dr. Mateus José Rodrigues Paranhos da Costa. Nesta mesma data se fez integrante do Grupo de Estudos e Pesquisas em Etologia e Ecologia Animal (ETCO). Em março de 2014, ingressou no Programa de Doutorado em Zootecnia (FCAV/UNESP), Campus Jaboticabal, com ênfase em Ecologia e Etologia animal, na qual foi orientada também pelo prof. Dr. Mateus Paranhos da Costa. Realizou estágio no Animal Welfare Science Centre (AWSC) – University of Melbourne - Austrália, sob orientação do Professor Paul Hamilton Hemsworth, exercendo atividades de pesquisas desenvolvidas pelo AWSC, recebendo a bolsa "Programa de Doutorado Sanduíche no Exterior" (PDSE) da CAPES. Sua atuação profissional é, principalmente, nas áreas de etologia aplicada, relação humano-animal e bem-estar dos animais domésticos.

"Compassion for animals is intimately connected with goodness of character, and it may be confidently asserted that he, who is cruel to living creatures, cannot be a good man."

Trecho do livro -On the basis of morality-

Arthur Schopenhauer, 1840

Dedico esta tese à minha avó Rosa, que tem participado da minha educação e do meu crescimento pessoal e profissional desde a minha infância, estando sempre comigo, me incentivando sempre a continuar melhorando a cada dia. Dedico também aos meus pais, Magaly e Jaime, por serem meu porto seguro cada segundo da minha existência, por me apoiarem em todas as minhas escolhas e sempre me incentivarem a seguir meus sonhos e a minha felicidade.

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SUMÁRIO

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Certificamos que o Protocolo nº 014198/14 do trabalho de pesquisa intitulado **"Acesso à informação sobre boas práticas de bem-estar animal: Efeitos sobre a qualidade do manejo, temperamento e bem-estar de bovinos de corte"**, sob a responsabilidade do Prof. Dr. Mateus José Rodrigues Paranhos da Costa está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 12 de agosto de 2014.

Jaboticabal, 12 de agosto de 2014.

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ACESSO À INFORMAÇÃO SOBRE BOAS PRÁTICAS DE BEM-ESTAR ANIMAL: EFEITOS SOBRE A QUALIDADE DO MANEJO, TEMPERAMENTO E BEM-ESTAR DE BOVINOS DE CORTE

RESUMO – O objetivo desta tese foi avaliar os efeitos do acesso de vaqueiros à informação sobre boas práticas de manejo de gado de corte na a qualidade do manejo, o temperamento e o bem-estar dos bovinos. Os objetivos específicos foram: 1) Contribuir para uma maior compreensão da relação entre a interação humano-animal, temperamento, resposta ao estresse e performance reprodutiva de novilhas nelore submetidas ao protocolo de inseminação artificial em tempo fixo; 2) Avaliar o potencial impacto do treinamento de vaqueiros em boas práticas de manejo sobre as atitudes e a qualidade de manejo dos mesmos e o bem-estar do gado; 3) Comparar fazendas onde todos os vaqueiros foram treinados com fazendas onde alguns foram treinados e fazendas onde ninguém foi treinado e; 4) Estudar se a qualidade do manejo do gado deteriora ao longo de um dia de trabalho. Para o primeiro objetivo específico, um total de 571 novilhas foram usadas para avaliar dois indicadores de temperamento (a velocidade de saída - VS e o escore composto de reatividade REA), cinco indicadores de interação humano animal (manejo negativo - MN, acidentes - ACIDENTES, defecação e micção – DEF-MIC, comportamentos indesejados - CI e tempo de entrada - TE) e a sujidade na região perineal - SUJIDADE. Todos esses indicadores foram avaliados no dia d0, d7 e d11 de um protocolo de inseminação a tempo fixo. Adicionalmente, dois indicadores de estresse (cortisol – CORT e relação neutrófilo: linfócito – N:L) foram avaliados em uma sub-amostra de 99 novilhas no dia d0 e d11 do protocolo. A ocorrência de MN foi associado com maiores graus de VS, REA, CI, DEF-MIC E SUJIDADE ($P < 0.05$). Novilhas mais reativas mostraram CI, DEF-MIC e ACIDENTES, e maiores concentrações de CORT e relação N:L ($P < 0.05$). Novilhas mais sujas na região perineal tiveram menor probabilidade de ficarem prenhas quando comparadas com as mais limpas ($\chi^2 = 6.75$; $P < 0.05$). O manejo negativo resulta em animais mais reativos, incrementando a expressão de comportamentos indesejados e o risco de acidentes nos animais, tornando o manejo mais difícil e arriscado. Novilhas mais reativas são mais sujas na região perineal, o que esteve associado com menor taxa de prenhes. O segundo estudo foi realizado em 24 fazendas comerciais de gado de corte, classificadas em três grupos, segundo o acesso ao treinamento dos vaqueiros: 1) regularmente treinadas, 2) ocasionalmente treinadas e, 3) não treinadas. Um total de 150 vaqueiros foram categorizados segundo ao seu treinamento como: i) treinados, ii) não treinados mas trabalham com alguém treinado e, iii) sem treinamento. Os indicadores de qualidade de manejo (incluindo comportamento dos animais, atitudes e comportamentos dos vaqueiros) foram medidos durante um dia de manejo de vacinação. Observamos que as fazendas sem nenhum tipo de treinamento teve a pior qualidade de manejo assim como comportamento dos animais durante o manejo ($P < 0.05$) quando comparada com os outros grupos de fazendas. Vaqueiros que foram treinados tiveram melhores comportamentos e atitudes perante o gado, quando comparados com vaqueiros não treinados. Observamos também que a medida que vai passando o tempo durante o dia de trabalho nas fazendas sem treinamento, o manejo se torna pior. Concluímos que treinar os vaqueiros em boas práticas de manejo de gado promove melhorias

nas suas atitudes e comportamentos perante o gado. Assim, programas de treinamento de vaqueiros que lidan diariamente com os animais são uma boa estrategia para promover interações humano-animal positivas, melhorando a qualidade de vida dos animais e vaqueiros.

Palavras chave: Comportamento do gado, fisiologia do estresse, manejo do gado, relação humano-animal.

ACESS TO INFORMATION ABOUT GOOD PRACTICES OF ANIMAL WELFARE: EFFECTS ON THE QUALITY OF HANDLING, BEEF CATTLE TEMPERAMENT AND WELFARE

ABSTRACT – The objective of this thesis was to assess the effects of the stockpeople access to information about good beef cattle handling practices on the quality of handling, cattle temperament and welfare. The specific objectives were: 1) To further contribute to the understanding of the relationships between human-animal interactions, temperament, stress response, and reproductive performance of Nellore heifers submitted to a FTAI protocol; 2) To evaluate the potential impacts of handling skills training on good practices of cattle handling on stockpeople' attitudes and behavior, and cattle welfare; 3) To compare farms where all stockpeople were trained, to farms where only some where trained, and farms where none where trained and, 4) To study whether the quality of cattle handling deteriorates as the working day time passes. For the first specific objective, a sample of 571 heifers was used to assess two temperament traits (flight speed - FS and the composite reactivity score - RS), five human-animal interaction traits (negative handling - NH, Accidents - ACCIDENT, Defecation-urination - DEF-URI, Undesirable behavior – UB and Entrance time – ET), and perineal region dirtiness – DIRTINESS. All variables were assessed on d0, d7 and d11 of a FTAI protocol. Additionally, two physiological indicators of stress (cortisol - CORT and neutrophil:lymphocyte ratio - N:L) were recorded in a subsample of 99 heifers on d0 and d11. The occurrence of NH was associated with the occurrence of ACCIDENTS and with higher grades of FS, RS, UB, DEF-URI, and DIRTINESS ($P < 0.05$). Excitable heifers showed more UB, DEF-URI and ACCIDENT, with highest CORT concentrations and N:L ratios ($P < 0.05$). Heifers with dirtier perineal region had lower chances of getting pregnant than cleaner ones ($\chi^2 = 6.75$; $P < 0.05$). Poor quality of handling often results in more reactive animals as well as more undesirable behaviors and an increased risk of accidents. More reactive heifers were dirtier in the perineal region, which in turn was associated with lower pregnancy rates. The second study was conducted in 24 commercial beef cattle farms, classified in three groups, determined by formal handling skills training, provided to the stockpeople: 1) regularly trained, 2) occasionally trained and, 3) never trained. A total of 150 stockpeople working on these farms were categorized according to training, as follow: i) Trained (TS); ii) Non-trained, but had close contact with a trained stockperson (CTS); and iii) Non-trained (NT). Indicators of quality of handling (including animals and stockpeople behaviors, as well as stockpeople attitudes) were measured during one workday, when carrying on vaccination handling procedures. We observed that non-trained farms had the poorest quality of handling, as well animal behaviors during handling ($P < 0.05$) when compared with the other farm categories. People who participated in a formal training course (TS) had the highest positive and the lowest negative behaviors' and attitudes scores, compared with the others (CTS and NT). We also observed an effect of the working day progresses within a day ($P < 0.05$) only on non-trained farms, where handling became worse over time. We conclude that training stockpeople on good cattle handling practices led to better attitudes and behaviors toward cattle. Thus, training the stockpeople can be an effective and practical strategy to promote positive

human-animal interaction in beef cattle farms, improving the quality of life of animals and workers.

Keywords: Cattle behavior, stress physiology, cattle handling, human-animal relationship

LISTA DE ABREVIATURAS

CAPÍTULO 2. Investigating the relationship between human-animal interactions, temperament, stress response and reproductive performance in Nellore heifers

FTAI: fixed-time artificial insemination
FS: flight speed
RS: composite reactivity score
NH: Negative handling
ACCIDENT: accidents
DEF-URI: defecation-urination
UB: undesirable behavior
ET: entrance time
DIRTINESS: perineal region dirtiness
CORT: cortisol concentrations
N:L: neutrophil:lymphocyte ratio
MCA: multiple correspondence analyses
BSC: body condition score

CAPÍTULO 3. Impact of training in good practices handling on beef cattle welfare and stockpeople attitudes and behaviors

TRAINED-R: farms with systematic training programs.
TRAINED-O: farms where at least one of the permanent employees attended to a formal training on good practices of beef cattle handling.
NON-TRAINED: farms where none of the workers received any training on good practices of beef cattle handling.
TS: trained stockpeople.
CTS: non-trained but had close contact with a trained stockperson.
NT: non-trained stockperson.
UBA: undesirable behaviors and accidents.
PB: stockpeople positive behaviors.
NB: stockpeople negative behaviors.
GATE: operation of the front gate of the squeeze chute.
RESTRAIN: frequencies of stockperson attempt to restraint each animal with the head bail.
REST.HB: speed and force used by the stockperson when restraining each animal with the head bail.
F.VACCINATION: failures in vaccination attempt.
SITE: wrong injection site.
PQI: poor quality of subcutaneous injection.
ESCAPE: frequency of animals' attempts to takes the head or the body out from the lateral window or gate of the squeeze chute during the handling at the squeeze chute.
PCA: principal components analyses.

CAPÍTULO 1 – Considerações Gerais

1. INTRODUÇÃO

O bem-estar animal, definido como o estado de um indivíduo em relação as suas tentativas de se ajustar ao ambiente (BROOM, 1986), é um tema complexo com implicações científicas, éticas, econômicas, culturais, sociais, religiosas e políticas (OIE, 2015), sendo de grande importância para a produção animal. Em 2012, a Organização Mundial de Saúde Animal (OIE) estabeleceu 10 princípios gerais para orientar o desenvolvimento de normas de bem-estar dos animais de produção onde, dentre eles, estão aqueles relacionados com o comportamento animal, assim como o estabelecimento de interação humano-animal positiva e a garantia de habilidade e conhecimento adequado aos trabalhadores rurais que lidam com animais (FRASER et al., 2013).

Estudos avaliando a influência da interação humano animal no bem-estar dos animais de produção tem demonstrado que este é um dos principais fatores que podem afetá-lo (RUSHEN et al., 1999; HONORATO et al., 2012; PROBST et al., 2012). Segundo Raussi (2003), a natureza das interações entre humanos e animais depende de fatores inerentes aos animais, relacionados às características dos humanos e também ao ambiente de criação e de trabalho. Esses elementos, em conjunto, determinam a qualidade do manejo dos bovinos nas fazendas, o que tem efeito direto na definição do temperamento dos mesmos (GRANDIN, 1993) e, consequentemente, interferem na produtividade e no bem-estar dos animais (GRANDIN, 2000; HOPPE et al., 2010; COOKE et al., 2011).

O temperamento é uma característica, bastante complexa, e de grande importância para a cadeia produtiva de bovinos, afinal animais de temperamento mais excitável podem apresentar reações extremas durante os manejos na fazenda, no transporte e nos momentos prévios ao abate, com efeitos importantes na eficiência durante a realização do trabalho, no desempenho dos animais e na qualidade das carcaças e da carne (FORDYCE et al., 1985; BOISSY, BOUISSOU, 1995; PARANHOS DA COSTA et al., 2002; HÖTZEL et al., 2004). Por exemplo,

os resultados obtidos nos estudos de Cooke et al. (2011) e Rueda et al. (2015) mostraram que vacas de temperamento mais excitável apresentam pior taxa de concepção (35 e 42%, respectivamente) quando submetidas a um protocolo de inseminação artificial em tempo fixo (IATF). Além disso, bovinos com temperamento ruim resultam ainda em reações negativas dos manejadores que, consequentemente, agravam a condição de medo dos animais comprometendo o bem-estar dos mesmos (HEMSWORTH, 2003; COLEMAN; HEMSWORTH, 2014). Vários estudos demonstram a existência de uma relação direta entre a produção animal, interações negativas e medo dos seres humanos (RUSHEN et al., 1999; BREUER et al., 2000; BOIVIN et al., 2003; WAIBLINGER et al., 2002; HEMSWORTH, 2003; ZULKIFLI, 2013).

A atitude e o comportamento dos vaqueiros têm um papel fundamental na redução do medo dos animais (HEMSWORTH, 2007), podendo promover a melhoria do temperamento dos mesmos, quando este é realizado de forma adequada. O comportamento dos trabalhadores com relação aos animais podem ser melhorados através da seleção criteriosa de pessoas ou com o desenvolvimento de programas de treinamento planejados para diminuir o estresse no manejo e melhorar o bem-estar dos animais (BOIVIN et al., 2007). A identificação das características humanas que interferem o desempenho no trabalho pode ter um valor prático para a seleção dos trabalhadores rurais e também na identificação de pessoas experientes e inexperientes que requeiram treinamento (HEMSWORTH, 2003). Por exemplo, estudos realizados em sistemas de produção de bovinos leiteiros e de suínos demonstraram o potencial das intervenções educativas com técnicas comportamentais e cognitivas desenvolvidas especificamente com o objetivo de melhorar as atitudes e, como consequência, o comportamento dos manejadores perante os animais (COLEMAN et al., 2000; HEMSWORTH et al., 2002; COLEMAN; HEMSWORTH, 2014).

É evidente que treinamentos são necessários para capacitar os trabalhadores rurais encarregados do manejo do gado nos sistemas de produção brasileiros, especialmente porque, apenas recentemente, a atividade de vaqueiro

foi reconhecida como profissão no Brasil pela Lei nº 12.870, de 15 de Outubro de 2013 (BRASIL, 2013). No entanto, no Brasil ainda não foram realizados estudos sistemáticos a fim de comprovar o impacto do acesso ao conhecimento a respeito do manejo racional de bovinos de corte sobre as atitudes dos vaqueiros, bem como suas consequências na qualidade do manejo, no comportamento animal e no bem-estar dos bovinos e dos manejadores. Assim, com os resultados do presente estudo espera-se revelar, de forma mais ampla, o alcance e os benefícios da capacitação formal dos vaqueiros, estimulando instituições públicas ou privadas a investirem em treinamento e capacitação dos funcionários que lidam com animais.

Por fim, esperamos que as informações obtidas nesse estudo possam embasar o desenvolvimento de materiais para treinamentos em manejo racional de bovinos, tonando mais eficiente a forma com que os profissionais ligados à pecuária de corte são capacitados nas fazendas.

2. REVISÃO DE LITERATURA

2.1. Bem-estar na produção animal

O bem-estar animal é um tema complexo e multifacetado com implicações científicas, éticas, econômicas, culturais, sociais, religiosas e políticas (OIE, 2015), sendo de grande importância para a área da produção animal. Nas últimas décadas tem acontecido um pronunciado aumento no interesse do público em geral com relação aos animais, tanto em questões morais quanto científicas.

No ano de 1964, Ruth Harrison, com seu livro "*Animal Machines*", foi a primeira a chamar atenção da população com respeito à produção e intensificação animal, assinalando que aqueles que estavam relacionados com esta indústria tratavam os animais como máquinas inertes, ao invés de indivíduos vivos (VAN DE WEERD; SANDILANDS, 2008). Como consequência disso, o Parlamento Britânico estabeleceu o "Comitê de Brambell", dirigido pelo professor F. Rogers Brambell no ano de 1965. Também fazia parte deste comitê o etólogo da

Universidade de Cambridge W.H Thorpe, quem enfatizou a importância em compreender a biologia e as necessidades dos animais para que fosse possível melhorar o bem-estar dos mesmos (BROOM, 2011). Esta proposta ficou registrada em um relatório onde foram apresentadas as primeiras diretrizes identificadas como condições mínimas que deviam ser asseguradas para que os animais de produção estivessem em boas condições, conhecidas como as "5 Liberdades de Brambell" (Conklin, 2014), sendo elas: assegurar que os animais tivessem liberdade para virar-se, deitar-se, levantar-se, estirar seus membros e cuidar do seu próprio corpo (BRAMBELL, 1965). Estes princípios foram posteriormente reformulados, em 1979, pelo Conselho para o Bem-Estar dos Animais de Produção (em inglês, *Farm Animal Welfare Council,* FAWC), as quais propõem que o animal deve estar livre de fome e sede; livre de desconforto; livre de dor, injúria e doença; livre de medo e estresse; e livre para expressar seus comportamentos naturais (FAWC, 1992). No entanto, o conceito de bem-estar animal baseado nas 5 liberdades promove algumas dificuldades de aplicação prática, já que é praticamente impossível cumprir algumas das condições nelas propostas. Por exemplo, como garantir que um animal esteja completamente livre de fome e sede, se fisiologicamente é necessário que ele sinta essas sensações para que tenha a motivação por buscar alimento e água? Por esse e outros questionamentos, hoje em dia, recomenda-se utilizar o conceito de necessidades, o qual é chave para compreender o bem-estar dos animais (BROOM, 2011).

O conceito de necessidade foi definido por Fraser e Broom (1990) como uma deficiência que só pode ser atendida pela obtenção de um recurso particular, ou com a apresentação de uma resposta a um estímulo ambiental ou corporal. As necessidades dos animais estão divididas em diferentes domínios, sendo eles: *i)* nutrição, relacionado à privação de água, alimento e má-nutrição; *ii)* meio ambiente, relacionado aos desafios ambientais aos quais os animais estão submetidos; *iii)* saúde, relacionado às doenças, lesões e ao comprometimento funcional que estas podem acarretar; *iv)* comportamento, relacionados com as dificuldade para expressarem seus comportamentos naturais ou com as restrições para estabelecerem interações sociais com outros animais; e finalmente *v)* estado

mental/experiência, relacionado às sensações e sentimentos (ansiedade, medo, dor, diestresse, sede, fome, aborrecimento, dentre outros), que o animal possa experimentar quando mantido em cativeiro (MELLOR, 2004). Deste modo, os primeiros quatro domínios representam os elementos físicos do bem-estar animal e o quinto engloba o elemento mental (MELLOR; STAFFORD, 2001). É de se considerar que um tipo de comprometimento em algum dos quatro primeiros domínios, acarretará, consequentemente, no comprometimento do quinto domínio, em que estão incluídos os componentes de sofrimento (MELLOR, 2004). O entendimento da complexa dinâmica entre os fatores envolvidos no bem-estar dos animais fornece ferramentas para identificar problemas de bem-estar associados aos sistemas de produção. Apesar dos vários desdobramentos relacionados à ciência do bem-estar animal e suas definições, a que continua sendo mais utilizada no âmbito da pesquisa é a clássica definição criada por Broom (1986): o estado de um indivíduo em relação às suas tentativas de se ajustar ao ambiente.

Além da responsabilidade moral com os animais de produção (BROOM, 2006), os fazendeiros e vaqueiros também possuem obrigações legais com relação ao bem-estar dos mesmos. Em vários países já existem legislações que condenam a crueldade com animais (HEMSWORTH, 2007), embora esta seja uma realidade extrema dentro das inúmeras preocupações relacionadas ao bem-estar animal. Além disso, legislações e normativas relacionadas especificamente com o bem-estar animal nos sistemas de produção, e direcionada aos pecuaristas, vem sendo desenvolvidas em alguns países (LUNDMARK et al., 2014) e são cada vez mais exigidas pelos consumidores. Sendo assim, cada vez em mais países, os proprietários e manejadores têm a obrigação legal de promover condição para que os animais supram suas necessidades, proporcionando sistemas de criação e manejos aceitáveis perante à comunidade (HEMSWORTH, 2007).

Em 2012, membros da OIE (Organização Mundial de Saúde Animal) estabeleceram 10 princípios gerais para orientar o desenvolvimento de normas de bem-estar dos animais nos sistemas pecuários de produção. Os oito primeiros princípios compreendem aspectos como alimentação, saúde, ambiente físico onde os animais estão alojados, seleção genética e comportamento. Enquanto que os

dois últimos, mas não menos importantes, estão relacionados à interação entre humanos e animais, são eles: 9 - estabelecimento de interação humano-animal positiva e, 10 - garantia de habilidade e conhecimento adequado aos trabalhadores rurais que lidam com animais (FRASER et al., 2013).

Ao longo dos anos, ao se avaliar diferentes fatores que influenciam o bem-estar dos animais domésticos, tem-se percebido que as preocupações levantadas com mais frequência sobre este tema estão relacionadas com o confinamento e as rotinas dos procedimentos de manejo. Contudo, a relação dos animais com o trabalhador rural tem recebido relativamente pouca atenção do público em geral ou da indústria pecuária, apesar de estudos demostrarem que o cuidado do manejador com o animal tem um grande impacto no bem-estar dos animais de produção (HEMSWORTH, 2007). Por isto, os últimos dois princípios apresentados pela OIE são de grande importância, pois a preocupação em oferecer uma melhor condição de bem-estar animal está diretamente ligada com a melhoria no manejo de rotina das fazendas; visto que, animais em condições confortáveis e expostos a experiências positivas com relação aos humanos tendem a reduzir o medo e, consequentemente, facilitar o manejo (PROBST et al., 2012). É evidenciado em muitos estudos que alguns dos principais fatores que podem influenciar o bem-estar dos animais de fazenda estão relacionados diretamente às práticas de manejo (HEMSWORTH; BARNETT; HANSEN, 1981, 1986; RUSHEN; TAYLOR; DE PASSILLÉ, 1999; HEMSWORTH et al., 2000; HEMSWORTH et al., 2002; BREUER; HEMSWORTH; COLEMAN, 2003; HEMSWORTH et al., 2011; HONORATO et al., 2012; PROBST et al., 2012; HEMSWORTH, 2014; PRUNIER; TALLET, 2015; DOYLE et al., 2016).

Há uma serie de implicações práticas, produto de problemas na relação humano-animal, que influenciam um ou mais dos "cinco domínios do bem-estar animal". Por exemplo, quando o manejo dos animais é realizado de forma agressiva, situação que pode ser caracterizada como um desafio ambiental (domínio dois: ambiente), é esperado um aumento da reatividade dos animais (promovendo uma alteração no domínio quatro: comportamento dos animais) que, por sua vez, resulta em maior dificuldade de manejo, aumentando ainda mais o

desafio ambiental. Esta situação, invariavelmente, resulta em maior estresse e, dependendo da intensidade deste desafio, há maior risco dos animais se ferirem, colocando, portanto, a saúde deles em risco (domínio três: saúde). Tudo isto, em conjunto, aumenta a probabilidade dos animais sentirem medo, dor e desconforto, influenciando, consequentemente, seu estado mental (domínio cinco); na Figura 1 é possível visualizar o exemplo graficamente. Esta situação caracteriza um ciclo negativo, onde aumenta o risco de haver reações negativas por parte dos manejadores que, por conseguinte, agravam a condição de medo dos animais (HEMSWORTH; COLEMAN, 2011), influenciando diretamente na piora do temperamento (PARANHOS DA COSTA et al., 2002).

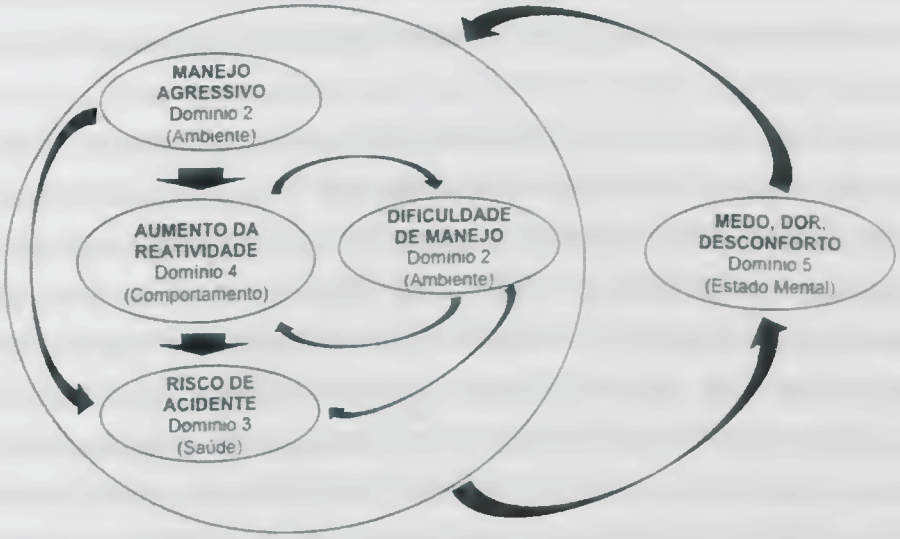

Figura 1. Representação gráfica com enfoque em sistema para analise de algumas das implicações práticas de um manejo ruim e sua influencia nos "cinco domínios do bem-estar animal" conforme proposto por Mellor e Stafford (2001). Adaptado de Paranhos da Costa (2016).

2.2. Temperamento Animal

2.2.1. *Conceito*

O temperamento animal é caracterizado pelas diferenças individuais do comportamento do indivíduo que se expressam no início da vida e são relativamente consistentes ao longo do tempo (BATES, 1989). Estas diferenças individuais podem ser caracterizadas pela expressão de várias reações dos animais, dentre elas: agressividade, tendência de evitar situações novas, disposição para assumir riscos, exploração e sociabilidade; cada qual expressada em um contexto particular (RÉALE et al., 2000; 2007). A grande variedade de características que modulam o temperamento dos bovinos dificulta a definição de uma única medida, que seja capaz de contemplar todos os aspectos do temperamento. Por conta disto, as avaliações são comumente realizadas levando-se em consideração mais de uma característica, já que é praticamente impossível definir uma única forma de medir o temperamento (PARANHOS DA COSTA et al., 2002).

A reatividade é a característica mais utilizada para a avaliar o temperamento dos bovinos, sendo mensurada com base no do nível de reação dos animais a algum tipo de manejo, em particular aos estímulos causados pela presença humana (BOIVIN et al., 1992). De forma a simplificar o conceito de temperamento em animais de produção, alguns autores têm criado definições operacionais como, por exemplo, a resposta a estímulos ambientais ou sociais (HASKELL; SIMM; TURNER, 2014), ou ainda, o conjunto de comportamentos dos animais em relação ao homem, geralmente atribuídos ao medo (FORDYCE; GODDARD; SEIFERT, 1982). Considerando que a variação individual na capacidade de resposta ao desafio reflete nas diferenças entre os animais enquanto à expressão do medo à estímulos ambientais ou sociais (VAN REENEN et al., 2005), as definições operacionais mencionadas anteriormente são de fato práticas e aplicáveis.

2.2.2. *Relação entre temperamento, estresse e produtividade*

Tem sido comprovado que o temperamento ruim de animais afeta negativamente características de importância econômica para o sistema de produção (PETHERICK et al., 2002; CAFE et al., 2011). As reações de bovinos de

temperamento ruim em diversas situações, como por exemplo, durante o manejo na propriedade, no transporte e nos momentos prévios ao abate, tem influência direta no ganho de peso, na qualidade da carcaça e da carne (FORDYCE et al., 1985; BOISSY; BOUISSOU, 1995; HÖTZEL et al., 2004; DEL CAMPO et al., 2010; CAFE et al., 2011; RIBEIRO et al., 2012). Existem também evidências da influência do temperamento sobre o desempenho reprodutivo de bovinos (COOKE et al., 2011; KASIMANICKAM et al., 2014; RUEDA et al., 2015), indicando que vacas e novilhas de temperamento mais excitável apresentam pior taxa de concepção (35, 51 e 42%, respectivamente) quando submetidas a um protocolo de inseminação artificial em tempo fixo (IATF).

Tais prejuízos no desempenho dos animais podem ser resultado de uma maior susceptibilidade ao estresse (CURLEY et al., 2006), o qual é "um estado que ocorre quando um animal está necessitando fazer ajustes anormais ou extremos em sua fisiologia ou comportamento, a fim de lidar com os aspectos negativos de seu ambiente" (FRASER et al., 1975). Desta forma, animais estressados apresentam respostas fisiológicas associadas a um estado emocional de medo, evidenciadas no aumento da atividade do sistema nervoso simpático e do eixo hipotálamo-pituitária-adrenal (HPA) (BURDICK et al., 2011; SÁNCHEZ-RODRÍGUEZ et al., 2013). A ativação do HPA resulta em uma cascata de respostas endócrinas que auxiliam o organismo a enfrentar estes fatores estressores (CURLEY et al., 2008). Bovinos mais reativos são mais susceptíveis ao estresse, quando comparados aos animais calmos (STAHRINGER et al., 1990; CURLEY et al., 2006; BURDICK et al., 2011; SÁNCHEZ-RODRÍGUEZ et al., 2013), e essa associação é frequentemente usada para explicar a relação entre temperamentos excitáveis e baixo desempenho reprodutivo.

Tem sido demonstrado que o estresse prolongado interfere na fertilidade do animal, atuando nos mecanismos que regulam os eventos envolvidos na fase folicular do ciclo estral (RIVER; RIVERST, 1991; DOBSON; SMITH, 2000). A influência do estresse na fertilidade ocorre por meio da ativação do HPA, afetando a secreção do hormônio liberador de gonadotrofinas (GnRH) pelo hipotálamo e consequentemente, as gonadotrofinas pela glândula pituitária (DOBSON; SMITH,

2000; VON BORELL; DOBSON; PRUNIER, 2007). A supressão da secreção pulsátil de GnRH promove a redução do suporte de hormônio luteinizante (LH) no folículo ovariano (DOBSON; SMITH, 2000; VON BORELL; DOBSON; PRUNIER, 2007), esta cascata de reações reduz também a produção de estradiol, resultado do crescimento mais lento dos folículos (DOBSON; SMITH, 2000). Em resumo, nos momentos da luteólise e da ovulação, o estresse pode acarretar diversos efeitos deletérios sobre a reprodução das fêmeas, em função do aumento de glicocorticóides adrenais (LIPTRAP, 1993), levando ao bloqueio da ovulação (RIVER; RIVEST, 1991), ovulações silenciosas (VON BORELL; DOBSON; PRUNIER, 2007) e estros de curta duração (SOEDE et al., 1997). No caso da aplicação de protocolos de IATF, que requer manejos sucessivos das fêmeas no curral, expondo estes animais a agentes estressores tais como, o manejo frequente no curral em um período de tempo curto, além da aplicação de hormônios tanto através de injeções, quanto da aplicação de dispositivos intra-vaginais; estes estressores podem ter efeitos ainda mais pronunciados, principalmente quando se trabalha com fêmeas bovinas mais reativas (KASIMANICKAM et al., 2014).

2.2.3. Cortisol e glóbulos brancos como indicadores fisiológicos de estresse

Qualquer estímulo eliciador de medo ativa a amígdala, estimulando diferentes respostas de estresse no corpo como o núcleo paraventricular do hipotálamo, ativando o fator liberador de corticotropina (CRF) que em seguida, causa a liberação do hormônio adrenocorticotrópico (ACTH), promovendo também a liberação de glicocorticoides. Outra resposta do estresse é a ativação do núcleo motor dorsal do vago, ativando o sistema nervoso parassimpático, o que faz com que o animal tenha respostas como defecação e micção. Um exemplo adicional é a ativação da área tegumental ventral e do locus coeruleus, que atuam em conjunto e liberam a dopamina, noradrenalina e a acetilcolina, os quais aumentam a resposta físicas de excitação (GREGORY; GRANDIN, 1998).

Desta forma, as principais respostas ao estresse são mediadas pelas catecolaminas e por glicocorticoides, a exemplo do cortisol, um dos hormônios esteroides que é fundamental para esta resposta fisiológica (SAPOLSKY et al., 2000). O aumento da concentração de cortisol no plasma sanguíneo tem um importante papel na regulação da locação física dos leucócitos no sangue (DHABHAR et al., 1994), induzindo a diminuição do número e porcentagem de linfócitos e monócitos (linfopenia) e o incremento dos neutrófilos no sangue (neutrofilia) (JAIN, 1993; DHABHAR et al., 1996). Essas mudanças ocorrem como parte da adaptação do organismo diante de potenciais estressores. Por isso, tanto a concentração de cortisol plasmático, como a contagem de glóbulos brancos no sangue são utilizados como indicadores fisiológicos de estresse (BOISSY; LE NEIDRE, 1996; BRISTOW; HOLMES, 2007; DAVIS et al., 2008).

2.3. Interação humano-animal nos sistemas de produção

Interações entre humanos e animais podem resultar em mudanças tanto fisiológicas quanto comportamentais no animal, influenciando consequentemente o desempenho e o bem-estar do mesmo (HEMSWORTH; COLEMAN, 2011). Para entender melhor essa interação, se faz necessário aprofundar-se um pouco na sua história (a partir do processo de domesticação), compreender como essa relação se estabeleceu e o que a caracteriza.

A domesticação de animais vem se desenvolvendo desde o período Neolítico e, ao longo do tempo, tem ocorrido com diversas populações de animais. Esta é definida como "o processo no qual uma população de animais se torna adaptada ao homem e ao ambiente de cativeiro, por meio da combinação de mudanças genéticas ao longo das gerações e eventos de indução por fatores ambientais ocorridos ao longo de cada geração" (PRICE, 1984, p. 3). No contexto histórico, as reações emocionais dos animais perante o homem, como por exemplo a tendência de agressão ou fuga, possivelmente desempenharam um papel importante na definição de quem seria domesticado (HEDIGER, 1964).

Apesar das inúmeras gerações de animais domésticos provenientes da reprodução seletiva por parte dos humanos, em que os acasalamentos ocorrem apenas com animais selecionados por características de seu interesse; respostas de medo dos animais perante os humanos não foram eliminadas, mas apenas reduzidas (HEMSWORTH, 2007). Isto pode acontecer, provavelmente, devido ao fato de que alguns dos eventos mais assustadores que muitos destes animais estão propensos a experimentar são a exposição ao ser humano (quando manejados de forma agressiva) e as mudanças bruscas no seu ambiente social e físico (BOISSY, 1995; HEMSWORTH; COLEMAN, 2011).

A interação humano-animal nos sistemas de produção está relacionada a todo e qualquer contato existente entre os manejadores e os animais. Segundo Waiblinger et al. (2006) estas interações podem envolver diferentes aspectos da percepção a estímulos táteis, visuais, olfativos e auditivos. Tais interações podem ser classificadas de cinco formas sendo elas: *i*) estacionária (com presença visual); *ii*) o manejador se movimenta entre os animais sem a ocorrência de contato tátil, mas pode haver interações auditivas; *iii*) contato físico; *iv*) alimentação (ou recompensa) e; *v*) invasivo, com manejo aversivo (WAIBLINGER et al., 2006). A interação pode também ser classificada quanto à sua natureza, como positiva, neutra ou negativa (WAIBLINGER et al., 2006; ZULKIFLI, 2013), dependendo da forma com que o tratador dos animais desenvolve suas tarefas de rotina na fazenda (ZULKIFLI, 2013). De acordo com Hemsworth (2007), os comportamentos de alguns manejadores dos animais não são corretos (da forma como deveriam ser) e estas situações exemplificam a inevitável relação desigual entre os animais domésticos e os humanos. O autor ressalta que tal desigualdade deve ser considerada em uma análise da fronteira entre o certo e o errado do comportamento dos seres humanos.

Os animais de produção podem reagir espontaneamente às características humanas, ou podem aprender a associar a presença e o comportamento de todos ou de alguns seres humanos com o tipo de manejo empregado (WAIBLINGUER, 2006). De forma geral, ações aversivas conduzem a respostas negativas, como por exemplo, o aumento do nível de medo dos animais em relação aos humanos

(PARANHOS DA COSTA et al., 2002). Esse aumento do medo não ocorre somente pelo fato da presença humana representar uma fonte de ameaça para eles (PARANHOS DA COSTA; TARAZONA, 2011), mas também porque algumas das tarefas de rotina das fazendas podem ter caráter aversivo, como por exemplo, a contenção para a vacinação, administração de medicamentos, intervenções cirúrgicas e o transporte (WAIBLINGER et al., 2006; HEMSWORTH; COLEMAN, 2011). Se estas atividades forem aliadas a elevação da voz, pancadas e utilização de choque na condução dos animais, podem fazer com que as experiências com interação direta dos bovinos com os humanos sejam, de um modo geral, agressivas (RUSHEN et al., 1999; WAIBLINGER et al., 2006; HONORATO et al., 2012) e claramente negativas.

2.3.1. Impacto das interações negativas na produção animal

Os animais aprendem a evitar estímulos relacionados a situações potencialmente perigosas, provenientes principalmente de eventos aversivos promovidos pelo manejador, e o medo desempenha um papel crucial neste processo de evitação (HEMSWORTH; VERGE; COLEMAN, 1996; RUSHEN; TAYLOR; DE PASSILLÉ, 1999). O medo, é definido como uma resposta emocional induzida pela percepção de perigo durante a exposição a um estímulo ameaçador (BOISSY; BOUISSOU, 1995; JONES, 1996; RUSHEN; TAYLOR; DE PASSILLÉ, 1999). Este tem sido amplamente considerado como um estado indesejável de sofrimento por vários membros da comunidade científica, grupos de bem-estar, assim como um crescente número de produtores pecuários (JONES, 1996).

Alguns autores confirmam a existência de uma relação direta entre as interações negativas e o medo dos animais com relação aos seres humanos, com consequente redução do desempenho produtivo em algumas espécies (RUSHEN et al., 1999; BOIVIN et al., 2003; HEMSWORTH, 2003; WAIBLINGER et al., 2006; ZULKIFLI, 2013). Segundo diversos estudos, as interações negativas estão relacionadas com: menores taxas de prenhez e menor produção de leite ou maior

retenção do mesmo em bovinos (HEMSWORTH et al., 2000; BREUER, 2000; HEMSWORTH et al., 2002; WAIBLINGER; MENKE; COLEMAN, 2002; MACEDO et al., 2011); menores taxas de crescimento e efeitos negativos na reprodução de suínos (HEMSWORTH; BARNETT; HANSEN, 1981, 1986); e menores taxas de conversão alimentar e menor quantidade de produção de ovos, em frangos e galinhas, respectivamente (JONES, 1993; HEMSWORTH et al., 1994a; BARNETT; HEMSWORTH; NEWMAN, 1992). Também têm-se demonstrado que interações negativas entre humanos e animais estão relacionadas com maiores níveis de cortisol em suínos (HEMSWORTH; BARNETT; HANSEN, 1981, 1986), bovinos (HEMSWORTH et al., 2000; HEMSWORTH et al., 2002; BREUER; HEMSWORTH; COLEMAN, 2003) e ovinos (HEMSWORTH et al., 2011) com consequente empobrecimento do bem-estar animal.

No entanto, o medo dos animais com relação aos humanos pode diminuir através de processos de aprendizado, por exemplo, bovinos manipulados com calma e silenciosamente tornam-se menos medrosos (PETHERICK et al., 2009). Isto indica que minimizar o uso de interações negativas não é só uma estratégia importante para melhorar o bem-estar dos animais, reduzindo a resposta ao medo, se não também, uma estratégia comercial útil (HEMSWORTH, 2007).

2.4. O vaqueiro

2.4.1. Atitude e comportamento

A Psicologia revela que um fator de disposição importante na previsão do comportamento humano é a atitude. As atitudes são aprendidas e depois podem ser modificadas, influenciando diretamente no comportamento dos humanos perante os animais (HEMSWORTH; COLEMAN, 2011). Por isso, a atitude do vaqueiro tem um papel fundamental também na redução do medo dos bovinos (HEMSWORTH et al., 2002).

É importante compreender que, na maioria dos casos, as atitudes e consequentes comportamentos ruins por parte dos trabalhadores nos sistemas de

produção pecuária, não ocorrem por crueldade intencional. A maioria desses comportamentos são, intuitivamente, considerados inofensivos por essas pessoas, geralmente executados pela falta de conhecimento e, por causa disso, acontecem frequentemente nos sistemas de produção animal (HEMSWORTH, 2007). Um fator agravante, é que o foco das pesquisas e desenvolvimentos na área produção pecuária é, na maioria das vezes, a inovação tecnológica, especialmente em áreas como alojamento, nutrição, genética e saúde (HEMSWORTH; COLEMAN, 2011); esquecendo-se dos vaqueiros (manejadores responsáveis pelos bovinos de uma propriedade), que não tem recebido o devido reconhecimento da importante tarefa que eles têm dentro do sistema de produção animal, e da grande influência dos mesmos no bem-estar e a produtividade dos animais.

A maioria das indústrias fazem programas de treinamento para supervisores e gerentes, sem se preocupar com aqueles que, de fato, trabalham diretamente com os animais (HEMSWORTH; COLEMAN, 2011). O que ocorre geralmente, é que os vaqueiros são considerados funcionários de alta rotatividade e por isso não se investe na capacitação desses profissionais. Isso acarreta em um ciclo problemático, no qual os funcionários que não são capacitados e nem valorizados (sendo consequentemente desqualificados), não permanecem no emprego, o que justifica ao gerente e proprietário o não investimento em capacitação (HEMSWORTH; COLEMAN, 2011). Passar a reconhecer o vaqueiro como peça fundamental na indústria da produção animal se faz necessário e requer uma mudança cultural. Tal mudança de paradigma promove também mudanças na vida do vaqueiro que, por conseguinte, modifica suas atitudes, as quais influenciam diretamente no bem-estar e desempenho dos animais de produção (HEMSWORTH; COLEMAN, 2011).

2.4.2. Desenvolvimento do trabalho

Através do aprendizado por condicionamento tanto clássico quanto operante, e da influência da variação do estado motivacional, os vaqueiros adquirem suas atitudes e comportamentos perante os animais que estão sob seus cuidados (HEMSWORTH; COLEMAN, 2011). Desta forma, as primeiras experiências

obtidas pelo vaqueiro quando o mesmo interage ou observa outra pessoa interagir com os animais, influencia na obtenção dessas atitudes e comportamentos perante os animais (HEMSWORTH; COLEMAN, 2011). Baseada na teoria da dissonância cognitiva proposta por Festinger (1957), existe uma relação recíproca entre as atitudes e o comportamento das pessoas. Quer dizer que, não só as atitudes influenciam o comportamento, mas também o contrário. Uma vez que uma pessoa realiza um comportamento particular, existe uma tendência a modificar as atitudes que são relevantes para a realização desse comportamento (HEMSWORTH; COLEMAN, 2011).

A atitude do vaqueiro perante o animal pode influenciar também algumas características relacionadas ao trabalho e, consequentemente, seu desempenho no mesmo (HEMSWORTH et al., 2002). Isto significa que uma atitude pobre do vaqueiro perante o animal criará dificuldades de manejo, o que consequentemente afetará negativamente a motivação no trabalho que, por sua vez, afetará a boa execução do trabalho do mesmo (ALENCAR et al., 2007; HEMSWORTH; COLEMAN, 2011). Uma pobre atitude do vaqueiro com os animais, por exemplo, pode afetar na disposição de inspecioná-los e intervir com rapidez quando os animais têm algum problema (HEMSWORTH, 2007).

Segundo Blumberg e Pringle (1982) existem três fatores que influenciam o desenvolvimento do trabalho das pessoas: a capacidade, a oportunidade e a boa vontade. A "capacidade" inclui variáveis como as habilidades, a saúde e o conhecimento; a "oportunidade" inclui as condições de trabalho, os equipamentos e ferramentas disponíveis, as ações dos companheiros de trabalho e as políticas e regras organizacionais; já a "boa vontade" inclui a motivação, a satisfação e a atitude para com o trabalho e com os animais (HEMSWORTH; COLEMAN, 2011; COLEMAN; HEMSWORTH, 2014). O desempenho no trabalho depende sobretudo de uma combinação de motivação, conhecimento técnico, habilidades e a oportunidade de desempenhar o trabalho. Uma baixa motivação vai limitar o desempenho no trabalho, independentemente das habilidades técnicas e do conhecimento do indivíduo (HEMSWORTH, 2007). Sendo assim, alguma deficiência nestas importantes características relacionadas com o trabalho do

encarregado dos animais pode afetar o seu comportamento quando os maneja e, consequentemente, afetar também o comportamento dos animais.

O impacto das características mencionadas anteriormente no desempenho geral do trabalho é óbvio, porém, o impacto que estas têm no comportamento do trabalhador rural, e o efeito deste no comportamento e desempenho dos animais é menos óbvio e precisa ser objeto de maior atenção (HEMSWORTH, 2007); apesar de já haver vários trabalhos demonstrando que as interações humano-animal afetam o rendimento do trabalho e a satisfação do manejador, com implicações diretas no bem-estar dos animais (HEMSWORTH, 2007). Na figura 2 é possível visualizar as relações conhecidas e previstas dentre as variáveis mencionadas anteriormente (COLEMAN; HEMSWORTH, 2014).

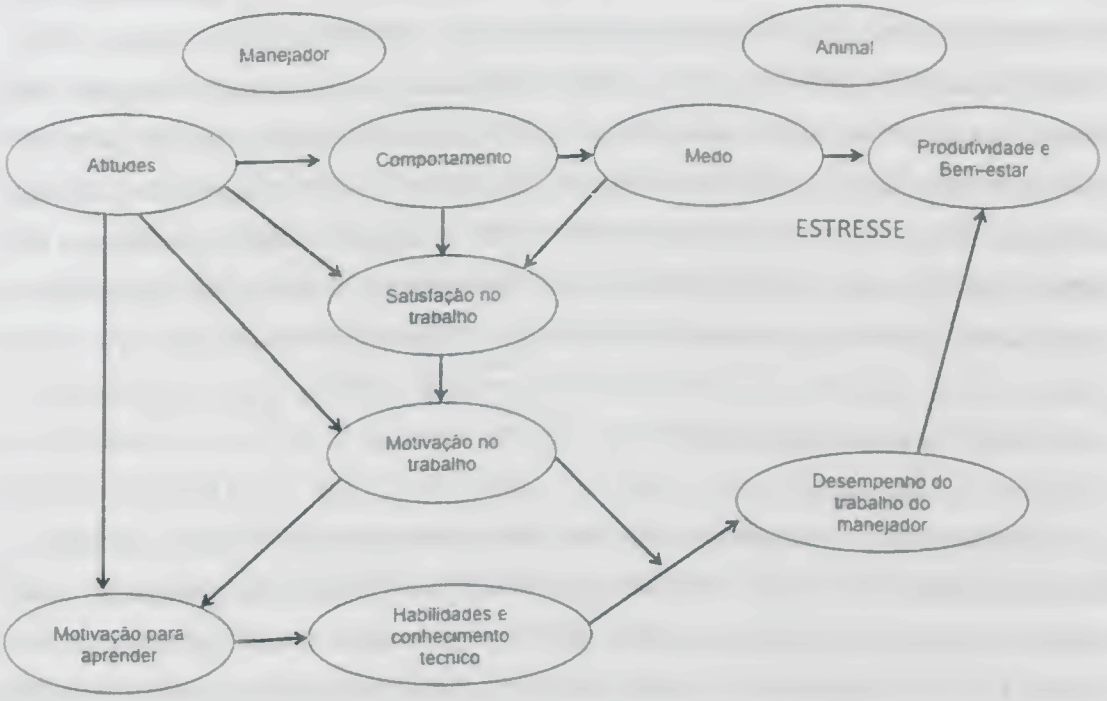

Figura 2. Características importantes relacionadas com o trabalho, e sua relação sequencial entre atitudes do manejador e bem-estar dos animais (COLEMAN; HEMSWORTH., 2014).

Fatores complexos que determinam o comportamento humano como, por

exemplo, a personalidade e a autoestima também devem ser considerados quando se quer contratar um vaqueiro em um sistema produção pecuária (BOIVIN et al., 2003). Estudos tem demonstrado que diferentes traços da personalidade dos vaqueiros estão diretamente relacionados com as atitudes dos mesmos perante os animais (COLEMAN et al., 2000; HEMSWORTH et al., 2002; COLEMAN; WAIBLINGER; MENKE, 2002; HANNA; SNEDDON; BEATTIE, 2009; COLEMAN; HEMSWORTH, 2014). Sendo assim, a identificação das características humanas que interferem no desempenho dos vaqueiros, pode ter um valor prático para a seleção dos trabalhadores rurais e também na identificação de pessoas experientes e inexperientes que requeiram treinamento (HEMSWORTH, 2003).

Apesar de existir evidência de que há características pessoais que predizem o bom desempenho dos vaqueiros (COLEMAN; HEMSWORTH; HAY, 1998; COLEMAN, 2004; HANNA et al., 2009), existe pouca pesquisa a respeito da seleção dos mesmos para este emprego (COLEMAN, 2004). Segundo Boivin et al. (2007) o comportamento dos trabalhadores com relação aos animais e as práticas de manejo do gado podem ser melhorados tanto através da seleção cuidadosa de pessoas, quanto pelo desenvolvimento de programas de treinamento desenhados para diminuir o estresse no manejo e melhorar o bem-estar dos animais.

2.4.3. Treinamento e capacitação

O comportamento humano é composto por quatro elementos: ação executada, alvo a qual a ação é dirigida, contexto em que a ação é realizada e tempo em que esta é executada (FISHBEIN; AJZEN, 2010). Sendo assim, para promover uma mudança no comportamento do ser humano, não é necessário apenas adquirir conhecimento e habilidades, mas também mudar hábitos estabelecidos; alterando atitudes e crenças que estão formadas na pessoa, para assim, preparar esta para lidar com as reações de outras pessoas. De acordo com Hemsworth e Coleman (2011) para provocar uma mudança de comportamento em uma pessoa, se faz necessário atuar em fatores pessoais e externos, que são relevantes para a

situação comportamental que se deseja mudar.

Trabalhos realizados em sistemas de produção de suínos e bovinos leiteiros, avaliando o efeito dos treinamentos dos funcionários (envolvendo técnicas comportamentais e cognitivas) nas atitudes e comportamento dos mesmos, reportam o potencial desses treinamentos na promoção da melhoria das interações humano-animal (HEMSWORTH et al., 2002; COLEMAN et al., 2000; HEMSWORTH, 2003). Alguns desses estudos demonstraram que as pessoas que tiveram esse tipo de treinamentos aumentaram o número de comportamentos positivos realizados por eles, perante os animais, assim como diminuíram os comportamentos negativos, quando comparados com vaqueiros que não receberam o mesmo treinamento (HEMSWORTH, COLEMAN; BARNETT, 1994b; COLEMAN et al., 2000; HEMSWORTH et al., 2002).

É comumente considerado que determinadas pessoas tenham naturalmente uma boa interação com os animais de fazenda. Algumas delas possuem maior afinidade, talvez porque possuam a capacidade psicológica de "se colocar no lugar do outro" e sentir o que o outro indivíduo está sentindo, caso estivesse em seu lugar (ter empatia), ou alguma habilidade de comunicação exclusiva que lhes permitam ser especialmente sensíveis às necessidades dos animais (COLEMAN, 2004). Por isso, se faz necessário conhecer previamente as pessoas (neste caso os vaqueiros) e suas atitudes (julgamentos e crenças) em relação aos animais, confrontando-as com seus conhecimentos e comportamento durante o manejo dos bovinos (BOIVIN et al., 2007). No entanto, a empatia e as atitudes são suscetíveis a mudanças e, por isso, processos de seleção de vaqueiros que identifiquem déficit nessas áreas podem ser utilizados, não só como uma ferramenta de seleção, mas também na identificação dos quesitos que necessitam de maior atenção durante os treinamentos (COLEMAN, 2004).

Programas de capacitação de vaqueiros, direcionados especialmente à mudança de atitudes e comportamentos, oferecem uma boa oportunidade para melhorar as interações humano-animal nas indústrias pecuárias (HEMSWORTH, 2007; COLEMAN; HEMSWORTH, 2014), com consequente benefício tanto para os animais quanto para os vaqueiros e produtores. Além desses benefícios, tais

treinamentos podem também promover a redução da rotatividade dos vaqueiros nos sistemas de produção animal (COLEMAN et al., 2000). Por este motivo, é evidente que treinamentos são necessários para capacitar os trabalhadores rurais encarregados do manejo do gado nos sistemas de produção brasileiros, especialmente porque, recentemente, a atividade de vaqueiro foi reconhecida como profissão no Brasil pela Lei nº 12.870, de 15 de Outubro de 2013 (BRASIL, 2013) e no Brasil não existem estudos que avaliem o impacto do acesso ao conhecimento de manejo racional de bovinos de corte sobre as atitudes dos vaqueiros e suas consequências na qualidade do manejo, no comportamento animal e no bem-estar de ambos, bovinos e vaqueiros.

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CAPÍTULO 2 – Artigo submetido à Livestock Science

Investigating the relationship between human-animal interactions, temperament, stress response and reproductive performance in Nellore heifers

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Abstract

The aim of this study was to contribute to further understanding of the relationships between human-animal interactions, temperament, stress response, and reproductive performance of Nellore heifers submitted to a fixed-time artificial insemination (FTAI) protocol. A sample of 571 heifers was used to assess two temperament traits (flight speed - FS and the composite reactivity score - RS), five human-animal interaction traits (negative handling - NH, Accidents - ACCIDENT, Defecation-urination - DEF-URI, Undesirable behavior – UB and Entrance time – ET), and perineal region dirtiness – DIRTINESS. All

variables were assessed on d0, d7 and d11 of a FTAI protocol. Additionally, two physiological indicators of stress (cortisol - CORT and neutrophil:lymphocyte ratio - N:L) were recorded in a subsample of 99 heifers on d0 and d11. To evaluate the relationships among all studied variables, we applied two different statistical approaches: a multivariate method (MCA, or multiple correspondence analyses) and linear models as confirmatory analyses. In the main data set, the MCA identified 11 correspondences, which we classified into two groups (undesirable vs. desirable aspects of the variables assessed). Another three correspondences were identified in the physiological data set: two indicated that the most reactive animals had the highest cortisol concentrations and the dirtiest perineal regions (and vice versa), and the other showed that animals facing negative handling display undesirable behaviors. Despite the significant effect of FS ($\chi^2 = 7.30$; $P < 0.05$) and RS classes ($\chi^2 = 4.61$; $P = 0.10$) on the chances of a heifer getting pregnant, no significant differences were observed between the calmest and most excitable heifers. Finally, heifers with dirtier perineal region (higher DIRTINESS score) had lower chances of getting pregnant than cleaner ones ($\chi^2 = 6.75$; $P < 0.05$). We conclude that poor quality of handling often results in more reactive animals as well as more undesirable behaviors and an increased risk of accidents. Although we did not observe a direct relationship between negative handling and excitable temperament with pregnancy rates, we note that heifers that experienced negative handling were more reactive during handling as well as dirtier in the perineal region, which in turn was associated with lower pregnancy rates.

Keywords: cattle behavior, cattle handling, animal welfare, stress physiology, pregnancy rate.

1. Introduction

One of the principal factors influencing cattle welfare is the quality of human-animal relationships (Hemsworth, 2007; Probst et al., 2012), which can also affect cattle performance (Hemsworth and Coleman, 2011; Coleman and Hemsworth, 2014). It is well known that the quality of handling practices depends on different stockperson characteristics (e.g. their attitudes and beliefs), as well as animal behavior (Waiblinger et al., 2006; Coleman and Hemsworth, 2014).

Temperament can be broadly defined as the individual behavioral differences that are repeatable over time and across situations, involving several phenotypic characteristics such as aggressiveness, avoidance of novelty, willingness to take risks, exploration and sociality (Réale et al., 2007). Its evaluation usually considers only one or a few of these traits, and reactivity to handling by humans is the trait most commonly-used to assess cattle temperament (Boivin et al., 1992). There is evidence that more reactive cattle are more susceptible to stress and present the highest blood cortisol concentrations (Stahringer et al., 1990; Curley et al., 2004; Curley et al., 2006; Cafe et al., 2011). Additionally, negative correlations have been found between temperament traits and serum concentrations of luteinizing hormone (LH) in heifers (Stahringer et al. 1990). Therefore, it is expected that temperament also affects cattle reproductive performance, as confirmed by previous studies reporting lower pregnancy rates among more excitable cows and heifers subjected to a fixed-time artificial insemination (FTAI) protocol (Cooke et al., 2011; Kasimanickam et al., 2014a; Rueda et al., 2015). Dirtiness in the perineal region has also been associated with higher cattle reactivity (Rueda et al., 2015), which in turn increases the risk of reproductive tract contamination during AI procedures.

Although some studies have evaluated the importance of cattle temperament and stress physiology during reproductive handlings (e.g., AI, FTAI, and embryo transfer) on reproductive performance (Stahringer et al., 1990; Cooke et al., 2009a,b), few studies have addressed the important impact of the human-animal interactions occurring during such handlings (Macedo et al., 2011, Rueda et al., 2015). Moreover, most of these studies used linear statistical methods, which makes it difficult to obtain an integrated view of the relationships between stockpeople practices, cattle temperament, behavior and reproductive performance. It is well known that cattle with excitable temperaments often provoke negative reactions from the stockperson, which in turn result in sudden and extreme reactions by the animals, making it very difficult to distinguish causes from consequences (Hemsworth, 2003; Coleman and Hemsworth, 2014). To address the issue of the limitations of using only linear statistical methods to understand the human-animal interaction (as discussed in Rueda et al., 2015), here we used a multivariate method, which we believe enables a broader understanding regarding the complex associations among all those variables.

In the present study, we aimed to further contribute to the understanding of the relationships between human-animal interactions, temperament, stress response, and reproductive performance of Nellore heifers submitted to a FTAI protocol. We hypothesized that rough handling is strongly associated with excitable temperament in cattle, and that both result in higher physiological stress responses and dirtier perineal regions, which in turn negatively affect the reproductive performance of Nellore heifers.

2. Materials and Methods

This research study was carried out in accordance with Brazilian legislation and was approved by the Committee for the Ethical Use of Animals at the Faculty of Agricultural and Veterinary Sciences of São Paulo State University, Jaboticabal, São Paulo, Brazil (Protocol n. 014198/14).

2.1. Animals and the FTAI protocol

The study was conducted on a commercial farm, located in northeastern Pará State, Brazil, with 571 Nellore heifers (mean age of 24 ± 2 mo). The heifers were distributed into five lots, herein referred to as lots 1, 2, 3, 4 and 5, with 157, 117, 110, 70 and 117 heifers, respectively. All lots were kept in tropical pastures, under a rotational stocking method, and with free access to water and mineral supplements.

The FTAI protocol was carried out in two corrals, which were between 250 and 2400-m from the pastures. Lot 1 was handled in a corral with a triangle-format crowding pen (without solid fences), leading to a 9.85-m solid side working chute, and then to the squeeze chute. Lots 2 to 5 were handled in another corral, which had a semi-circular crowding pen (with solid fences) leading to a 5-m solid side working chute, which in turn led to a squeeze chute.

All heifers were subjected to the same FTAI protocol, which consisted of four handling procedures over a period of 11 days. Two workdays were necessary to carry out each handling procedure for all lots: one work day was spent handling the animals from lots 1 and 2 and the other day was spent handling animals from lots 3, 4 and 5. Animals from the same lot (1 to 5) were always handled on the same day and period (morning or afternoon).

A team of four stockpeople was responsible for all handling procedures. They were informed that the observers were there to evaluate cattle behavior and were thus partially aware of the aims of the study. All stockpeople were previously trained on good practices of beef cattle handling by a professional with expertise in animal welfare and ethology. The training includes two main components (similarly to the methodology described by Hemsworth et al., 2002): 1) a cognitive behavioral session that aims to modify stockpeople's fundamental beliefs and behaviors, and 2) a technical training session on the best practices for cattle handling.

Briefly, the handling routine for each protocol day consisted of stockpeople on horses (one in the front and two in the back of the cattle lots) driving the heifers from pasture to the corral using voice commands to stimulate the animals to walk. After entering the corral, the heifers were held in the holding pens for around 1 hour. Then, the stockpeople (now on foot) drove small groups of animals (6-8 heifers) into the crowding pen using handling flags and voice commands, and then into the working chute. Finally, one stockperson stimulated each heifer individually to walk along the working chute and to enter into the squeeze chute, where the FTAI procedures were applied. Entry order was collected for each lot on each handling day. The heifers' previous handling experience with humans was mainly in the pastures, when changing paddocks in the rotational system (almost every 4 days); they experienced less frequent handling in the corral (around 6 times in their lives).

On the first handling day of the FTAI protocol (d0), the females received an estradiol benzoate injection (2.0 mg RIC-BE®, Vetecia Lab. Prod. Veterinários; São Paulo, Brazil) and an intravaginal progesterone device (1.9 g progesterone, CIDR®, ZOETIS Indústria de Produtos Veterinários, São Paulo, Brazil) was inserted. On the seventh day

(d7), the heifers received an injection of dinoprost tromethamine - PGF2α (2.5 mg LUTALYSE®, ZOETIS Indústria de Produtos Veterinários), and their body conditions were assessed using a visual scale (the body condition score, BCS), with scores from 1 (very thin) to 5 (very fat) and fractions of 0.5 points. In the lots assessed, the BCS ranged from 2.5 to 4.0. On the ninth day (d9), injections of estradiol cypionate (0.3 mg ECP®, Ouro Fino Saúde Animal Ltda) and equine chorionic gonadotropin (1.5 mg NOVORMON®, Syntex Industria Bioquímica & Farmaceutica S.A) were administered, and the progesterone device was removed. The AI was performed on the 11th day (d11) using semen from four purebred Nellore bulls and one purebred Angus bull distributed among the five lots. Following the procedures, heifers returned to the pastures. No additional handling was conducted after finishing the FTAI protocol until 30 days after AI, when pregnancy was determined via ultrasonography.

2.2. Temperament assessment

Heifers' temperament was assessed on d0, d7 and d11 of the FTAI protocol without changing the farm handling routine and always by the same trained observer, using the reactivity in the squeeze chute (crush test) and flight speed. The crush test (adapted from Fordyce et al., 1985) was performed immediately upon animals' entrance into the squeeze chute (during the first 4s) without using any restraining devices (head bail or squeeze sides), and prior to applying the FTAI protocol procedures. The crush test measured the degree of movement, tension, body posture, breathing, kicking, and vocalizations, as described in Table 1. Later, these scores were summed, resulting in a single measure of reactivity: the composite reactivity score (RS), as described by Ceballos et al. (2016). In this study, RS ranged from 6 to 14, which we later converted to a scale of 1 (least reactive)

to 9 (most reactive). The flight speed (FS), defined by the speed at which each animal exited the squeeze chute (Burrow et al., 1988), was assessed after the FTAI protocol interventions, using a stopwatch to record the time taken by each animal to cover a known distance (1.48 m for lot 1 and 1.96 m for the other lots). Then, time data were converted to speed (m/s), and faster animals were considered to be more reactive (Burrow et al., 1988).

Table 1. Description of the traits included in the composite reactivity score (RS)

Traits	Scores
Movement	1 - no movement, 2 - few movements, for less than half of the observation time 3 - frequent but not vigorous movements, for half of the observation time or more 4 - constant and vigorous movements 5 - constant and vigorous movements, animal jumps and raises its forelimbs off the ground
Tension	1 - the animal did not exhibit sudden movements of the tail, head and neck, no muscle tremors, and eye whites were not visible 2 - the animal exhibited few sudden movements of the tail, head and neck, no muscle tremors, and eye whites may or may not have been visible 3 - the animal exhibited continuous and vigorous movements of the tail, head and neck, no muscle tremors, eye whites visible 4 - the animal appeared paralyzed or in freezing, muscle tremors were visible
Body posture	1 – standing: when the animal stands upright with all four feet in contact with the ground, still or moving 2 – kneeling: when the animal stands on its rear feet but rests on its front carpal joints, 3 – lying: when the ventral part of the animal's body is in contact with the ground
Breathing	1 - normal, rhythmic and non-audible breathing 2 - easily audible breathing, or puffing and blowing, but not rhythmic
Kicking	1 - when the animal does not exert a vigorous blow with its hind foot 2 - when the animal exerts a vigorous blow with its hind foot
Vocalization	1 - absence of vocalizations 2 - presence of vocalizations

Source, Ceballos et al. (2016)

2.3. Assessment of human-animal interactions during the FTAI procedures

Human-animal interaction assessments were also conducted at d0, d7 and d11 of the FTAI protocol, while heifers were handled in the crowding pen and the working chute. The following indicators were assessed: ***a)*** Negative handling (NH), measured as the sum of negative stockperson actions toward the animal, including: hitting the gate against the animal's body, hitting or prodding the heifer with a wooden stick, and twisting the animal's tail; ***b)*** Accidents (ACCIDENT), measured as the number of times the animal fell (when any part of the animal's body, except the hoofs, touched the ground as a result of a sudden movement), slipping (when the heifer slid any limb on the ground, without touching the ventral region of its body on the ground), bumping (when the heifer hit or stumbled on any structure in the facilities), and being trampled by another animal (measured only in the working chute, when a lying or kneeling heifer was trampled by another individual); ***c)*** Defecation-urination (DEF-URI), defined by the sum of each heifer's occurrences of defecation and urination; ***d)*** Undesirable behaviors (UB), composed by the sum of the frequencies of kicking (when a heifer exerted a vigorous blow with its hind foot), jumping (when a heifer pushed itself off the surface while keeping its front legs in the air), mooing (when the heifer vocalized), lying (the animal lay down smoothly, maintaining sternal recumbence), kneeling (the animal stood on its rear feet while resting on its front carpal joints), returning/balking (when the stockperson tried to drive the heifer in a particular direction, but the animal moved backwards or avoided entering the working or squeeze chutes), and attacking (when a heifer showed aggressive behavior towards the stockperson); ***e)*** Entrance time (ET; measured for lots 2 to 5), defined by the time (in seconds) it took to drive each heifer into the squeeze chute (measured from the time the squeeze chute gate is opened until the heifer's entire body is inside).

2.4. Physiological indicators of stress: Cortisol and the neutrophil:lymphocyte ratio

Blood samples from 99 heifers were collected twice (on d0 and d11 of the FTAI protocol) by coccygeal venipuncture. In all five lots, one in every six animals was randomly selected as it entered the squeeze chute during the d0 assessment. Two collection tubes (BD, Vacutainer™) with a rubber cover which were previously identified and not siliconized were used for each heifer, one containing K2 EDTA to make blood smears (for subsequent differential white blood cell count in the laboratory) and the other containing SST II Advance (separating and activating coagulant gel), used to measure cortisol concentrations. All samples were frozen at -20° C until they were analyzed in the laboratory.

The cortisol concentration analyses were conducted with an enzyme-linked immunosorbent assay (ELISA) following the instructions provided with the AccuBind ELISA Microwells kit (Monobind Inc., Lake forest, CA, USA). The inter-assay coefficient of variance (CV) for cortisol concentration was 5% and the intra-assay CV was 5.6%.

The neutrophil:lymphocyte ratio (N:L, expressed as a percentage) was used as a complementary method to assess the physiological stress response (Davis et al., 2008). It was calculated by manually conducting the differential leukocyte count from 100 cells in stained blood smear at the lab, as described by Thrall (2007).

2.5. Perineal region dirtiness (DIRTINESS)

DIRTINESS was graded on a 5-point scale, as follows: 1 = no dry or fresh feces in the perineal region, 2 = less than 25% of the area covered by feces, 3 = about 25% of the area covered by feces, 4 = between 25 and 50% of the area covered by feces, and 5 = more than 50% of the perineal region covered by feces (Rueda et al., 2015).

2.6. Statistical analysis

We first visually verified the distributions of all the dependent variables. RS was considered continuous, following the assumption that 'ordered categorical traits are often supposed to be controlled by a continuous underlying variable' (Van Tassell et al., 1998). Cortisol concentration values were log-transformed (CORT = $\log_e$) to follow a normal distribution ($P > 0.05$, Kolmogorov-Smirnov test). ET presented a lognormal distribution, NH, UB, DEF-URI and ACCIDENT presented a Poisson distribution, DIRTINESS presented a multinomial distribution and pregnancy rate presented a binomial distribution. Pearson's correlation coefficients were calculated between RS and FS on d0, d7 and d11.

We then analyzed the variation of temperament, human-animal interactions, and physiological traits over the FTAI protocol (d0, d7 and d11). For the temperament traits (FS and RS), linear mixed models for repeated measures were fitted using PROC MIXED in SAS (version 9.3, SAS Institute Inc., Cary, NC, USA). For human-animal interaction traits (NH, UB, ACCIDENT, DEF-URI and ET) generalized linear mixed models were fitted using PROC GLIMMIX in SAS. Raw and standardized residuals were plotted, and their distributions were examined to determine the distributions of the following dependent variables and respective link functions: Poisson distribution and log link function for NH, UB, ACCIDENT, DEF-URI; and lognormal distribution with identity link function for ET. All models included day of assessment (d0, d7 and d11 separately) as a fixed effect and heifer and lot (lots 2 to 5 for ET and 1 to 5 for all other variables) as random effects. Linear mixed models (PROC MIXED, SAS) were used for the physiological stress indicators (CORT and N:L), considering day of assessment (d0 and d11), period of blood sampling (morning or afternoon) and their interaction as fixed effects. Lot (1 to 5) and heifer were considered as random effects. Means were compared using *post hoc* Tukey tests. A

probability level of $P < 0.05$ was chosen as the limit for statistical significance and tendencies were discussed when $0.05 < P < 0.10$.

Subsequent statistical analyses were performed after categorizing the continuous variables, as follows: *i)* NH and ACCIDENT classes were categorized as binomial variables (occurrence = 1 and no occurrence = 0); *ii)* FS, CORT and N:L classes were categorized into three scores (low = 1, average = 2 and high = 3), by using the following criteria: low (< mean - 0.5 SD), average (= mean ± 0.5 SD) and high (> mean + 0.5 SD). *iii)* RS class was categorized based on the visual analyses of its distribution (low = scores 1 and 2, average = scores 3 and 4, and high = scores 5 to 9); *iv)* DIRTINESS class, for which only three scores were recorded (1, 2 and 4), was categorized as 1 (= low), 2 (= average) and 3 (= high), respectively. *v)* DEF-URI class, included four categories (no occurrence = 0; one occurrence = 1; two occurrences = 2, and three or more occurrences = 3); *vi)* UB class, included three categories (no occurrence = 0, one occurrence = 1 and two or more occurrences = 2); *vii)* Entry order class, used the quartiles to define four categories; and *viii)* ET class, used the tertiles to define three categories. Pregnancy was a binomial variable (pregnant = 1 or not pregnant = 0).

Respecting the dependence structure among the variables, a multiple correspondence analysis (MCA) was applied to identify the dependencies contained in the categorical variables of human-animal interactions, temperament, physiological stress traits, and pregnancy. The MCA uses the chi-square test to standardize the frequencies and to build the base for the correspondences (Takane and Hwang, 2006; Hair et al., 2009). It also assigns scores to rows (representing the subjects) and columns (representing the response categories) of a data matrix, yielding a graphical display (Takane and Hwang, 2006). Each correspondence could have more than two associated variables (Hair et al., 2009). ET,

BCS, entrance order and pregnancy were excluded from the final MCA after a first exploratory analysis revealed they did not show any significant correspondence with any of the variables. FS, RS, DIRTINESS, NH, ACCIDENT, DEF-MIC, and UB were included for the main data set analyses on d11 (n = 571). The same variables plus CORT classes were considered when using the subsample data set (n = 99) for the MCA analyses. After a first exploratory analysis of this data set, N:L was excluded from the final MCA because it did not show significant correspondence with any other variable. These analyses were processed with the Burt table (X'X) using Statistica 7® software (version 7.0) and significance was set to $P < 0.05$.

Linear mixed models and generalized linear mixed models were then used, as confirmatory analyses, to assess the relation of negative handling (NH, assessed on d0 and d11) with temperament (RS, FS), behavioral (UB, ACCIDENT, DEF-URI and ET) and physiological (CORT and N:L) traits, considering NH class and period (only in the CORT and N:L analyses) as fixed effects, and lot and entrance order as random effects. The PROC MIXED command in SAS was used for variables with normal distributions (RS, FS, CORT and N:L) and PROC GLIMMIX was used for UB, ACCIDENT, DEF-URI and ET, fitting generalized linear mixed models. The models were calculated separately for each day of assessment (d0 and d1).

The relation of temperament (FS and RS classes, assessed on d0 and d11) with behavioral (UB, ACCIDENT and DEF-MIC) and physiological (CORT and N:L) traits was also assessed, considering both temperament traits (FS and RS classes) and period (only in the CORT and N:L analyses) as fixed effects, and lot as a random effect. Again, the PROC MIXED command in SAS was used for variables with normal distributions (CORT and

N:L) and PROC GLIMMIX was used for variables with a non-normal distribution (UB, ACCIDENT, DEF-URI).

Finally, a logistic regression model was fitted to calculate the chance of heifers getting pregnant as a function of temperament traits (FS and RS), negative handling (NH), dirtiness (DIRTINESS) and BCS class. This was done with the PROC GENMOD command in SAS, with binomial distribution for the response variable (pregnancy) and logit link function. Lot was considered a fixed effect for control purposes. Other variables such as bulls, inseminator, entrance order, and insemination time showed no significant effects on pregnancy ($P > 0.05$), and were thus excluded from the final model. Results were expressed in odds ratios (OR) calculated by exponentiating the regression coefficients (β). The OR refers to the number of times the chance of pregnancy increases or decreases for each independent variable category in comparison to a reference category with OR = 1. Odds ratios with 95% confidence intervals (95% CI) and P-values were estimated for the following independent variables: FS, RS and DIRTINESS classes measured on d11. Reference classes (RC) were defined as the highest FS, RS and DIRTINESS classes.

3. Results

3.1. Characterization of heifer temperament, human-animal interactions and physiological indicators throughout the FTAI protocol

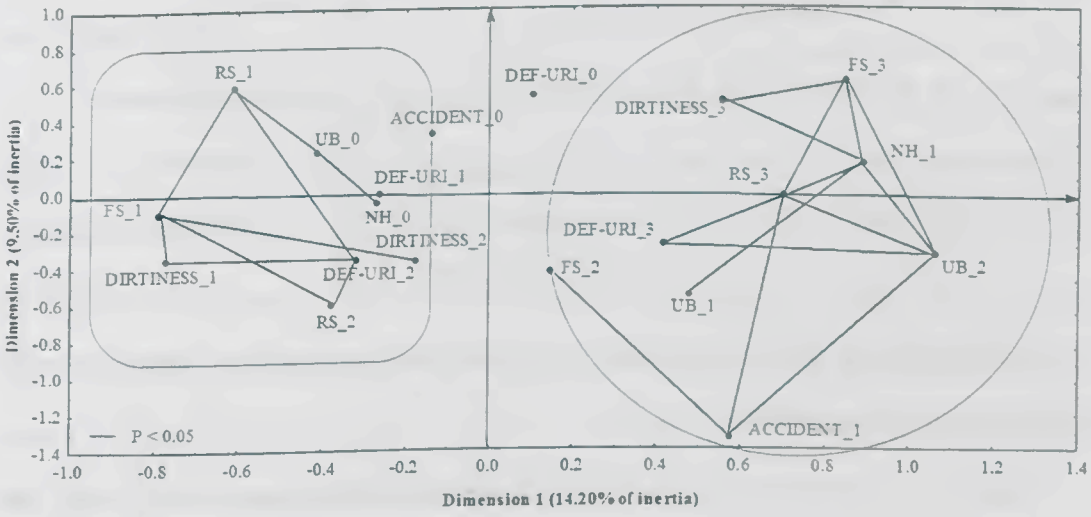
We observed variations in both temperament traits (FS and RS) across the FTAI protocol time points ($F_{2,1689} = 53.22$, and $F_{2,1685} = 8.92$, respectively; $P < 0.0001$ for both). The FS mean was significantly higher on d0 (2.67 ± 0.14 m/s) compared with d7 (2.06 ± 0.14 m/s) and d11 (2.35 ± 0.14 m/s), and these last two time points also significantly differed from each other. The same tendency was observed for RS, with a significantly

allowed us to define two groups of correspondences. The right side of Figure 1 shows the correspondences determining the undesirable aspects of human-animal relationships (circle), and the left side shows the complementary information associated with the desirable aspects of these relationships (square). The categorical scores for each variable were defined as follow: three classes for FS, RS, CORT and DIRTINESS (low = 1, average = 2 and high = 3); two for NH and ACCIDENT (occurrence = 1 and no occurrence = 0); four for DEF-URI (no occurrence = 0; one occurrence = 1; two occurrences = 2 and three or more occurrences = 3); and three for UB (no occurrence = 0; one occurrence = 1 and two or more occurrences = 2).

The following correspondences, which can be classified as a negative cycle, were identified on the right side (circle) of Figure 1: (***a***) FS_3 – NH_1 – UB_2 ($P < 0.05$), (***b***) RS_3 – FS_3 – UB_2 – NH_1 – DEF-URI_3 – ACCIDENT_1 ($P < 0.05$), and (*c*) FS_2 – ACCIDENT_1 ($P = 0.022$). These correspondences indicate that the animals experiencing poor human-animal interactions are the most reactive, display more undesirable behaviors, defecation and urination, and experience more accidents. In line with these findings, other correspondences also support the idea of a negative cycle, including (***d***) NH_1 – UB_1 – UB_2 ($P < 0,01$), showing that animals facing negative handling present undesirable behavior, and (***e***) UB_1 – ACCIDENT_1 ($P = 0.005$) and (*f*) UB_2 – ACCIDENT_1 ($P = 0.003$), indicating that heifers presenting undesirable behaviors suffer accidents. Two additional correspondences support the notion that undesirable behavior and negative handling are associated with stress: (***g***) DEF-URI_3 – UB_2 ($P < 0.020$), showing that the animals with the most defecation and urination have the highest frequency of undesirable behaviors; and (***h***) DIRTINESS_3 – FS_3 – NH_1 ($P < 0.01$), showing that dirtier animals experience NH and were more reactive.

We also found correspondences that can be classified as a positive cycle (Figure 1, left side; square): (*i*) NH_0 – UB_0 ($P < 0.0001$), revealing that animals that do not face negative handling do not show undesirable behaviors; (*j*) DIRTINESS_1 – FS_1 – DEF-URI_1 ($P < 0.05$), showing that calmer animals display cleaner perineal region and have low defecation and urination; and (*k*) RS_1 – FS_1 – UB_0 ($P < 0.05$), showing an association among the lowest classes of RS and FS (the calmest animals) and the absence of undesirable behaviors.

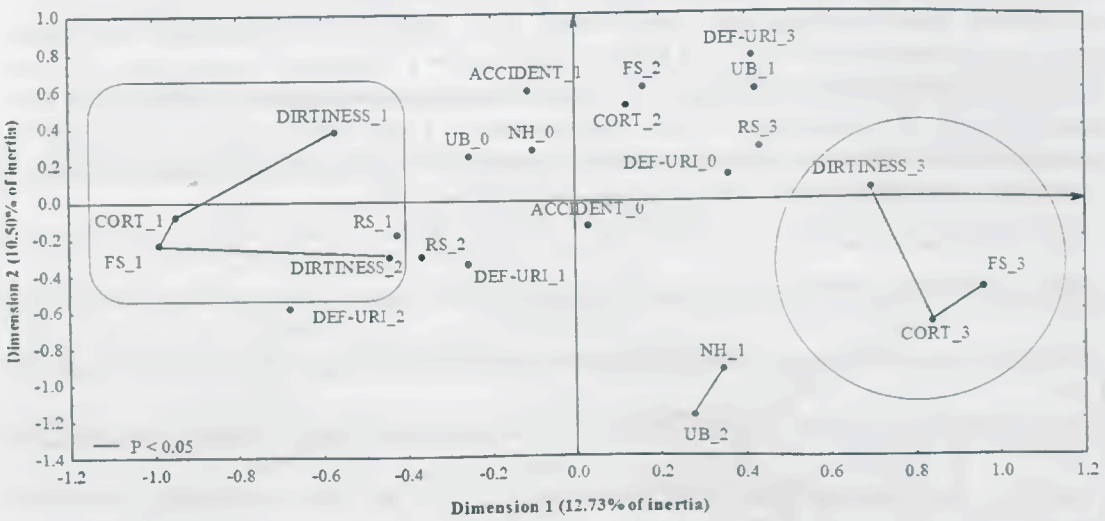
Figure 1. Perceptual map of multiple correspondence analyses for the categorical variables (classes) of flight speed (FS), reactivity score (RS), DIRTINESS, negative handling (NH), ACCIDENT, defecation-urination (DEF-URI), and undesirable behavior (UB). Three classes were defined for FS, RS, and DIRTINESS (low = 1, average = 2 and high = 3); two for NH and ACCIDENT (occurrence = 1 and no occurrence = 0); four for DEF-URI (no occurrence = 0; one occurrence = 1; two occurrences = 2 and three or more occurrences = 3); and three for UB (no occurrence = 0; one occurrence = 1 and two or more occurrences = 2). Analyses conducted on the complete sample data (n = 571).



Finally, using the subsample data set, the following correspondences were found: (*l*) CORT_3 – FS_3 – DIRTINESS_3 ($P < 0.05$), and (*m*) CORT_1 – FS_1 – DIRTINESS_1 ($P < 0.05$). These correspondences indicate that the most reactive animals also have the highest cortisol concentrations and dirtiest perineal regions (and vice versa). The last

correspondence found, (*n*) NH_1 – UB_2 (P = 0.015), corroborates the main data set findings, that animals with negative handling have undesirable behaviors. The subsample data set correspondences are represented in Figure 2.

Figure 2. Perceptual map of multiple correspondence analyses for the categorical variables (classes) of flight speed (FS), reactivity score (RS), cortisol (CORT), DIRTINESS, negative handling (NH), ACCIDENT, defecation-urination (DEF-URI), and undesirable behaviors (UB). Three classes were defined for FS, RS, CORT and DIRTINESS (low = 1, average = 2 and high = 3); two for NH and ACCIDENT (occurrence = 1 and no occurrence = 0); four for DEF-URI (no occurrence = 0; one occurrence = 1; two occurrences = 2 and three or more occurrences = 3); and three for UB (no occurrence = 0; one occurrence = 1 and two or more occurrences = 2). Analyses conducted on the subsample of data with physiological measures (n = 99).



3.3. Relation of negative handling with temperament traits, heifer behaviors and physiological indicators of stress

Significant relationships of NH class were found with FS ($F_{1,547}$ = 6.55, P = 0.011) and RS ($F_{1,546}$ = 6.09, P = 0.014) only on day 11. On that day, heifers that faced NH had higher means for both temperament traits than those that did not face NH (Table 4).

Among the behavioral indicators, NH was related only with UB (d0: $F_{1,546}$ = 14.46 and d11: $F_{1,548}$ = 126.37, P < 0.0001 for both), with animals who experienced NH showing

higher UB frequencies (Table 4). ACCIDENT, DEF-URI, ET and the physiological indicators of stress were not related with NH ($P > 0.05$).

Table 4. Adjusted means (± SE) of the temperament traits (flight speed - FS and reactivity score - RS), and undesirable behaviors (UB) on days 0 and 11 when heifers did or did not encounter negative handling (NH). Arithmetic means are shown within parentheses.

Indicator	Non-occurrence of NH	Occurrence of NH
d0		
Behavior		
UB	$-0.37^{b} \pm 0.28$ (0.81)	$-0.004^{a} \pm 0.28$ (1.06)
d11		
Temperament		
FS (m/s)	$2.30^{b} \pm 0.18$ (2.18)	$2.56^{a} \pm 0.20$ (2.67)
RS	$3.73^{b} \pm 0.13$ (3.74)	$4.20^{a} \pm 0.19$ (4.22)
Behavior		
UB	$-0.53^{b} \pm 0.17$ (0.60)	$0.50^{a} \pm 0.17$ (1.85)

$^{a-c}$ Means followed by different lower case letters in the same row are statistically different ($P < 0.05$), according to Tukey tests.

3.4. Relations between temperament traits with heifer behavior and physiological indicators of stress

There were significant relations between FS class and ACCIDENT on d0 ($F_{2,554} = 3.96$, $P = 0.025$) and d11 ($F_{2,558} = 11.96$, $P < 0.0001$). Likewise, RS class was significantly associated with UB (d0: $F_{2,554} = 35.67$ and d11: $F_{2,558} = 32.17$, $P < 0.0001$ for both) and ACCIDENT (d0: $F_{2,554} = 4.97$, $P = 0.008$ and d11: $F_{2,558} = 4.04$, $P = 0.018$) (Table 5). Less reactive animals had lower accident frequencies and UB. RS class was also significantly related to DEF-URI on d11 ($F_{2,558} = 3.97$, $P = 0.019$), with the lower DEF-URI means appearing together with low RS scores (Table 5).

In terms of physiological measures, CORT was related to FS class on d11 ($F_{2,87} = 5.99$, $P = 0.003$) and there was a tendency for a relation between RS class and CORT ($F_{2,87} = 2.58$, $P = 0.081$), with the most excitable individuals (high FS and RS classes) showing the highest CORT means (Table 5). Regarding the N:L ratio, we observed a relation

tendency of FS class ($F_{2,89}$ = 2.58, P = 0.082), with high FS class animals showing relatively greater N:L ratios on d11.

Table 5. Adjusted means (± SE) of undesirable behaviors (UB), accidents (ACCIDENTS), defecation-urination (DEF-URI), cortisol (CORT), and neutrophils: lymphocyte ratio (N:L) for the categorized classes (low, average and high) of flight speed and reactivity score classes on days d0 and d11 of the FTAI protocol. Arithmetic means are shown within parentheses.

	Flight speed class			Reactivity score class		
d0	**Low**	**Average**	**High**	**Low**	**Average**	**High**
Behavior						
UB	-0.49±0.29 (0.58)	-0.37±0.29 (1.02)	-0.48±0.29 (1.08)	-0.82^{b}±0.30 (0.52)	-0.65^{b}±0.29 (0.63)	-0.13^{a}±0.28 (1.20)
ACCIDENTS	-1.42^{b}±0.17 (0.24)	-0.92^{a}±0.14 (0.45)	-0.94^{a}±0.16 (0.46)	-1.29^{b}±0.20 (0.26)	-1.21^{b}±0.17 (0.29)	-0.78^{a}±0.13 (0.50)
DEF-URI	0.86±0.09 (2.44)	0.88±0.06 (2.39)	0.78±0.07 (2.16)	0.84±0.07 (2.37)	0.88±0.06 (2.44)	0.81±0.05 (2.23)
Physiology						
CORT	4.25±0.19 (3.98)	4.36±0.19 (4.44)	4.41±0.19 (4.52)	4.25±0.19 (4.24)	4.31±0.19 (4.18)	4.46±0.18 (4.46)
N:L	0.68±0.12 (0.77)	0.59±0.10 (0.60)	0.68±0.11 (0.74)	0.56±0.12 (0.63)	0.63±0.11 (0.71)	0.76±0.10 (0.72)
d11	**Low**	**Average**	**High**	**Low**	**Average**	**High**
Behavior						
UB	-0.35±0.24 (0.59)	-0.31±0.23 (0.90)	-0.22±0.23 (1.28)	-0.83^{c}±0.25 (0.44)	-0.25^{b}±0.24 (0.78)	0.20^{a}±0.23 (1.30)
ACCIDENTS	-2.06^{b}±0.28 (0.18)	-1.08^{a}±0.25 (0.44)	-1.27^{a}±0.27 (0.29)	-0.86^{b}±0.29 (0.16)	-1.35^{a}±0.26 (0.29)	-1.21^{a}±0.25 (0.41)
DEF-URI	0.48±0.09 (1.79)	0.55±0.06 (1.82)	0.59±0.06 (1.81)	0.41^{b}±0.06 (1.55)	0.58^{a}±0.06 (1.83)	0.63^{a}±0.05 (1.98)
Physiology						
CORT	3.97^{ab}±0.17 (3.73)	3.82^{b}±0.17 (3.91)	4.17^{a}±0.17 (4.23)	3.84^{y}±0.17 (3.82)	4.03^{xy}±0.17 (3.89)	4.09^{y}±0.16 (4.04)
N:L	0.37^{y}±0.05 (0.42)	0.47^{xy}±0.05 (0.48)	0.55^{y}±0.06 (0.57)	0.39±0.06 (0.37)	0.47±0.05 (0.47)	0.52±0.04 (0.54)

$^{a-c}$ Means followed by different lower case letters in the same row are statistically different ($P < 0.05$), according to Tukey tests.

$^{x-y}$ Means followed by different lower case letters in the same row has a tendency to be statistically different ($0.05 < P < 0.1$), according to Tukey tests.

3.5. Relation of temperament and human-animal interactions with heifers' reproductive performance

The chance of any heifer getting pregnant (expressed in OR) was related with FS class (χ^2 = 7.30; P = 0.026) and RS class (χ^2 = 4.61; P = 0.099), but no significant difference was found between the extreme classes (low vs. high) for either FS or RS ($P >$

0.05), indicating that the chance of getting pregnant did not differ significantly between the calmest and most excitable heifers (Table 6). A greater chance of pregnancy was observed in heifers classified as low (OR = 2.09) or average DIRTINESS (OR = 1.42) relative to high DIRTINESS ($\chi^2 = 6.75$ $P = 0.034$). No relation was observed between pregnancy rates and NH, BCS and lot ($P > 0.05$).

Table 6. Number of cows (N), pregnancy rate (in % and total number N), odds ratio (OR) with standard error (SE) of pregnancy and confidence interval (CI) for each class of flight speed (FS), reactivity score (RS) and dirtiness score (DIRTINESS). RC = reference class.

Traits	N Total	Pregnant heifers, % (N)	OR (SE)	CI (95%)	Chi-square	*P*-value
FS						
Low	213	37.09 (79)	0.73 (0.25)	0.00 to 1.19	1.58	0.21
Average	190	34.21 (65)	0.54 (0.23)	0.35 to 0.85	7.13	**0.008**
High	167	47.90 (80)	RC	RC	.	
RS						
Low	171	40.94 (70)	0.90 (0.22)	0.58 to 1.40	0.23	0.63
Average	161	32.30 (52)	0.62 (0.23)	0.40 to 0.98	4.26	**0.04**
High	237	43.04 (102)	RC	RC	.	
DIRTINESS						
Low	124	41.94 (52)	2.09 (0.29)	1.18 to 3.68	6.48	**0.02**
Average	202	39.60 (80)	1.42 (0.22)	0.93 to 2.18	2.58	0.10
High	242	37.19 (90)	RC	RC	.	.

4. Discussion

In this study, we used an integrated view to assess the association between cattle temperament, stress response and human-animal interactions, as well as their possible relation with the pregnancy rate of Nellore heifers. We observed a complex relationship among all traits, and only some of the studied traits were related to pregnancy rates. Additionally, we detected that most of these traits varied over the FTAI protocol period, mostly reducing over time.

4.1. Variation of the assessed traits over the FTAI protocol period

Heifers' reactivity decreased from the first to the last assessment of the FTAI protocol, despite a slight increase in FS from d7 to d11. A similar trend was observed for the behavioral measurements of ACCIDENT and DEF-URI. Physiological indicators of stress were also significantly different between d0 and d11, with the lowest cortisol and L:N ratio values recorded on d11. We expected that those variables would vary in the same direction, since most reactive animals show higher cortisol concentrations, as previously reported by other authors (Stahringer et al., 1990; Curley et al., 2006; and Cafe et al., 2011). The reduction in the N:L ratio was also expected because cortisol (produced in response to an acute stressor) decreases the number of lymphocytes and monocytes and increases the number of neutrophils in the blood, as reviewed by Jain (1993) and Dhabhar et al. (1994, 1996). According to these authors, adrenal steroids play an important role in regulating the physical location of leukocytes, which leads to physiological changes that prepare the body for facing potential stressors.

One interpretation could be that decreased behavioral and physiological responses to handling reflect the animals' habituation during the FTAI protocol. However, the fact that ET (the time it took to enter the squeeze chute) increased across the FTAI protocol suggests this is not the case. Thus, heifers presented increasing resistance to enter the squeeze chute across days, even without showing greater physiological stress or reactivity. Heifers may have actually learned and memorized the experiences in the squeeze chute, which would have provided an element of predictability. One could assume that slightly aversive yet predictable experiences (such as physical restraint and injections during FTAI) may cause some reluctance but would be less stressful than unpredictable experiences (as occurred in d0). According to Petherick et al. (2009), successive visits in the corral

involving slight negative handlings reduced behavioral reactivity, presumably because the handling procedures became predictable.

Unlike our own results, Rueda et al. (2015) reported increasing crush scores throughout the FTAI protocol, with higher means on d11 than d0 and d9. On the other hand, FS in that study remained constant over time in most of the farm units, showing it was not affected by repeated handlings. However, the fact that the authors did not measure physiological indicators of stress or the time it took females to enter the squeeze chute makes it difficult to interpret the results.

It is important to emphasize that, in the present study, neither NH nor UB (usually expressed by animals in response to stockpeople practices) changed across the FTAI protocol. Stockpeople behavior was consistent over time, which probably reflects the fact that the same team conducted all of the work and was previously trained in good practices of beef cattle handling (Ceballos et al., 2017).

4.2. Relationships between the assessed traits

It is well known that the quality of human-animal relationships depends on behavioral aspects of both humans and animals, and that when either behaves badly during handling procedures, the other is likely to react negatively, causing a negative feedback cycle (Waiblinger et al., 2006; Hemsworth and Coleman, 2011). This raises the concern that linear analysis methods may not be powerful enough to analyze these complex human-animal relationships, as already suggested by Rueda et al. (2015). In order to address this concern, here we used multiple correspondence analyses (MCA) in addition to some linear analyses.

As expected, the MCA revealed a complex association among all assessed traits (human-animal interactions, cattle temperament, and physiological indicators of stress), as represented in Figures 1 and 2, indicating that this method is a useful tool for these types of analyses. Some authors had previously reported association patterns among some of the studied traits (Macedo et al., 2011; Rueda et al., 2015), but to the best of our knowledge, this is the first study combining all variables in a single model.

In the present study, we observed that the occurrence of NH was associated with the occurrence of ACCIDENTS, as well as higher scores for UB, DEF-URI, DIRTINESS and temperament traits (FS and RS; see Figure 1, right side). Note on the MCA graphic that higher DIRTINESS was related to higher FS and the presence of NH, which in turn was directly related to DEF-URI; due to this, DEF_URI and DIRTINESS were also associated. We therefore conclude that negative handling has a marked relationship with cattle responses, as already reported for other species. For example, pigs afraid of handling were wary of moving to an unfamiliar situation and were more likely to show exaggerated responses to handling (Hemsworth, 2014). In dairy cattle, higher negative actions by humans were strongly correlated with more stepping or kicking of the cows during milking (Waiblinger et al., 2002), and with fewer cows approaching an observer in an approximation test (Hemsworth et al., 2000).

Based on this, animals experiencing NH should display the worst behaviors in the crowding pen and working chute, be more likely to suffer accidents, and show more reactive behavior during handling. This would fit well with the model proposed by Hemsworth and Coleman (2011), which shows that the occurrence of negative handling leads to fear responses in animals. Furthermore, fearful animals' reactions also affect stockpeople's behavior, since such reactions make handling more difficult, exacerbating the

problem. The results shown on the left side of Figure 1 confirm this expectation: heifers that did not face NH were classified as less reactive, with no UB and lower DEF-URI and DIRTINESS scores.

Results from the linear analyses also corroborated the notion that heifers facing poor quality of handling tended to be more reactive (higher FS and RS) and to present more UB, as previously reported by Macedo et al. (2011) and Rueda et al. (2015). We did not observe any significant relation between NH and cortisol concentrations or N:L ratios, which differs from previous studies conducted with dairy cattle (Hemsworth et al., 2000, Breuer et al., 2003) and Nellore females (Macedo et al., 2011).

We also observed that NH was associated with higher URI-DEF and DIRTINESS scores, and that the animals with the highest cortisol concentrations were also the dirtiest (and vice versa). These results are in line with those reported by Rueda et al. (2015), who showed that dirtiness in the perineal region was associated with higher agitation in cows, which in turn may be explained by the higher frequency of defecation and urination in response to stress (Rushen et al., 2001). A common sign of fear in cattle is defecation, which occurs due to vagus nerve activation (de Passillé et al., 1995; Gregory and Grandin, 1998). According to Mason (2000), defecation is a valid measure of emotional reactivity, which would explain why the heifers that faced NH were classified with higher URI-DEF and DIRTINESS scores.

The MCA and linear statistical analyses evinced the relationship between the excitable heifers' temperament and some human-animal interaction traits (UB, DEF-URI and ACCIDENT), showing significant relations of FS and RS with ACCIDENT on d0 and d11, and of RS with UB and DEF-URI. It is usually assumed that the degree to which an animal reacts to a stressful situation is influenced by its temperament, and that even

seemingly harmless handling practices may result in stress (Curley et al., 2004). So, fear-provoking stimuli are likely to elicit extreme behavioral responses by the excitable animals which, in turn, can make handling more difficult (Hemsworth, 2014).

It is worth noting that FS and RS were not equally related with heifer behavior, and animal reactivity within the squeeze chute was more strongly related to all previously-assessed behavioral traits. This could be explained by the conditions under which these traits were measured: ACCIDENTS, UB and DEF-URI were measured with heifers kept under physical restraint (in the crowding pen and in the working chute), which was similar to how RS was recorded. These results and the low correlations between FS and RS suggest that these traits assess different aspects of cattle temperament, as suggested by Curley et al. (2004) and Rueda et al. (2015). As mentioned by Ceballos et al. (2016) and corroborated by the present results, some aspects of cattle temperament may be ignored if only one test is used. Therefore, it is recommended that more than one trait be used to assess cattle temperament.

As expected (see Curley et al., 2004; Curley et al., 2006 and King et al., 2006), in both statistical analyses (MCA and linear models), higher cortisol concentrations and higher N:L ratios were found in more reactive animals (higher FS). This result corroborates the findings reported by Hulbert et al. (2011) that relative to calmer bulls, temperamental ones had greater neutrophil:mononuclear cell ratios. Additionally, this result suggests an association between cattle temperament and immune function, as previously reported by Fell et al. (1999) and Hulbert et al. (2011), who described that less reactive animals have better immune responses and are thus less susceptible to bacterial infections.

4.3 Relation of temperament and human-animal interaction traits with pregnancy rates

While most previous studies have reported that excitable females have the lowest pregnancy performance (Cooke et al., 2011, 2012; Kasimanickam et al., 2014a,b and Rueda et al., 2015), we observed that the least and most reactive heifers (for both FS and RS classes) had the same chance of getting pregnant, which was greater than that for animals with average reactivity scores.

There is evidence that more excitable cattle display higher physiological responses to stress (Curley et al., 2008; Cafe et al., 2011; Kasimanickam et al., 2014a), and this association is frequently used to explain the relationship between excitable temperament and lower reproductive performance. The main explanation for this phenomenon is that a stressor can disrupt the events surrounding ovulation in two ways (Dobson and Smith, 2000; Sarma, 2011): *i*) the activation of HPA may block successful ovulation through the secretion of CRH, which inhibits the secretion of gonadotropin-releasing hormone (GnRH) (Moberg, 2000; Etim et al., 2013); and ii) increased cortisol secretion is linked to a reduction in LH secretion (Moberg, 2000, Toufexis et al., 2014). Most likely, if an acute stressor occurred at another time in the reproductive cycle (not during pre-ovulation) it would have no effect on reproduction and might not even represent a significant biological cost to the animal (Moberg, 2000).

Review papers on rodents (Brann and Mahesh., 1991) and other mammal species (Tilbrook et al., 2000) conclude that chronic stress normally inhibits reproductive function, but that the effects of acute stressors are variable, and some stressors may sometimes even facilitate reproduction (Tilbrook et al., 2000). Thus, one could hypothesize that under good handling conditions, cows and heifers might react to the squeeze chute the way they would to short-term stressors. According to von Borell et al. (2007), short-term stressors are not intense enough to affect cattle reproduction. Comparable results have been reported for

pigs: in one study in which gilts were subjected to acute stress (with nose-sling and an unpredictable feeding scheme during the follicular phase and/or during early pregnancy), reproduction was not affected (Soede et al., 2007). Similarly, Turner et al. (1998a,b) exposed gilts to repeated acute stress, which resulted in increased plasma cortisol but had no effect on reproduction. Finally, as discussed by Cooke et al. (2011), the exogenous hormones administered to the cattle during the FTAI protocol could counteract the detrimental effects of an excitable temperament on the synthesis and release of stress hormones.

Additionally, the cumulative effects of multiple stressors (e.g. psychological, thermal, and nutritional) have a stronger effect on reproduction than any one single stressor (Sejian et al., 2012). This may explain why excitable temperaments are more prone to affect reproduction only under certain circumstances. For example, in the present study, BCS did not affect the pregnancy rate, differing from Rueda et al. (2015), who found that cow pregnancy was affected by both low BCS and excitable temperaments (high FS). It should be noted that almost all heifers in our study were in good body condition (BSC mean = 3.6 ± 0.5), with higher mean BCS scores than the cows tested in Rueda's study.

Based on previous studies, we hypothesized that NH would negatively affect reproductive performance (Dobson and Smith, 2000; Hemsworth et al., 2000; Macedo et al., 2011). However, similarly to Rueda et al.'s (2015) findings, NH had no significant relation with pregnancy. It is possible that the handling practices applied in the present study were not stressful enough to generate a physiological reaction capable of compromising reproduction (Turner et al., 1998b).

Finally, we did not find any study addressing the relation of cow/heifer perineal dirtiness with the chance of getting pregnant. Our results showed that dirtier heifers had a

lower chance of getting pregnant than cleaner ones. This could result from poor hygiene during the AI procedure, which would imply in a higher risk of reproductive tract contamination, and consequently, lower pregnancy success. Only one study investigated the effect of dirtiness on uterine tract contamination and found that during calving, cows with higher dirtiness scores show a greater incidence of metritis (Schuenemann et al., 2011).

5. Conclusions

A marked association between bad handling (in the form of aggressive actions by the stockpeople) and animal behavior was identified, which often resulted in more reactive animals. In the same way, highly reactive animals can incite aversive actions by the stockpeople; thus, this cycle could begin from either side (animal reactivity as well as negative stockpeople actions). This, in turn, resulted in more undesirable behaviors as well as an increased risk of accidents, making handling more difficult and risky for stockpeople as well. Although there was no direct relation between negative handling and excitable temperament on the pregnancy rate of Nellore heifers submitted to a FTAI protocol, the heifers that faced negative handling and were more reactive during handling also had higher dirtiness scores, which were in turn associated with the lower pregnancy rates. Based on this, we recommend that farmers and farm managers adopt a fine-tuned approach to improve the handling routines of cattle under their responsibility by training their stockpeople on good practices of cattle handling and systematically monitoring the quality of labor.

Conflict of interest statement

The authors declare that there are no conflicts of interest associated with this publication.

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CAPÍTULO 3 – Artigo a ser submetido à Livestock Science

Impact of training in good practices handling on beef cattle welfare and stockpeople attitudes and behaviors

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Abstract

The aims of this study were to evaluate the potential impacts of stockperson training in good practices of cattle handling on their attitudes and stockhandling behavior and on cattle welfare in Brazilian beef farms. Additionally, we also aimed to study whether the quality of cattle handling deteriorates as the working day progresses. The study was conducted in 24 commercial beef cattle farms (located in Northeastern Pará State, Brazil), classified in three groups, determined by formal handling skills training, provided to the stockpeople: regularly trained (TRAINED-R, n = 9), occasionally trained (TRAINED-O, n = 9) and never trained (NON-TRAINED, n = 6). A total of 150 stockpeople working on these farms were categorized according to training, as follow: i) Trained (TS, n = 43), those who

attended a formal handling skills training; ii) Non-trained, but had close contact with a trained stockperson (CTS, n = 62), those who did not attend any formal training, but had worked with a colleague who attended training; and iii) Non-trained (NT, n = 45), those who did not attend any training and no-one in the working group was trained on good practices of cattle handling. Indicators of quality of handling (including animals and stockpeople behaviors, as well as stockpeople attitudes) were measured during one workday, when carrying on vaccination handling procedures in around 236 ± 65 (mean ± SD) heads of cattle in each farm. We observed that NON-TRAINED farms had the poorest quality of handling, as well animal behaviors during handling ($P < 0.05$) when compared with the other farm categories (TRAINED-R, TRAINED-O). The stockpeople attitude and behaviors varied with their access to training or information about good practices of beef cattle handling ($P < 0.05$). People who participated in a formal training course (TS) had the highest positive and the lowest negative behaviors' and attitudes scores, compared with the others (CTS and NT). We also observed an effect of the working day progresses within a day ($P < 0.05$) only on NON-TRAINED farms, where handling became worse over time. Our results support previous research using cognitive-behavioral training in other farm species, showing that training stockpeople on good cattle handling practices led to better attitudes and behaviors toward cattle. Thus, training the stockpeople can be an effective and practical strategy to promote positive human-animal interaction in beef cattle farms, improving the quality of life of animals and workers.

Key words: Zebu cattle behavior, quality of handling, working day time

1. Introduction

There is a concern about the welfare of farm animals, partially in relation to handling procedures (Hemsworth and Coleman, 2011). In spite of that, the stockmanship is still receiving relatively little attention from the livestock industries notwistanding the fact that there is evidence demonstrating that animal care has a major impact on their welfare (Coleman and Hemsworth, 2014).

It is well known that stockperson attitudes in relation to their animals is directly associated with their behaviors during handling and, when rough practices are used, the animal welfare can be negatively affected, resulting in an increase of fear towards humans (Hemsworth, 2007; Hemsworth and Coleman, 2011). On the other hand, when the animals experience positive interactions with humans, they become less fearful, which, in turn, facilitates easier handling (Schmied et al, 2010; Probst et al., 2012).

In most of the cases, stockpeople negative attitudes and behaviors that are aversive to the animals cannot be characterized as intentional cruelty (Hemsworth, 2007). Most of the time, stockpeople may believe that their behaviors are harmless for the animals, and this assumption is very common in the daily routine of livestock production, where handling practices are learned from working "on the job" (Hemsworth, 2007). Many farmers can consider beef cattle as dangerous and not easy to handle (Boivin et al, 2007), which may underlying difficulties of people towards handling these animals, and could increase their stress during handling. Additionally, workload and the risk of accidents are considered to be cause of stress to stockpeople (Douphrate et al., 2013; Lindahl et al., 2013; Menger et al., 2016). Stress, in turn, influences stockpeople decision-making, since when stressed, the willingness to adopt better handling practices decreased (Burnett, 2014). Therefore, working time and tiredness may also be related to their behavior. Nevertheless, to our

knowledge, the role of long lasting handling, with repetitive, dangerous and exhausted tasks, such as during beef cattle handling with large herds, has been poorly investigated to date. In a completely different research area (medical nursing), it has been proposed and studied the concepts of compassion discomfort, compassion stress and, at a final step, compassion fatigue, leading to poor quality of care (Coetze and Klopper, 2010). This research could be source of inspiration for understanding stockpeople behavior towards farm animals. Thus, complex factors determining human behavior including beliefs, job satisfaction (Boivin et al., 2003, Coleman and Hemsworth, 2014) and workload should be further considered.

Stockpeople behavior during livestock handling can be improved by a careful selection of people and through the development of training programs designed to reduce animal stress during handling and improve the animal's welfare (Boivin et al., 2007). Thus, training programs especially targeted to change stockperson attitudes and behaviors offers a good opportunity to improve the human-animal interactions in livestock industries (Hemsworth and Coleman, 2011), using behavioral modification rather than just skills transfer (Coleman and Hemsworth, 2014). Indeed, there are already some studies with dairy cattle and pigs, demonstrating the potential of interventions using cognitive and behavioral techniques to improve the attitudes and, consequently, the workers' behavior (Hemsworth et al. 1994; Coleman et al., 2000; Hemsworth et al, 2002). Nevertheless, these authors highlighted that the efficacy of the training can depend on the interactions between all trained and non- trained stockpeople on the same farm, thus, developing or not a common culture towards the animals within the farm (Coleman and Hemsworth, 2014). They stated that "an explicit attention is given to barriers to change, such as pressure to conform from their co-workers and incorrect beliefs about perceived barriers to change, such as poor facilities, poor animal temperament and lack of time."

It is evident that handling skills training programs are needed to improve animal handling in livestock production, especially in Brazil, the country with the largest commercial cattle herd in the world, with 215.2 million head (IBGE, 2016) and which is projected to be the top global beef exporter in 2017, overtaking India and Australia (USDA, 2016). Additionally, there is a growing demand for training of stockpeople from Brazilian farmers (Zuin et al., 2014). Training on good practices of beef cattle handling for those workers has been already carried out (Zuin et al., 2014), however, there has been no research addressing the effects of this kind of training on the stockperson attitudes and behavior, and on beef cattle welfare in Brazil particularly, but also, to our knowledge, in any part of the world to date. Investigating training in beef productions is also interesting, compared to previous studies in dairy production (Hemsworth et al, 2002) as there is no daily handling for milking and, so, much less possibilities for the trainees to daily practice after they followed their training sessions.

Thus, the aims of this study were to evaluate the potential impacts of handling skills training on good practices of cattle handling on stockpeople' attitudes and behavior, and cattle welfare in Brazilian beef farms. Our survey aimed to compare farms where all stockpeople were trained, to farms where only some where trained, and farms where none where trained. Finally, we also aimed to study whether the quality of cattle handling deteriorates as the working day time passes. We hypothesized that trained stockpeople has better attitudes and behaviors toward cattle, handling the cattle always the same way over time, during a working day in a corral.

2. Materials and Methods

This research was carried out according to Brazilian legislation and was approved by the Committee for the Ethical Use of Animals from the Faculty of Agricultural and Veterinary Sciences of São Paulo State University (Protocol n. 014198/14), Jaboticabal, SP. Brazil.

2.1. Farms, stockpeople, training strategies and animals

The study was conducted in 24 commercial beef cattle farms, located in Northeastern Pará State, Brazil. Three groups of farms were identified, defined according to their access in training on cattle handling, as follows: *i*) Farms with systematic training programs (TRAINED-R, n = 9), represented by farms assisted systematically by an expert in applied ethology and animal welfare who, through periodic visits (at least once every six months), trained and advised the stockpeople about good practices of beef cattle handling for more than one year (in this case, some of the workers were day laborers, therefore, not trained); *ii*) Farms with occasional training (TRAINED-O, n = 9), farms where at least one of the permanent employees attended to a formal training on good practices of beef cattle handling; and *iii*) Non trained farms (NON-TRAINED, n = 6), farms where none of the workers received any training on good practices of beef cattle handling.

A total of 150 stockpeople (all men), responsible for cattle handling in the studied farms, were categorized according to their previous access to information or training on good practices of cattle handling, as follows: i) Trained stockpeople (TS, n = 43), those who had attended to a formal training; ii) Non-trained but had close contact with a trained stockperson (CTS, n = 62), those who did not attend any formal training, but worked with a colleague who attended a training; and iii) Non-trained stockperson (NT, n = 45), those who did not attend any training and nobody from his working group was trained on good

practices of cattle handling. The distributions of the stockpeople categories within each farm class are presented in Table 1.

Table 1. Distribution of the type of stockpeople (TS = Trained; CTS = Non-trained, but had close contact with a trained stockperson; NT = Non-trained) on each group of farms: farms with regular training = TRAINED-R, farms with punctual training sections = TRAINED-O and farms without any type of training = NON-TRAINED.

Type of stockpeople	TRAINED-R	TRAINED-O	NON-TRAINED
TS	69.8% (n=30)	30.2% (n=13)	0
CTS	37.1% (n=23)	46.8% (n=29)	16.1% (n=10)
NT	15.6% (n=7)	17.8% (n=8)	66.7% (n=30)

The activities carried out during the stockpeople formal training in "good practices of beef cattle handling" involved theoretical lectures and practical classes, aiming to encourage the stockpeople to improve cattle handling and minimize animal stress by improving their interaction with cattle. The training emphasized the importance of using the knowledge about cattle behavior to define low stress handling strategies. The course also addresses the concept of animal welfare, human-animal interactions and its implication on cattle production, presenting a series of recommendations about beef cattle handling. All this information is based in guidelines developed by our research group in Brazil, addressing, among other subjects: (e.g. calves handling at birth and weaning, identification, cattle loading and transport), and beef cattle vaccination procedures (Paranhos da Costa et al., 2006). It was emphasized that aversive handling (highly or moderately repeatedly) has a negative influence on cattle behavior, making them become fearful of humans. The course was offered over three continuous days and, on the last day, there were practical classes on cattle handling.

A total of 5,659 animals where handled in all farms, with an average of 236 ± 65 (mean ± SD) animals handled per day in each farm. The animals were classified in three

groups, according to the breeds and animal categories (herein referred as animal breed-type): 1 = Nellore calves, 2 = Nellore cows and heifers, 3 = Predominantly crossbred adult bulls. These groups of animals were represented in all categories of farm training.

2.2 Assessing the stockpeople and animals behaviors during the handling

All assessments were carried out during one day of vaccination procedures (subcutaneous injection of vaccine against foot-and-mouth disease) at the corral. Three trained observers, always the same through the whole study, recorded the behaviors when animals were handled by stockpeople in three corral sections: the crowding pen, the working chute and the squeeze chute. Stockpeople were informed previously that the observers were there to evaluate cattle behavior, so they were partially aware about the purpose of the study.

2.2.1 Assessment of cattle behavior and accidents

Cattle behavior and accidents were assessed in the crowding pen and working chute during the handling for vaccination procedure. The following behavioral categories were recorded: a) *jumping* – the animal pushed itself off the surface and kept its front legs into the air; b) *lying down* – the animal lay down smoothly, maintaining sternal recumbence; c) *return/balking* – when the stockperson tried to drive the animal in a particular direction, but it moved backwards or avoided entering in the working chute or in the squeeze chute; d) *attack* – the animal showed aggressive behavior toward the stockperson (head front down, move toward the human, sometimes air blowing or charging); e) *falls* – when any part of the animal body, except the hoofs, touched the ground as a result of a sudden movement; f) *bump on the facilities* – when cattle hit or stumbled in any structure of the facilities and; g)

being trampled by another animal in the chute – a lying down or keeling animal was trampled by another individual during handling. The frequencies of all these categories were summed to compose a new variable, as an indicator of cattle behavior, named *'undesirable behaviors and accidents' (UBA).*

2.2.2 Assessment of stockpeople behaviors and attitudes

The quality of handling was assessed by recording the behaviors of each stockperson, individually, during handling procedures in the crowing pen, working chute and squeeze chute. In the crowding pen and the working chute the following behavioral categories were recorded: *correct use of handling flag*, recorded when the handler applied the concept of flight zone to move the animals, without physical contact with them; *positive tactile interactions* (adapted from Waiblinger et al., 2002), recording all occurrences of gentle touch on the animals' body; *negative tactile interactions* (adapted from Waiblinger et al., 2002), recording the occurrences of hitting the gate against the animal, hitting and prodding the animals with the hands or any implement, e.g. wooden stick and electric prod, (positive and negative tactile interactions are described in Table 2); *positive vocalization* (adapted from Waiblinger et al., 2002), recording the use of voice commands in low tone and soft whistles; *negative vocalization and other acoustic sounds* (adapted from Waiblinger et al., 2002), by measuring the frequency of shouts, loud whistles, and noises when hitting any tool on the corral structure; *negative contact with the animals' tail*, when twisting the tail to stimulate the animal to move. Later, the frequencies of these behaviors were summed, resulting in two variables of quality of stockpeople handling, as follow: *Stockpeople positive behaviors (PB)* = sum of the frequencies for correct use of handling

flag; positive tactile interactions, and positive vocalization; and *Stockpeople negative behaviors (NB)*: sum of the frequencies for negative tactile interactions; negative vocalization and other acoustic sounds and negative contacts with the animals' tail.

Table 2. Definitions of tactile interactions with the animal made by the stockperson.

Category	Definition
Touch	Act of softly touch the animal with the hands and fingers or with any kind of tool like flag, stick, stinger, etc. This contact is not violent and does not cause any kind of injury to the animal.
Hitting and prodding the animal	Act of brusquely hit the animal with the hands, feet or any kind of tool like flag, stick, stinger, etc. This contact is violent and may cause injury to the animal.
Hitting the gate against the animal	Brusquely hit the animals with a gate (corral structure with the function of close or split environments).
Use of electric prod	Act of touching the animal with a prod that promotes electric discharges.

Positive and negative behaviors (PB and NB) were also recorded in the squeeze chute, where additional handling actions were recorded, considering three situations: i) when the animals enters into the squeeze chute, where was assessed the operation of the front gate of the squeeze chute (GATE), by assigning two scores: (1) correct = when the door was kept closed or opened just to stimulate the animal to entry, being closed before trying to restraint the animal with the head bail, and (2) wrong = when the door was kept open or closed only after restraint the animal. The relative frequencies of score 2 (worst) per farm was used in the statistical analyses.

The second situation (ii) when restraining the animal with the head bail, we recorded the frequencies of stockperson attempts to restraint each animal with the head bail (RESTRAIN), and the speed and force used by him during the performance of that action (REST.HB), assigning the following scores: (1) = when the head bail was operated slowly

when approaching it to the animal's neck, without hitting it, (2) = when the head bail was operated quickly, but without hitting the animal's neck, and (3) = when the head bail was operated with quick and vigorous movements, hitting animal's body with force). The statistical analyses of RESTRAIN was carried out considering the relative frequency at which the stockperson did three or more attempts to restrain an animal; and for REST.HB we used the relative frequencies of the worst scores (3) per farm.

Finally, during vaccination (iii), we recorded the failures in vaccination attempt (F.VACCINATION), measuring the frequency of needle insertions to deliver one dose of vaccine; wrong injection site (SITE), registering where the vaccine was injected (e.g. neck shoulder, hump, ribs, rump and base of the tail), considering the relative frequency of animals that received the injection outside of the neck; poor quality of subcutaneous injection (PQI), assigning two scores: (1) = when the handler pulls the skin of the animal and inserts the needle in the skin fold (recommended procedure for subcutaneous injection), and (2) = when the needle insertion is performed without pulling the skin; and attempt to escape (ESCAPE), measuring the frequency of escape attempts during the handling at the squeeze chute, where ESCAPE was defined by the times each animal takes the head or the body out from the lateral window or gate of the squeeze chute. The relative frequencies (within each farm) of fails in vaccination (defined by the occurrence of two or more needle insertions to deliver one dose of the vaccine), animals vaccinated outside of the neck, score 2 of PQI, and animals attempting to escape, were assumed as dependent variables for the statistical analyses.

Between one week and one month after assessing the quality of cattle handling, each stockperson participated in a structured interview, aiming to assess their attitudes towards cattle, work satisfaction and their perceptions about handling practices. The

interview consisted in 26-items (adapted from Boivin et al., 2007) divided in three parts, as presented in Table 3. The first part, addressing attitudes toward cattle, comprised 11-items; the second part (also with 11-items) focused on the stockperson attitudes toward handling practices; and the third part (with five items) was related to work satisfaction. Before starting the interview, we informed all stockpeople that their opinion about working with cattle would be useful addition to our study. As not all stockpeople were able to read the questionnaire, interviewers read each question without any comment and recorded their responses in categories, from 1 (fully disagree) to 5 (fully agree). At the end of the study, all the interviewed stockpeople were informed about the full purpose of the study, detailing that all data would be processed and treated anonymously when doing data analysis and results interpretation for publication.

2.3. Statistical analysis

Initially a multivariate factor analyses was applied to questionnaire data, extracting the factors by the principal components analyses (PCA) methodology, with varimax rotation, using Statistica software (Statsoft, version 7). To do it, all questionnaires answers were summed on each response (number of answers from 1 to 5 for each question). It was possible to identify, among the 26 items, two components that represented positive and negative stockpeople attitudes. These components comprised 16 and 10 questions respectively with loadings above 0.5 (Table 3). Based on the factor analyses results, the responses of each stockperson were summed to produce composite scores for the 16 positive and 10 negative attitudes questions (adapted from Breuer et al., 2000).

For the subsequent statistical analyses, two sets of data were created, one considering data from stockpeople as experimental unit and the other with farm as experimental unit.

2.3.1. Effects of stockpeople training groups on their individual attitudes and behaviors

To evaluate the effects of stockpeople training on "good practices of beef cattle handling" on their attitudes (positive and negative), linear mixed models were used, via PROC MIXED of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA), with positive and negative attitudes as response variables, stockperson training category (TS, CTS and NT) as fixed effect and farm as random effect. Normal distribution was visually tested by plotting raw and standardized residuals.

To assess the effects of stockperson access to training on "good practices of beef cattle handling" on their positive and negative behaviors, generalized linear mixed models were fitted, with lognormal distributions of the response variables (PB and NB), using the PROC GLIMMIX of SAS. Models included the fixed effect of stockperson training category (TS, CTS and NT), and farm, as a random effect. Raw and standardized residuals were plotted, and their distributions were examined to determine the distributions of the response variables.

In all analyses, the post hoc Tukey test was applied for means comparisons and significance of P-values was set to $P < 0.05$. Additionally, characteristics of stockpeople such as age, time of experience working with cattle, and time spend working in the assessed farm were previously tested, and no significant effects ($P > 0.05$) were found, so they were excluded of the final models.

2.3.2. Effects of farm training groups on stockpeople quality of handling

The response variables in the farm dataset were: GATE, RESTRAIN, REST.HB, F.VACCINATION, SITE, PQI, ESCAPE and UBA, besides *farm positive behaviors (FPB*, characterized by the sum of PB from all stockpeople within each farm during the whole handling sections); and *farm negative behaviors (FNB*, defined by the sum of NB from all the stockpeople within each farm). Use of electric prod was not included in these analyses, because only three farms (of NON-TRAINED group) used it. Raw and standardized residuals were plotted, and their distributions were examined to determine the distributions of the dependent variables: FPB, FNB, RESTRAIN, F.VACCINATION and ESCAPE following lognormal distributions; REST.HB and UBA, Poisson distribution; and GATE, SITE, PQI normal distributions.

The effect of farm categories of training on farm handling variables (FPB, FNB, GATE, RESTRAIN, REST.HB, F.VACCINATION, SITE and PQI) was assessed. Models included category of farm (TRAINED-R, TRAINED-O and NON-TRAINED) as a fixed effect, and handling speed (number of animals per minute) as a covariate with linear effect. The effects of number of stockpeople at each handling session and breed-type of animals were previously tested for each response variable, and were not included in the final models because no significant effects were found ($P > 0.05$). The PROC MIXED of SAS was used for variables with normal distribution (GATE, SITE, and PQI) and PROC GLIMMIX for non-normal variables, fitting generalized linear mixed models, specifying the respective distributions of response variables.

To test the relationship between farm training category with ESCAPE and UBA, generalized linear mixed models were fitted, using PROC GLIMMIX of SAS, with the

fixed effects of farm category (TRAINED-R, TRAINED-O and NON-TRAINED), breed-type of animals (1-Nellore calves, 2-Nellore cows and heifers, and 3-Predominantly crossbred adult males) and their interaction. Handling speed (number of animals per minute) was included as a covariate with linear effect.

2.3.3. *Effect of the time elapsed within a workday on the quality of handling and cattle behavior*

Finally, we tested the hypothesis that the quality of handling deteriorates as time elapses within a workday, and a simple regression analyses was done for each farm training category, using handling quality and cattle behavior as the dependent variables (FPB, FNB and UBA) and the effect of time of handling (each 15 min of work). To do this, we summed all those variables (FPB, FNB and UBA) independently, on every 15 minutes, until the day work finished on each farm. For these analyses, the response variables were log-transformed (on a $\log_e$ basis) to obtain normal distributions, and then the PROC GLM of SAS was used.

3. Results

3.1. Effects of stockpeople training groups on their individual attitudes and behaviors

Questionnaire data generated two main factors, explaining 93,43% of the variation in the data set (Table 3). In the first factor (with 64.43% of the variation), 16 questions had positive loadings above 0.50, and all of them were characterized as indicative of stockpersons positive attitudes. In the second factor (with 29.00% of the variation), 10 questions had negative loadings above 0.50, and these questions were characterized as

indicative of negative attitudes. Most of the stockpeople showed higher agreement with the questions indicating positive attitudes and disagreement with the questions indicating negative attitudes toward cattle (see Table 3).

Table 3. Percentages of responses (from 1 - fully disagree to 5 - fully agree) characterizing the stockpeople (n = 150) attitudes toward cattle, handling behavior and work satisfaction. Loadings of the two factors estimated by the Factor Analysis for questionnaire data.

	1. Fully disagree	2	3	4	5. Fully agree	Factor 1 Positive Attitudes	Factor 2 Negative Attitudes
Attitudes toward cattle							
Cattle have good memory	5	0	6	12	78	0.99200*	0.102538
Recognize the stockperson	5	1	4	7	83	0.99418*	0.083420
Have learning capacity	1	1	4	6	89	0.99209*	0.114302
Easily scares	2	1	13	3	81	0.99203*	0.084987
Are afraid of unknown people	6	1	14	10	69	0.99351*	0.074933
Are sensitive to pain	0	0	0	2	98	0.99123*	0.096451
Can suffer and feel pain when hits it	1	0	1	2	97	0.99208*	0.090411
Flees for fear	2	1	3	9	84	0.98987*	0.123546
Are stubborn and hard to handle	29	6	43	9	12	-0.17231	-0.592567*
Are afraid of known people	64	6	19	3	8	-0.17752	-0.979287*
Are dangerous	34	1	29	9	27	0.40104	-0.809054*
Attitudes toward handling behavior							
You need to hit the cattle to handling it	66	12	18	3	1	-0.34091	-0.926566*
Iron stick is useful	77	3	9	6	5	-0.20006	-0.941974*
Handling flag disrupts handling	81	3	10	1	5	-0.18402	-0.956604*
You need to jump and shake the hands	57	5	14	8	16	-0.03768	-0.978164*
You need to poke whit a stick to make them enter into the squeeze chute	36	8	34	8	14	-0.14944	-0.832649*
You need to increase your voice to be obeyed	73	9	14	2	2	-0.27101	-0.939056*
You need to apply a lot of effort	50	10	13	3	24	0.18340	-0.949710*
You talk softly to be obeyed	0	0	5	6	88	0.99217*	0.121102

Handling flag is useful	5	0	3	10	82	0.99176*	0.094482
You need to use softy touch to be obeyed	6	4	30	10	49	0.88150*	0.082275
Facilities influence the handling	3	1	1	3	93	0.99160*	0.080999
Work satisfaction							
You want to work for long time	10	2	14	10	64	0.99763*	0.026931
Like work with cattle	1	1	8	13	77	0.98542*	0.159934
Training is important	1	0	0	12	87	0.98418*	0.135872
If you had the opportunity, you would participate on training	1	1	2	4	92	0.99151*	0.100503
Eigenvalues						16.75218	7.53908
% Total variance						64.43	29.00

Loadings with * represent the questions with the most positive contributions on factor 1, representing the stockpeople positive attitudes; and the most negative contributions on factor 2, representing the stockpeople negative attitudes.

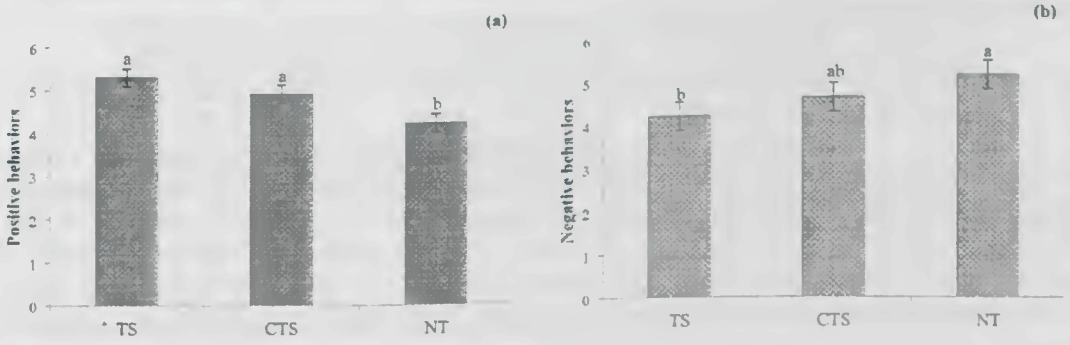
Questionnaire responses, for positive and negative attitudes, were summed for each stockperson to produce composite measures of positive and negative attitudes for each stockperson (adapted from Breuer et al., 2000). The average score of all stockpersons' positive attitudes was 74.0 ± 4.5 (mean ± SD), ranging from 56 to 80; and the average score of their negative attitudes was 21.4 ± 6.7, ranging from 10 to 41. The percentage of stockpeople showing positive attitudes scores above the average was higher for trained stockpeople group (65.5, 61.2 and 28.1%, for TS, CTS and NT, respectively) and, as expected, the percentages of stockpeople showing negative attitudes scores above the average was lower for the trained stockpeople group (27.3, 59.7 and 68.8% for TS, CTS and NT, respectively).

There was a significant difference between stockpeople training groups on their positive and negative attitude scores ($F_{2, 131} = 4.50$, and $F_{2, 131} = 16.30$, $P < 0.01$). Trained stockpeople had significantly higher ($P < 0.01$) positive attitudes (mean ± SD) (75.41 ± 0.71) than non-trained ones (72.40 ± 0.72), with no difference between these two groups

and the stockpeople who had occasional access to the information (74.14 ± 0.60) ($P > 0.05$). On the other hand, negative attitudes were significantly higher for non-trained group (25.45 ± 0.99), followed by occasionally informed (21.26 ± 0.80), and lower for trained stockpeople (17.54 ± 0.97), which significantly differed among each other ($P < 0.01$).

Significant differences between stockpeople training groups were found for both PB and NB ($F_{2, 135} = 7.32$, $P < 0.01$ and $F_{2, 132} = 3.38$, $P < 0.05$, respectively). As expected, non-trained stockpeople showed lower PB (adjusted mean ± SE) (4.22 ± 0.20) than trained (5.30 ± 0.21) and the occasionally informed stockpeople (4.91 ± 0.17), who did not differ between each other (Figure 1a). Negative behaviors were more frequent for non-trained stockpeople (5.12 ± 0.32), compared to trained stockpeople (4.18 ± 0.33), but did not differ from occasionally informed (4.60 ± 0.31), which, in turn, did not differ from the trained stockpeople (Figure 1b).

Figure 1. Adjusted means (± SE) of positive behaviors (a) and negative behaviors (b) according to the stockpeople access to good practices of beef cattle handling information or training (Trained stockperson= TS, Non-trained but had close contact with trained stockperson= CTS and Non-trained stockperson= NT). Same case letters are not statistically different ($P > 0.05$), by Tukey test.



3.2. Effects of farm training groups on stockpeople quality of handling

A significant effect of the farm training category was found on FPB ($F_{2,20} = 3.65$, $P = 0.045$), REST.HB ($F_{2,17} = 65.16$, $P < 0.001$) and PQI ($F_{2,20} = 7.68$, $P = 0.003$) (Table 4). Post-hoc tests showed that FPB was higher for TRAINED-R than for NON-TRAINED farms, but did not differ from TRAINED-O (Table 4). The REST.HB was higher for NON-TRAINED, followed by TRAINED-O and TRAINED-R. The variable PQI had higher values for NON-TRAINED, with a 100% of those farms vaccinating the animals in the wrong way, followed by the TRAINED-O and TRAINED-R, which did not differ between from each other (48.25 and 22.78%, respectively). No significant effects of farm training category were found for FNB, GATE, RESTRAIN, F.VACCINATION, SITE, and ESCAPE ($P > 0.05$). Nevertheless, an interesting numeric difference was found for ESCAPE, having around 6 times more attempts of escape on the squeeze chute for NON-TRAINED (1.41 ± 0.75) when compared with TRAINED-R (0.24 ± 0.61).

Table 4. Adjusted means (± SE) of the effect of each farm training group (farm training group = TRAINED-R, farms with at least one employer trained in an official training course = TRAINED-O and Farms without any type of training = NON-TRAINED) on the farm handling variables. Farm positive behaviors (FPB), the speed and force used by the stockperson when restraint the animal with the head bail (REST.HB), and poor quality of subcutaneous injection (PQI).

Variable	TRAINED-R	TRAINED-O	NON-TRAINED
FPB	$7.43^{a} \pm 0.19$	$7.03^{ab} \pm 0.19$	$6.57^{b} \pm 0.25$
REST.HB	$2.49^{c} \pm 0.10$	$3.20^{b} \pm 0.07$	$3.84^{a} \pm 0.07$
PQI	$22.78^{b} \pm 11.96$	$48.25^{b} \pm 11.85$	$100.60^{a} \pm 15.25$

$^{a-c}$ Means followed by the same uppercase letters in the same row are not statistically different ($P > 0.05$) by Tukey test.

For UBA, we found a significant interaction between category of farm x animal breed-type ($F_{4,14} = 10.48$, $P < 0.01$), with higher UBA means for the crossbred adult males

on the NON-TRAINED group and lower for TRAINED-R with Nellore cows and heifers, as shown on Table 5.

Table 5. Adjusted means (± SE) of undesirable cattle behavior and accident (UBA) according to the farm training group (TRAINED-R, TRAINED-O and NON-TRAINED) and breed-type (Nellore calves, Nellore cows and heifers and crossbred adult males) interaction. Breed-type on the column and farm training group on the rows.

Training/animal category	Nellore calves	Nellore cows and heifers	Crossbred adult males
TRAINED-R	$4.15^{Bb} \pm 0.14$	$3.66^{Cb} \pm 0.07$	$5.16^{Ab} \pm 0.06$
TRAINED-O	$3.94^{Bb} \pm 0.10$	$3.85^{Bab} \pm 0.09$	$4.65^{Ac} \pm 0.05$
NON-TRAINED	$4.87^{Ba} \pm 0.06$	$4.02^{Ca} \pm 0.09$	$5.80^{Aa} \pm 0.06$

$^{A-C}$ Means followed by the same uppercase letters in the same row are not statistically different ($P > 0.05$), by Tukey test. $^{a-c}$ Means followed by the same lower case letters in the same column are not statistically different ($P > 0.05$), by Tukey test.

3.3. Effect of the time elapsed within a workday on quality of handling and cattle behavior

For the TRAINED-R and TRAINED-O farm groups, no significant effect ($P > 0.05$) of pass of time (working day progresses) was found on handling variables (FPB, FNB and UBA). Nevertheless, for the NON-TRAINED group, significant effects were found on FPB ($F_1 = 9.06$, $P < 0.01$) and UBA ($F_1 = 5.45$, $P < 0.05$) and a trend on FNB ($F_1 = 3.52$, $P = 0.065$). As 15 min of handling passes within a day, positive behaviors and undesirable behavior decreases (0.06 ± 0.02 and 0.08 ± 0.03, respectively) as well as negative behaviors increases (0.04 ± 0.02) (Table 6).

Table 6. Relation of the time of handling within a day on stockpeople and cattle behavior for NON-TRAINED farm group: $Y = a + bx + SE_{x,y}$, where Y is FPB (Farm positive behaviors), FNB (Farm negative behaviors) and UBA (undesirable cattle behaviors and accidents), a = intercept, b = estimated time, x = measured time and $SE_{x,y}$ = standard error of prediction.

Variable	R^2	Intercept	Time estimated	$SE_{x,y}$	P-value
FPB	0.133116	4.402579669	-0.059310290	0.01970464	0.0038
FNB	0.056322	4.610554309	0.037497334	0.01998245	0.0655
UBA	0.098362	2.736607042	-0.081841124	0.03504197	0.0236

4. Discussion

The stockperson attitudes and behavior may be the most influential factors affecting animal handling, welfare and productivity (Coleman and Hemsworth, 2014). For that reason, it is important to understand how to improve the human-animal relationships during routine handling in beef cattle farms. Several stockpeople training programs have been developed in Brazil (Zuin et al., 2014), but there is no scientific information about its real impact on the quality of cattle handling and animal welfare. To our knowledge, this is the first published study in any country aiming to investigate a potential impact of different accesses to information about good practices of beef cattle handling on stockpeople attitudes and quality of handling.

In general, most of the stockpeople assessed in our study had higher percentages of agreement with the positive attitudes questions and lower with the negative attitudes ones. Similar findings were reported by Boivin et al. (2007), working with French cattle farmers. These results suggest that a majority of beef stockpeople has adequate attitudes about cattle behavior and handling. However, it is not possible to interpret these scores in absolute terms, because the number of studies of this kind is very limited with no standardized evaluation methodologies. Moreover, it is not known the extent to which slight variations in positive attitudes, for example, might affect behavior.

Nevertheless, our study reveals differences between the stockpeople training categories, and potential room for improvement. Although the training program studied

here did not target attitudes specifically related to fear provoking handling behavior improved, we found that stockpeople attitudes were associated with their access to handling skills training, suggesting that training programs of this nature can be effective in improving stockpeople attitudes and behaviors. Stockpeople who participated in this formal training course had the highest positive and the lowest negative attitudes scores. Hemsworth et al. (2002) reported that a training based on cognitive-behavioral interventions improved stockpeople attitudes leading to a reduction in their use of negative interactions with cows, with consequent reduction in dairy cows' fear of humans. After a similar intervention with stockpeople working on pigs' commercial farms, Coleman et al. (2000) found that after the stockpeople training procedure, improved attitudes were directed towards handling pigs. Although we did not carry out a longitudinal assessment of stockpeople attitudes and behaviors before and after training sections, our results are consistent with those studies.

In dairy and pig production, daily handling following immediately the training sessions often occurs. Practicing animal handling immediately and regularly allows stockpeople experiencing every day the benefit of the training session, by observing consequences of their behavior, on animal behavior. Thus, attitudes toward the animal, in particular behavioral attitudes, could be improved by a feedback loop (Hemsworth and Coleman, 2011). This is a common situation in dairy and pig production systems where humans and animals have close contact every day; however, such situation usually does not occur in beef cattle production. Despite this difference, our results would suggest a possible positive impact of the handling skills training on stockpeople attitudes. Nevertheless, we need to be careful with any conclusion, even if they are promising, since they are explorative, and there are possible confounding factors in the present study. According to

our factor analysis results, higher positive and lower negative attitudes toward cattle were also associated with job satisfaction. It has been previously shown that job satisfaction and job performance are partly related (Judge et al, 2001). In addition, the managing style in the farms that organized the training sessions could have been different from the one who did not, making them dissimilar in other aspects than the training. Human resources management, farm management practices, handling facilities, etc., may have varied between the three group of studied farms; thus, a better managing style within the farm that organized regular training could have been more favorable to the development of positive attitudes among the stockpeople toward the animals, or could have reinforced the beneficial effect of the training.

Another important result of our study was that stockperson access to training and farm training category were related to more positive stockpeople behaviors. Indeed, it was expected that trained people behave better toward animals than those ones that did not have any training or information about good cattle handling practices, in consistency with other studies made in dairy cattle and pork industry (Hemsworth et al., 1994; Coleman et al., 2000; Hemsworth et al., 2002). Interestingly, stockpeople in contact with trained ones but not trained themselves also performed more positive behaviors toward cattle, suggesting a possible social facilitation or transmission for these types of behaviors. Social facilitation can be defined by the process by which the presence of another person affects performance (Guerin, 2010). However, other mechanisms can also be evolved, as stated by Kanekar (1976, p. 1) "attitudinal responses can be developed by conditioned emotional responses acquired through Pavlovian or classical conditioning". It means that humans can experience the emotions of others and, through the observation of attitudes reactions of other persons' to an specific object or being (the animal, for example), the others' actions can act as

reinforcement to the subject, behaving in the similar way (Kanekar, 1976; Hemsworth and Coleman, 2011). Further, human behavior is a composition of four elements: "the action performed, the target at which the action is directed, the context in which it is performed, and the time at which is performed" (Fishbein and Ajzen, 2010, p 29). So, the presence of someone who has being trained, during handling, has not only the potential to change the normal context of the handling in the farm, but also the behavior made by those trained people can affect the behavior of the non-trained people, possibly influencing them to behave more properly.

In relation to the other handling variables, at the NON-TRAINED farms we found higher percentage of rough restraining of the animals with head bail and poor quality of subcutaneous injection. Non-trained people usually handled the animals in a way that they have learned along their lifetime, culturally. According to Hemsworth et al. (2007), stockpeople normally may not appreciate that some handling practices are stressful and painful for the animals. Therefore, this may be the reason for stockpeople from the NON-TRAINED farms using more aversive practices when handling the cattle. A Brazilian study about quality of cattle vaccination, showed that when people do not use good handling practices during vaccination procedures (for example, vaccinating the animals at the wrong place or in a wrong way) the animals were more stressed, displayed more undesirable behaviors, and consequently suffered more welfare problems (Chiquitelli Neto et al., 2015). Indeed, our results also showed higher frequencies of undesirable animal behaviors' and accidents for the crossbred adult males, especially in NON-TRAINED farms, as well more escape behavior, this may confirm that human behavior affects animals' behaviors (Ellingsen et al., 2014), with negative consequences for animal welfare, due to the stressful situations that they may experience (Hemsworth et al., 1994). Animals learn to avoid

conditioned stimuli related with aversive events and, through this kind of learning (conditioning), the farm animals' behavioral reactions may be regulated by the nature of previous experiences when interacting with humans (Hemsworth et al., 2002). Moreover, for adult cattle, previous specific interactions, and their associated predictability during handling, can influence their behavior (Waiblinger et al., 2006).

One important and new result of our study is that we observed a relationship between the duration of handling within a day and the occurrence of positive and negative behaviors, but only on the NON-TRAINED farms. This suggests that handling deteriorated over time in this farm group. It has been stated that people understanding and applying the knowledge acquired in a training course about animal handling, with information about animal behavior, results in better handling of the animals, and consequently the animals behave better, leading people to work less stressed (Lindahl et al., 2013). When people do not know how best to handle animals, they could become more stressed, and consequently, their behavior could get worst (M.C. Ceballos, personal conversation with some stockpeople). Weakening attention, desensitization, weariness, but also irritability and poor judgment (Coetze and Klopper, 2010) might have occurred, explaining the reduction in positive stockpeople behaviors and the increase in negative ones, in the non-trained farms. It is also plausible that when people act with negative behaviors (hitting, shouting, jumping, etc.) during handling, they feel more tired. Grandin (2015) mentioned that tired people will abuse animals and that, in poultry and pig companies, injuries and deaths of the animals may double after the truck loading team works more than 6 hours. Our results are only explorative in this study and we need to develop new indicators of these dimensions for later studies. However, taking all of this into account, we suggest that training the stockpeople could help them to be more conscientious for longer periods, despite the

fatigue. There is also the alternate possibility that trained stockpeople feel less exhausted by the way they handle the animals, resulting in less deterioration in their handling over time.

According to our results we recommend that beef cattle farmers invest in stockperson training, since is possible, through this intervention, to improve their performance when handling animals. Nevertheless, our study is just a stationary comparison. It would be interesting in future studies to do a longitudinal research, measuring and comparing the people attitudes and behaviors, as well as the animals' behavior before and after the stockpeople training interventions. Another interesting future study could be the evaluation of stockperson opinions about the behavioral-cognitive training interventions, aiming to better understand the impact on aspects related to their personal and professional life.

5. Conclusion

Together with explicit cognitive-behavioral training research, this study supports the principle, for the first time in beef cattle, that training stockpeople on good practice of beef cattle handling is associated with better attitudes and behaviors towards cattle. Furthermore, trained stockpeople maintained their good practice handling over time, compared to untrained stockpeople. Good handling practices are associated with better animal behaviors (being less reactive) and likely to be less stressed, not only because the animals behave better and are less prone to have accidents, but also because they are handled with more positive and less negative behaviors by the stockpeople. Finally, training stockpeople is an effective and practical strategy to promote positive human-animal interaction in beef cattle farms, improving the quality of life of animals and workers, with consequently improvement of farms welfare (animals and workers).

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CAPÍTULO 4 – Considerações finais

O treinamento dos vaqueiros nas fazendas de gado de corte tem papel importante na relação humano-animal. Uma boa relação reflete diretamente em benefícios para ambos, vaqueiros e bovinos, promovendo a diminuição do medo dos animais perante o homem, com consequente redução no risco de acidentes de trabalho, aumento da produtividade e melhoria do bem-estar dos animais. Além disso, uma boa relação humano-animal pode levar ao aumento na motivação dos trabalhadores e, consequentemente, melhoria no desempenho do seu trabalho, além da diminuição da rotatividade dos vaqueiros dentro da fazenda.

Adicionalmente aos benefícios do treinamento de vaqueiros, evidenciados no presente estudo, os relatos dos trabalhadores e as experiências vivenciadas nas fazendas treinadas nos levaram a constatar ganhos além daqueles mensurados pela pesquisa. Por exemplo, para os vaqueiros que receberam treinamento, o trabalho passou a ser menos estressante, sendo reconhecido por eles que no passado o manejo dos animais era mais difícil e resultava, muitas vezes, em acidentes. Durante as coletas de dados, foi possível observar também que os vaqueiros das fazendas treinadas trabalhavam com atenção e comprometimento, resultando em menor risco de ocorrência de animais agredidos, machucados ou conduzidos de forma inadequada. Foi relatado também pelos vaqueiros, que a realização de um manejo adequado reduz o cansaço durante um dia de trabalho. De modo geral, estas mudanças trouxeram melhorias nas condições de trabalho, o que refletiu em maior satisfação dos trabalhadores, que em alguns casos foi transmitida também para as suas relações familiares, como relatado por alguns deles.

Durante as coletas de dados, realizadas em dias de manejo de vacinação, percebemos que grande parte das fazendas treinadas contratavam vaqueiros diaristas, que na maioria dos casos nunca tiveram acesso a treinamento em boas práticas de manejo. Esta prática é muito comum nas fazendas de gado de corte brasileiras, onde se vacinam aproximadamente 400 animais por dia, e existe a crença de que ter mais trabalhadores no manejo faz com que este seja mais

rápido. No entanto, vaqueiros não treinados manejam pior do que os treinados, e isto pode influenciar negativamente o comportamento de todas as pessoas envolvidas no trabalho. Isso pode explicar o fato das interações negativas não terem diferido entre os três grupos de fazendas (treinadas, com treinamento pontual e não treinadas). Assim, acreditamos que a contratação de vaqueiros diaristas não treinados pode ser negativa para os produtores que investem em treinamentos para os vaqueiros de suas fazendas, com o intuito de melhorar o manejo. Caso os gerentes considerem necessário fazer isto, devido ao reduzido número de pessoas na equipe da fazenda, recomenda-se que estes diaristas sejam previamente orientados sobre as boas práticas de manejo, ou que realizem funções que envolvam menor contato com os animais (como manejar porteiras, por exemplo) e sob a supervisão de um responsável da equipe da fazenda. Normalmente, os diaristas são posicionados na entrada da seringa do curral, por ser um trabalho de bastante atividade e esforço. Porém, para realizar um bom manejo nessa área é indispensável entender sobre o comportamento dos animais, pois se ocorrer uma falha de manejo na entrada da seringa, há um grande risco de promover dificuldades de manejo nas etapas subsequentes (tronco coletivo e de contenção), pela promoção do aumento da reatividade dos animais. É evidente que a capacitação dos vaqueiros é importante para a transformação do manejo com os animais. No entanto, um ponto chave para aproveitar esse benefício dentro das fazendas é ter a capacidade de identificar as afinidades e as facilidades dos funcionários para realizar as diferentes funções dentro da mesma.

Além da preocupação com a capacitação dos funcionários que lidam diretamente com os animais, é necessário valorizar o bom trabalho desempenhado por eles dentro do sistema de produção, já que é um trabalho árduo e que exige extrema responsabilidade e atenção. Quando este trabalho é realizado por pessoas que gostam de animais e, principalmente, quando há o apoio e a valorização dos gestores do empreendimento, o trabalhador fica mais satisfeito, e trabalha com mais dedicação. É necessário entender que vaqueiros submetidos a situações precárias de trabalho e sem o devido direcionamento e capacitação não realizarão um serviço que atenda às necessidades dos animais,

prezando pelo bem-estar dos mesmos. O bem-estar nas fazendas deve ser o termo utilizado na produção animal, considerando todos os elementos da cadeia, pensando em promover condições para melhoria do bem-estar dos animais e dos trabalhadores. Questões como: *o funcionário que está trabalhando na minha propriedade tem uma moradia digna? O salário é adequado? Ele recebe hora extra? A minha propriedade oferece os equipamentos necessários para que este desenvolva seu serviço em segurança?* devem ser consideradas pelos gestores. Antes de cobrar desempenho dos funcionários vale a pena se perguntar sobre esses pontos. Caso haja falha em alguns deles, precisam ser levadas em consideração alternativas para melhorar as condições de trabalho e, assim, alcançar resultados satisfatórios nos sistemas de produção.

No Brasil, a contratação de profissionais para realizar treinamentos sobre boas práticas de manejo nas propriedades, já é uma realidade. A demanda por este tipo de serviço vem crescendo, motivado pelas novas exigências do mercado consumidor de carne e leite bovino, além da conscientização dos produtores sobre a sua importância. Uma alternativa econômica para a difusão destes treinamentos a um maior número de vaqueiros, passa pela associação dos produtores, seja por intermédio de cooperativas ou de sindicatos rurais, para contratar este tipo de treinamento. Enviar os vaqueiros mais proativos ou líderes da equipe de cada fazenda, para que eles transmitam depois o conhecimento adquirido aos demais companheiros da fazenda, pode ser uma opção viável.